BIOAVAILABILITY OF CONTAMINANTS IN URBAN SOILS

by

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B. Sc., University of Peradeniya, 2007
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Agronomy
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Abstract

Urban soils may contain harmful levels of potentially toxic contaminants. These contaminants transfer to humans via two exposure pathways: direct transfer (soil-humans by soil ingestion, dermal exposure and inhalation) and food chain transfer (soil-plant-humans). Soil amendments alter the speciation of the contaminants in soils and thereby modify their bioavailability. The objectives of this research were to access the plant availability of lead (Pb), arsenic (As), and polycyclic aromatic hydrocarbons (PAHs); bioaccessibility and speciation of soil Pb, and As; and dermal absorption of soil PAHs in contaminated urban soils; and effectiveness of soil organic amendments on reducing contaminant bioavailability. Two field experiments were conducted in Kansas City, MO and Indianapolis, IN. Both sites had elevated concentrations of Pb in soils (Kansas City site: 30-380 mg kg^{-1} and Indianapolis site: 200-700 mg kg^{-1}). Indianapolis site's soils also had elevated concentrations of As (40-100 mg kg^{-1}) and PAHs (benzo[a]pyrene: 1-10 mg kg^{-1}). A control treatment (no-compost) and compost-types (leaf compost and/or composted biosolids, non-composted biosolids, mushroom compost) were used as treatments. A leafy vegetable, a fruiting vegetable and a root crop were grown for two growing seasons. The treatments were arranged in split-plot design (main plot factor: compost; sub-plot factor plant-type). An *in vitro* steady fluid experiment was conducted using human skins to examine the dermal transfer of soil PAHs. The concentrations of Pb, As, and PAHs in the vegetables were low, except Pb in root crops. Compost reduced the bioaccessibility of Pb, but did not change the bioaccessibility of As. Selected soil samples were analyzed for speciation of Pb using extended x-ray absorption fine structure spectroscopy. The predominant Pb species were Pb sorbed to Fe oxy(hydr)oxide and to organic C. Stable Pb phosphates (pyromorphite) was

formed during the *in vitro* extraction. Dermal transfer experiments showed PAHs in the contaminated soils did not transfer through the skin. Stratum conium of the skin acted as a barrier for dermal transfer of soil PAHs. In general, the risk of food chain transfer of soil Pb, As, and PAHs were low in the studied sites and can be further reduced by compost addition. Bioaccessibility of Pb and As in urban soils were low. Dermal absorption of soil PAHs was insignificant.

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Dedication

To my loving parents for showing me the path of success in life

And

My husband, who has always being there to cheer me up and stood by me

through the good times and bad

Chapter 1 - Introduction

Urban agriculture is gaining attention as a means of revitalization of brownfields in urban areas. U. S. Environmental Protection Agency (USEPA) has given a special care about safeness of gardening in these sites, due to the presence of contaminants in soils that originated from past and nearby industrial and residential activities. There can be various inorganic and organic contaminants in these soils. Some of the common inorganic contaminants are lead (Pb), arsenic (As), Cadmium (Cd), chromium (Cr), and Copper (Cu) (Mielke et al., 1983; USEPA, 2011). Examples for common organic contaminants are polycyclic aromatic hydrocarbons (PAHs), chlordane, and dioxins (USEPA, 2011).

People can be exposed to these contaminants via direct and indirect exposure pathways. Direct exposure pathways address potential means of transferring these contaminants directly from the soil to humans. Soil ingestion is a predominant direct exposure pathway (Lanphear and Roghmann, 1997; Beak et al., 2006) and have gained extensive research attention. Some of the above contaminants, like PAHs and As can potentially absorb into humans via skin (i.e., dermal absorption) (ASTDR, 1995; ASTDR, 2007). Indirectly, these contaminants can transfer to humans via food chain (i.e., consumption of food crops grown in these contaminated sites). The significance of the food chain transfer of the contaminant is low. However, it is important to address the risk of this exposure pathway in the gardening context, because food chain transfer is an obvious potential pathway in gardening and also food crops can be superficially contaminated with aerial dust deposition which could make food chain transfer of contaminants significant. Due to insignificant plant absorption and/or accumulation of many of the soil contaminants not much research data are available on this regard. Further, very less is known on dermal absorption

of contaminants under natural environmental conditions. With increasing number of urban gardens filling of such research gaps has become a critical requirement.

The bioavailability of these contaminants in soils depends on site specific soil characteristics and speciation (i.e., chemical forms) of the contaminants in soil. Speciation of the contaminants depends on various factors such as source and total concentration of the contaminants, soil pH, soil Eh, soil organic C concentration, soil metal oxide concentration, soil available P concentration, clay fraction, and clay mineralogy. Therefore, site specific studies need to be conducted to evaluate bioavailability of the contaminants and risk of exposing to the contaminants.

The bioavailability of soil contaminants can be reduced by modifying their speciation by *in situ* amendments. Several *in situ* amendments have tested and proven to be effective on minimizing bioavailability of soil Pb and As. Phosphorous fertilizer and metal oxides are two such amendments used to reduce bioavailability of soil Pb. Soil As can be stabilized *in situ* by metal oxides. Further, compost act as another potential amendment that is capable of stabilizing soil contaminants. However, the effectiveness of compost on reducing the bioavailability of soil amendments depends on the properties of compost, and variable results have been reported by the past researches (Sauve et al., 1998; Brown et al., 2003; Vega et al., 2009; Brown et al., 2012). A better understanding of underlying mechanisms responsible for the effectiveness of traditionally used soil amendments in gardening situations on contaminant stabilization is needed to understand the variable results. Elemental speciation in complex environments such as soils has been successfully achieved by using X-ray absorption fine-structure spectroscopy (XAFS), coupled with statistical analysis via linear combination fitting (LCF) or principal component

analysis (PCA) (Isaure et al., 2002, Roberts et al., 2002, Scheckel and Ryan, 2004, Baker et al., 2014).

As explained above, being the predominant exposure pathway, soil ingestion has gained much research attention. Various *in vivo* and *in vitro* method have been developed to estimate the bioavailability of contaminants, especially soil Pb and As, upon direct soil ingestion. Although *in vivo* animal models are superior in estimating the reliable bioavailability of contaminants, their high cost and time consumption; and negative public opinion towards animal tests raise the importance of *in vitro* methods. However, most of the validated *in vitro* methods with animal feeding studies have been validated using highly contaminated soils or mine waste materials. Mine waste materials possess very different chemistry comparing to moderately contaminated urban soils. So applicability of these *in vitro* methods to assess the bioaccessibility of contaminants in urban soils is questionable. Further, the ability of the *in vitro* methods to assess bioaccessibility of contaminants in amended soils needs to be tested.

This research addressed bioavailability of three most commonly found contaminants in urban soils. They are Pb, As and PAHs. Risk of exposing to these contaminants via soil ingestion, plant uptake, and dermal absorption was assessed using a combination of field and laboratory evaluations. Effectiveness of different compost types on reducing the bioavailability of these contaminants is assessed. Using direct soil Pb speciation via XAFS, attempts were taken to support the *in vitro* bioaccessibility measurements of urban soil Pb. Further, change of soil Pb speciation during an *in vitro* bioaccessibility extraction method was studied using Pb XAFS to explain bioaccessibility results of soil Pb in depth and to provide insight to the performance of the *in vitro* method for compost amended urban soils. This thesis consists of four studies. Their specific objectives are given below:

1. The objectives of the first study, Field Evaluations on Soil Plant Transfer of Lead from an Urban Garden Soil (Chapter 3), were to assess vegetable crop uptake of Pb; effectiveness of compost addition on Pb concentrations in vegetables; effects of vegetable-cleaning methods on Pb concentrations in vegetables; and the risk of Pb transfer via direct ingestion of garden soil

2. The objectives of the second study, Lead speciation in urban garden soils with and without compost and its' changes during *in vitro* bioaccessibility extraction test (Chapter 4), were to assess the change of Pb speciation upon addition of organic amendments *in situ* and how it relate to the bioaccessibility of Pb in three urban soils; change of Pb speciation and bioaccessibility over time; and change of Pb speciation during the bioaccessibility extraction test.

3. The objective of the third study, Potential for transfer of lead, arsenic, and polycyclic aromatic hydrocarbons from amended urban soils to humans and vegetables (Chapter 5), were to assess the effectiveness of adding four compost-types on reducing bioaccessibility of soil Pb and As; and vegetable Pb, As and PAHs concentrations.

4. The objective of the fourth study, Dermal absorption of PAHs in a urban soil: A qualitative study of the effect of the soil matrix and aging (Chapter 6), was to determine the soil matrix effect on dermal absorption of 16 priority PAHs (suggested by USEPA) using field contaminated urban soil and to assess the short-term aging effect of PAHs in soil on dermal absorption.

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Chapter 2 - Literature Review

Urban soils and significance of urban gardening

Deindustrialization of metropolitan areas has resulted in a large amount of vacant urban brownfields. A Brownfield means real property, the expansion, redevelopment, or reuse of which may be complicated by the presence or potential presence of a hazardous substance, pollutant, or contaminant (USEPA, 2014b). The United States has an estimated 450,000 to 1 million brownfields many of which are often considered as potential gardening sites (USEPA, 2014b). Nearly 15% of urban lands or approximately 1,800 hectares per city in the United States is vacant or abandoned (Pagano and Bowman, 2000). Urban gardening has been identified as a reuse options for vacant urban brownfield (USEPA, 2011). Currently, about 15% of worlds' food production occurred in urban areas (USDA, 2012). Urban agriculture has ability to cater substantial proportion of local food demand as most of the produce is used for household consumption, and the rest is sold in the local market. There are a variety of health, environmental, economic and social benefits that can be gained by urban agriculture: Urban agriculture increases surrounding property values, beautifies vacant properties, increases a sense of community, and provides recreational and cultural uses; increases infiltration of rainwater, reducing storm-water overflows and flooding, decreases erosion and topsoil removal, improves air quality, and reduces waste by the reuse of food and garden wastes as organic material and compost; increases physical activity and educates new gardeners on the many facets of food production from food security to nutrition and preparation of fresh foods (USEPA, 2011).

Challenges of urban gardening

Using urban lands for food production is challenging, since the quality of urban soil often is poor with regards to growing plants. Generally, urban soils have a high bulk density, low nutrient and organic C content, and low biological activity (Jim, 1998). The main challenge of urban agriculture is the potential of containing harmful levels of toxic contaminants that are originated from past or nearby industrial or residential activities (Mielke et al., 1983). United States Environmental Protection Agency has given attention for the safeness of gardening and consumption of produce grown in these sites (USEPA, 2011).

Contaminants in urban soils

Common contaminants, their sources, and exposure hazards

Urban lands have been used for various activities including residential, industrial, and commercial land uses. Therefore, urban soils may contain potentially toxic contaminants that are originated from these human activities. Some common contaminants, their sources, exposure hazards are summarized in Table 2.1.

Table 2.1 Common contaminants in urban soils, their sources, and exposure hazards (sources: ATSRD, 1994; ATSDR, 1995; ATSDR, 2007a; ATSDR, 2007b; USEPA; 2005; USEPA, 2011b; USEPA, 2014a; WHO, 2010)

Contaminant	Source/past activity	Major health hazards
Lead (Pb)	Leaded paint, leaded gasoline old residential buildings, mining, leather tanning, landfill activities, pesticides, coal burning	Damage nervous system, liver, and kidneys, increases in blood pressure, anemia, miscarriage, In children: behavioral problems, hearing loss, developmental delays, damage to brain, and

		hyperactivity
Arsenic (As)	Pesticides, Lumber treatment facilities	Cancer in the liver, bladder, skin, and lungs, decreased production of red and white blood cellschange in skin pigmentation, neurotoxicity, developmental defects, and cardiovascular diseases
Cadmium (Cd)	battery manufacture, metal welding, coal burning	Kidney damage, high blood pressure, iron-poor blood, liver disease, nerve damage or brain damage, and bone weakening
Zinc (Zn)	Mining, production and use of zinc in brass, bronze, die, castings metal, alloys, rubbers, and paints	Hematology, damage to the kidneys, pancreas, and gastrointestinal tract
Copper (Cu)	Contaminated manure	Liver, kidney damage, and gastrointestinal distress
Mercury (Hg)	Pesticides, paints	Impaired neurological development, speech, hearing, walking; and muscle weakness
Chromium (Cr)	Lumber treatment facilities, cement-producing plants, leather tanneries	Gastrointestinal, liver, kidney, immune systems and neurological effects; and skin burns
Polycyclic aromatic hydrocarbons (PAHs)	Partial burning activities, gasoline and industrial emissions.	Possible carcinogen, birth defects and decreased body weight
Dioxins	Landfill operations, burning wastes	Carcinogen, reproductive and developmental problems, damage the immune system, and interfere with hormones

Chlordane	Pesticide, manufacturing and application places	Possible carcinogen, Harmful effects in nervous system, liver damages

Lead in soils

Lead occurs naturally in soils. The general concentration of Pb in soils ranges from 1 to 200 mg kg^{-1} (Zimdahl and Skogerboe, 1977). There are many soil minerals that could present in soils under variety of environmental conditions: a complete set of minerals and their equilibrium constants (log K at 25^{o}C) can be found in Lindsay (1979) and Hettiarachchi and Pierzynski (2004).

The common Pb ore mineral is galena (PbS) (Hettiarachchi and Pierzynski, 2004). This is a stable mineral (log K_{sp}=-27.51) in reduced, high sulfur environments. Under oxidized environments, PbS can be oxidized to $PbSO_4$ (log K_{sp}= -7.79), which has several times higher solubility. When soil pH is high, $PbCO_3$ can be precipitated under adequate CO_2 pressure. Pyromorphites ($Pb_5(PO_4)_3OH$: K_{sp}= -4.14; $Pb_5(PO_4)_3Br$: K_{sp}= -19.49; $Pb_5(PO_4)_3F$: K_{sp}= -12.98; $Pb_5(PO_4)_3Cl$: K_{sp}= -25.05) are the most stable Pb mineral under a wide range of soil pH (Hettiarachchi and Pierzynski, 2004; Lindsay1979). Formation of pyromorphite is facilitated by high concentration of soluble inorganic P, but the Pb concentration in soil solution is also important (Scheckel et al., 2013). Pb in solution occurs mainly as divalent cation (Pb^{+2}) (Lindsay, 1979).

Generally, soils have a high capacity to adsorb Pb, because of its high affinity to organic C and metal (Fe, Al and Mn) oxides (McKenzie, 1980; Martinez and McBride, 1999). Therefore, the activity of Pb in soil solution is mostly undersaturated with respect to many of the stable Pb minerals mentioned above, especially under low to moderate soil Pb (<200 mg kg^{-1})

concentrations (Zimdahl and Skogerboe, 1977; Hettiarachchi and Pierzynski, 2004; Scheckel et al., 2013). Metal oxides bind Pb via specific adsorption and coprecipitation (Hettiarachchi and Pierzynski, 2004). Inner-sphere and outer-sphere adsorption mechanisms are involved in Pb adsorption in soils (Zimdahl and Skogerboe, 1977; Appel and Ma, 2002). Because of the importance of this adsorption processes Pb solubility can mainly be explained by soil pH and cation exchange capacity (CEC) (Zimdahl and Skogerboe, 1977). At low pH, Pb solubility is relatively high, because of protonation of adsorption sites of organic and inorganic soil constituents and reduction of CEC.

Arsenic in soils

The concentration of As in the soils ranges from 0.1 to 40 mg kg^{-1} (Bowen, 1979). A complete set of arsenic minerals that could present in a variety of environmental conditions and their log K can be found in Sadiq (1997). Arsenic exists as As(V) and As(III) under natural Eh and pH conditions. In soils solution, As occurs mainly as arsenate and arsenic. Arsenite (As[III]) is more toxic, soluble, and mobile compared to arsenate (As(V)). Mostly adsorption of arsenic onto Fe and Al oxides and clay minerals controls As solubility in soils. Iron and Al oxides have high affinity to As(V) (Redman et al., 2002; Beak et al., 2006). Adsorption of As(V) onto metal oxides highly depend on soil pH: when soil pH increases the adsorption of As(V) is decreased because of negative surface potential of the surface of absorption created by deprotonation and increase of negative As(V) species concentration in soil solution (Smith et al., 1999). It has been found that inner-sphere adsorption mechanisms are involved, when binding As(V) onto metal oxide surfaces (Waychunaset al., 1996; Fendorf et al., 1997) and As(V) have potential to coprecipitate with Fe oxides (Masscheleyn et al., 1991), whereas, Adsorption of As(III) onto

metal oxide surfaces could happen via both inner-sphere and outer-sphere mechanisms (Arai et al., 2001).

Because As(III) is more soluble in soils compared to As(V), alterations in the oxidation state of arsenic, as influenced by redox potential (and pH), greatly affects its solubility in soil (Masscheleyn et al., 1991). At higher soil redox levels (500-200 mV), a majority of As exists as As(V), and arsenic solubility is low. The reduction of As(V) to As(III), releases substantial proportions of arsenic into solution. Under moderately reduced soil conditions (0-100 mV), arsenic solubility seems to be controlled by the dissolution of Fe oxyhydroxides. Coprecipitated As(V) with iron oxyhydroxides can be released upon Fe dissolution (Masscheleyn et al., 1991).

In oxidized acidic soils, the dominant mineral form of As is $AlAsO_4$ (log K =-4.70; Sadiq, 1997), $FeAsO_4$ (log K=-9.45; Sadiq, 1997), whereas in calcareous alkaline soils more soluble Ca_3AsO_4 (log K= -1.91; Sadiq, 1997) is the most dominant As mineral (Sadiq, 1997; Mandal and Suzuki, 2002). In acidic reducing soil conditions, As-S association (e.g. orpiment [As_2S_3: log K=-180.43; Sadiq, 1997], realger [AsS: log K=-83.13; Sadiq, 1997]) precipitate, under high sulfur environments (Ware et al., 2005). Although these As-S minerals are formed under reduced conditions they have been found in oxidized soils as well (Brown et al., 2013), because of their low solubility.

Organic forms of As can also present in the soil, but in a lesser extent compared to the inorganic forms of As. Methylarsonates are the common organic As forms in the soil. Several strains of soil microorganisms can transform AsO_4^{-3} to methylarsonates, under reduced conditions, if appropriate methyl donor becomes available. Methylarsonates (mono-, di-, and tri-) can either biotransform to methylarsines or biodegrade to AsO_4^{-3}. Methylarsines (mono-, di-, and tri-) are volatile and can escape to the atmosphere (Sadiq, 1997).

Polycyclic aromatic hydrocarbons (PAHs) in soils

Polycyclic aromatic hydrocarbons are a group of organic contaminants that consist of two or more fused benzene rings (ATSDR, 1995). Polycyclic aromatic hydrocarbons in soils undergo numerous biochemical transformations such as volatilization, biodegradation, photolysis, hydrolysis, abiotic oxidation, and adsorption to organic C (ATSDR, 1995). The behavior of PAHs in the environment largely depends on their physiochemical properties such as octanol-water partitioning coefficient (K_{ow}: ability of an organic molecule to partition from water to lipid), organic C partitioning coefficient (K_{oc}), volatilization, water solubility, and Henry's constant. The USEPA priority 16 PAHs can be divided into three molecular weight categories and their physicochemical properties can be explained according to those categories (ATSDR, 1995). Low molecular weight (128-178 g mol^{-1}) PAHs are naphthalene, acenaphthene, acenaphthylene, anthracene, fluorene, and phenanthrene. High molecular weight (202-278 g mol^{-1}) PAHs are fluoranthene, pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-c,d]pyrene. In general, all the PAHs are lipophilic and have low water solubility. High molecular weight PAHs have higher K_{ow} (log K_{ow}= 5-6.8; ATSDR, 1995), and they are more lipophilic than the low molecular weight PAHs (log K_{ow}= 3-4.5; ATSDR, 1995). Generally, PAHs have high affinity to soil organic matter. The log K_{oc} of PAHs ranges from 3-6 (ATSDR, 1995), but low molecular weight PAHs have lower affinity to soil organic matter compared to high molecular weight PAHs. Further, the volatility of low molecular weight PAHs is higher than that of high molecular weight PAHs (ATSDR, 1995). It has been demonstrated that low molecular weight PAHs tend to volatilize from soil more compared to high molecular

weight PAHs because of the high volatility and low adsorption capacity of low molecular weight PAHs (ASTDR, 1995). Further, low molecular weight PAHs are more prone to biodegradation than the high molecular weight PAHs because low molecular weight PAHs show higher accessibility to microorganisms via lower adsorption capacity to soil constituents (Haritash and Kaushik, 2009). The low molecular weight PAHs tend to show significantly lower half-life in soils than the high molecular weight PAHs. Park et al. (1990) conducted an experiment with artificially contaminated soil with PAHs and estimated the half-lives (days) of the PAHs in soil: naphthalene, 2.1-2.2; anthracene, 50-l34; phenanthrene, 16-35; fluoranthene, 268-377; pyrene, 199-260; chrysene, 371-387; benz[a]anthracene, 162-261; benzo[b]fluoranthene, 211-294; benzo[a]pyrene, 229-309; dibenz[a,h]anthracene, 361-420; dibenzo[a,i]pyrene, 232-361; and indeno[l,2,3-c,d]pyrene, 288-289.

In addition to the inherent properties of PAHs, soil properties such as moisture content, organic C concentration, dissolved organic C concentration, clay percentage, aeration, pH, temperature, redox conditions, and concentration of toxic metal also affect the transformation and partitioning of PAHs (Thomas et al. 1989; Wilson and Jones 1993; Bengtsson and Zerhouni, 2003). These soil properties affect biodegradation of PAHs via affecting microbial structure and activities, and providing suitable sorptive sites for binding PAHs. Well aerated soils with high porosity could have high loss of PAHs from soil via volatilization and enhanced microbial degradation (ASTDR, 1995). Soils with high organic C concentrations tend to show more biodegradation of soil PAHs as compared to the soils with low organic C. Wong et al. (2002) described that enhancing dissolved organic C via pig manure application enhanced easily accessible C source for soil microbes and stimulates microbial degradation of soil PAHs. In

addition, dissolved organic C could enhance the desorption of PAHs from soil constituents and enhance biodegradation (Bengtsson and Zerhouni, 2003).

Exposure pathways of soil contaminants

People can expose to soil contaminants via several pathways. They can be categorized into two: direct and indirect pathways. Examples for direct exposure pathway are soil/dust ingestion, inhalation and dermal absorption. Soil ingestion is considered as one of the main exposure pathway many of the soil contaminants (Lanphear and Roghmann, 1997; Beak et al., 2006). Main indirect exposure pathway is the transfer of soil contaminants to humans via consumption of food crops grown in contaminated sites. Some of the contaminants (e.g.Cd) have high potential to be absorbed to plant (Voutsa et al., 1996), whereas plant absorption of Pb and PAHs could be relatively low. However, in the gardening context, this is considered as an important exposure pathway that needs to be addressed in risk assessment procedures. Some of the contaminants have potential to absorb via skin, especially organic chemicals like PAHs and As. Therefore, dermal absorption is another critical exposure pathway that needs to be aware in safe gardening.

Direct exposure pathways

Soil ingestion is the most significant exposure pathway for many soil contaminants, including Pb and As. Children are more susceptible to this pathway than adults because of their hand to mouth behavior (ASTDR, 1991). Accidental ingestion of soil by gardeners can also happen while eating, drinking, or smoking during working in the garden (USEPA, 2011). Lead is one of the main toxic contaminants that transfer via direct soil ingestion. This direct soil/dust ingestion due to hand to mouth behavior of children is now recognized as a major contributor to the total body burden of Pb in children (Bornschein et al., 1986). Different investigators have

found widely varying relationships between levels of Pb in soil and dust and children's blood lead levels: Blood lead levels generally rise 3-7 µg/dL for every 1,000 mg kg^{-1} increase in soil or dust lead concentrations (USEPA, 1986; Bornschein et al., 1986; ATSDR, 1988). The bioavailability of Pb (possible other contaminants too) in children's gastrointestinal system is much higher than that of the adults (USEPA, 2007). Therefore, precautions need to be taken to minimize children's direct exposure for soil contaminants. Inhalation of dust carries a minor risk of contaminants transfer (Davies et al., 1990).

Dermal absorption of soil contaminants such as PAHs (Yang et al. 1989), Polychlorinated biphenyls (Wester et al., 1993a) and As (Wester et al., 1993b), has been studied by past research. The extent of absorption depends on various factors such as dose and exposure area (Duff, 1996); contaminant properties such as volatility and capacity to partition into lipids (Turkall et al., 2009).

Indirect exposure pathways

Absorption, translocation, and accumulation of Pb and As in plants

Absorption and accumulation of Pb in plants have not gained much research attention. It could be mainly because the absorption of Pb by plants is very limited and much lower than some other potentially toxic trace elements such as Cd, Zn, and Se. Studies on phytoavailability of As has been widely focused on As contamination in rice grown under reduced soil environments and As phytoextraction aspects with hyperaccumulators. Arsenic absorption and accumulation by other food crops, including vegetables, have not been studied much.

Phytoavailability of trace elements like Pb and As depends on several factors such as solubility/speciation of the trace elements in soil, plant nutrient status in soil (Sterrett et al, 1996), characteristics/physiology of plant species/cultivars (Zhao et al., 2009), and plant

heat/moisture stress (Merry et al., 1986). In general except in acidic soils, the concentration of Pb^{+2} in soil solution is relatively low, where protonation of adsorption sites of clay minerals and oxides possess low binding capacity for cations. Therefore, plant availability of soil Pb is relatively low. Under well aerated soil environments, majority of As in soil exist as oxidized As (i.e., arsenate). Arsenate has high affinity to bind into mineral surfaces and hence maintain low As solubility and low phytoavailability. Because of the low solubility of Pb and As in soil, the absorption and accumulation of soil Pb and As by plants mainly depend on the other factors mentioned above.

Availability of adequate amounts of nutrients in the soil improves plant growth and leads to high biomass production. High biomass content dilutes the absorbed potentially toxic elements in the plants (Ekvall and Greger, 2003). It has been found that increase of plant nutrients (N, P and K) in Pb contaminated urban soil reduced Pb concentrations of lettuce (Sterret et al., 1996). Similarly, low concentration of Pb, Cd, and Hg were found in water spinach grown in high nutrient medium compared to that of water spinach grown in low nutrient medium (Gothberg et al., 2004). Some plant species and cultivars show higher potential to absorb and accumulate potentially toxic elements from the soil (Alexander et al., 2006; Liu et al., 2010; Price and Hettiarachchi, 2012). Particularly, As absorption, translocation, and accumulation by plants seems to be much variable among species and genotypes (Zhao et al., 2009; Zhao et al., 2010).

When Pb is absorbed by plant roots, it can precipitate as Pb-phosphate forms in root cells and xylem because of high P concentration and moderate pH in the root cells. Therefore, a majority of absorb Pb tends to retain in plant root cells, with limited capacity to transfer to above ground plant parts. In P-deficient soils, Pb precipitation in the root cells is limited, and Pb is transferred to shoot efficiently. Under such conditions, plants may show high Pb absorption (Foy

et al. 1978), In contrast, plants absorb As in As(V) form and absorbed As(V) is rapidly reduced into As(III) in plant root cells. These As(III) can complex with thiol-peptides and stored in vacuoles, efflux to the external medium, and translocate to shoot via xylem (Zhao et al., 2009). Therefore, accumulation of As in roots is not prominent as in Pb.

Not much research was done to test how plants respond for external stresses, in terms of absorption of potentially toxic trace elements. Merry et al. (1986) showed that, when plants were grown under high temperature, they tend to absorb and accumulated more potentially toxic trace elements. Although the mechanism was unclear, Merry et al. (1986) postulated that the high temperature increases the uptake of Pb, Cu and As in vegetable crops by increasing the permeability of root cells to absorb more ions and by increasing the diffusion rate on ions in soils.

In addition to the absorbed potentially toxic trace elements in plant tissues, plants grown in contaminated soils may be superficially contaminated by soil particles. Especially, in urban areas, plants may have atmospheric deposition of toxic trace elements containing particulates originated from contaminated soils or other atmospheric emissions. Nabulo et al. (2006) analyzed Pb concentrations of vegetables grown in roadside farming sites along major highways (Kampala City, Uganda). They found that leaves of the leafy vegetables have more Pb concentrations than the Pb concentrations in their roots, due to deposition of Pb bearing particles on the leaves. In that study, the highest ratio between the Pb concentrations in the leaves and roots was 3.4:1and found in *Brassica oleracea* (common name: cabbage) acephala group. This ratio for *Amaranthus dubius* (common name: Red spinach) and *Beta vulgaris Cicla* (common name: Chard) was approximately 1.2:1 at soil Pb concentration of 30 to 65 mg/kg. Further, a significant difference of Pb concentrations in unwashed and washed leafy vegetables, grown in

urban gardens, was observed by Nabulo et al. (2010). They analyzed Pb concentrations of 15 leafy vegetables in several urban gardens that have Pb concentrations ranging from 9 to 770 mg kg^{-1} near Kampala City, Uganda. Washed leafy vegetables had 35% lower Pb concentration compared to the Pb concentrations in unwashed vegetables. The highest unwashed: washed ratio for Pb concentrations in leaves was 9.1, and it was in *Cucurbita maxima* (common name: pumpkin) grown in soils that have 770 mg kg^{-1} of total Pb concentration.

In the above context, the risk of transfer of soil Pb and As via consumption of food crops grown in moderately contaminated soils would be low. Nevertheless, need for filling research gaps related to this aspect is greater now than ever, since food chain transfer is an obvious pathway for soil Pb and As transfer in gardening settings, and concerns for subtle levels of Pb and As to humans health is increasing. Further, superficial contamination of vegetable crops by contaminated soils could be a significant source of toxic trace elements.

Absorption of PAHs by plants

Uptake of PAHs by plants depends on physiochemical properties of PAHs, besides plant physiology (Wagrowski and Hites, 1997). It has been found that organic compounds that have log K_{ow} higher than 4, are poorly absorbed by plant roots (Simonich and Hites., 1995), because of their low water solubility. Low molecular weight PAHs are more water soluble than the high molecular PAHs; hence low molecular PAHs are more phytoavailable compared to the high molecular weight PAHs. Supporting this fact, Wild and Jones (1992) found that the concentration of low molecular weight PAHs are 3-4 times higher than the concentration of high molecular weight PAHs in carrot roots grown in PAHs contaminated soils.

However, because of low water solubility and high affinity to soil organic matter, the uptake of PAHs from soil to plant root is not the dominant pathway of transferring soil PAHs

into plants. The dominant pathway is entering of gaseous and particulate PAHs in the atmosphere into the plants via plant foliage (Simonich and Hites, 1995). Gaseous PAHs easily enter the plant leaves whereas, the particulate PAHs deposit on the foliage and only smaller particulates may enter the plant.

The concentration of low molecular weight PAHs in the air is comparatively high, because of their high volatility. Plants tend to have a high concentration of low molecular weight PAHs, especially in the foliage. Partitioning of gaseous molecules into the leaf surface depends on octanol-air partitioning coefficient (K_{oa}). The log K_{oa} of PAHs ranges from 6-12 (Odabasi et al., 2006). Octanol-air partitioning coefficients are high in higher molecular weight PAHs. Therefore, if high molecular weight PAHs are in the atmosphere in the gaseous phase, they efficiently partition into the waxy cuticle layer on the leaf surface. However, transportation of these molecules into the plant through the phloem or the xylem is limited, because of their low water solubility (Simonich and Hites, 1995).

Dermal transfer of soil PAHs

Dermal absorption of soil PAHs has been widely studied (Storer et al., 1984; Kao et al., 1985; Dankovic et al., 1989; VanRooij et al., 1993). In general, PAHs readily absorb via skin, due to their high K_{ow}. Stratum corneum is the topmost layer of the skin which acts as a barrier for solute transfer into deeper layers (Bouwstra et al., 2003). Stratum corneum consists of inactive, keratinized cells surrounded by extracellular lipids (ASTDR, 1995). Lipophilic compounds tend to show higher potential to absorb via skin, because the lipids in the stratum corneum act as the route of absorption for such compounds (Albery and Hadgraft, 1979; Raykar et al., 1988). Therefore, high molecular weight PAHs have higher penetration capacity into skin than the low molecular weight PAHs. Turkall et al. (2009) showed higher penetration and accumulation of

benzo[a]pyrene (molecular weight: 252 g mol^{-1}) in human skin compared to that of naphthalene (molecular weight: 128 g mol^{-1}), in an *in vitro* study, conducted with and without soil matrix.

A majority of the studies that evaluate dermal absorption of PAHs had been done using pure/single chemicals of PAHs in simple solvents (Kao et al., 1985; Ng et al., 1992; Withey et al., 1993). This approach ignores how PAHs behave when there are several of PAH together in a reactive matrix such as soil and the effect of vehicle of transfer/matrix in dermal absorption of PAHs. Dankovic et al. (1989) studied dermal absorption of benzo[a]pyrene and benzo[a]pyrene with the presence of eleven other PAHs using a mice model. They found that the half-life of benzo[a]pyrene in skin is lower when it would absorb alone. This could indirectly affect the amount of benzo[a]pyrene that partition into the skin, since dermal metabolism of benzo[a]pyrene in the skin was found to be the rate limiting factor of its dermal absorption (Kao et al., 1985). PAHs in soils show relatively low dermal absorption compared to the PAHs in pure solvents (Yang et al. 1989; Wester et al. 1990; Turkall et al., 2009), because of sorption of PAHs by soil organic and inorganic constituents. Dermal absorption of soil PAHs could tremendously influence by soil matrix and its biochemical reactions. Further, aging of PAHs in soils tend to reduce the dermal absorption of soil PAHs (Turkall et al., 2009). Turkall et al. (2009) reported that total penetration (in the receptor fluid and accumulated in the skin) of benzo[a]pyrene reduced by about 56% when the PAHs were aged for 3 months in soil compared to the total penetration of benzo[a]pyrene immediately added to soil. In the above context, risk of exposing to PAHs in naturally contaminated soil via dermal absorption could be largely deviated from the data produced pure chemicals in simple organic solvents.

Reducing risk of urban soil contaminants

Remedial Options

There are many remediation technologies available for contaminated sites. They are excavation and surface restoration, landfilling, bioremediation, bioremediation, phytoremediation, phytoextraction, and *in situ* stabilization of contaminants by amendments. Excavation and surface restoration; and landfilling are practiced in highly contaminated sites, and these methods are costly. Bioremediation (i.e., the use of microbes to clean up contaminated soil) and phytoremediation (i.e., use of green plants and their associated microorganisms to stabilize or reduce contamination in soils) methods can be effectively used for remediating sites with elevated organic pollutants (USEPA, 2012b; USEPA, 2014b), but extra precautions need to be taken to provide a suitable environment for right microbes to grow. Phytoextraction (i.e., use of living plants, especially hyperaccumulators to remove toxic metals from soil) is an environmental friendly technology, but it usually takes longer time to remove contaminants from soil and may not be effective for the metals that have low phytoavailability. Given the above issues with common remediation technologies, *in situ* stabilization has been identified as a cost effective, and attractive remediation method for metal contaminated sites (Scheckel et al., 2013; Hettiarachchi and Pierzynski, 2004). Since most urban soils carry only low to moderate concentrations of contaminants, *in situ* stabilization may be successfully used.

In situ stabilization of soil Pb and As

The bioavailability of soil Pb and As can be reduced by stabilizing them in the field. Several amendments have been tested for their effectiveness of stabilizing soil Pb and As *in situ* via changing their chemical speciation into more stable/sparingly soluble chemical forms. Scheckel et al. (2013) showed that the Pb minerals that are most stable under a wide range of pH

are galena (PbS; Ksp=10-27.51; Hettiarachchi and Pierzynski, 2004), wulfenite (PbMoO4; Ksp= 10-16.04; Hettiarachchi and Pierzynski, 2004), and chloropyromorphite (Pb5(PO4)3Cl; Ksp=10-25.05; Hettiarachchi and Pierzynski, 2004). Galena can be oxidized and transformed into anglesite (PbSO4; Ksp=10-7.79; Hettiarachchi and Pierzynski, 2004) and anglesite is several orders more soluble than galena and also has much higher bioaccessibility in the acidic gastrointestinal environment (Hettiarachchi and Pierzynski, 2004). Formation of wulfenite can be induced by adding Mo into soils, which could lead to Mo toxicity issues (Scheckel et al., 2013). In this context, formation of pyromorphite by adding P fertilizer is considered as the most acceptable approach to *in situ* immobilization of soil Pb. Solubility of pyromorphite under natural soil conditions and human stomach conditions are very low or negligible (Nriagu, 1974; Lindsay, 1979; Scheckel et al., 2005). Many researches proved that the addition of P fertilizer increases soil-available P and results in the formation of Pb-phosphates, especially pyromorphite-like minerals (Ma et al., 1993; Cotter-Howells and Caporn, 1996; Hettiarachchi et al., 2001; Brown et al., 2003; Scheckel et al., 2013). Plant Pb uptake could also reduce by apatite or triple superphosphate (TSP) addition to Pb-contaminated soils (Laperche et al., 1997; Brown et al., 1999; Hettiarachchi and Pierzynski, 2002). Soil pH is critical for induction of formation of pyromorphite in soil, as it affects the solubility of soil Pb and phosphates, which is the rate limiting factor of the pyromorphite formation (Scheckel et al., 2013). Ideally, pH <4 is reported to be the pH that facilitate dissolution of both Pb and phosphate in the soil (Scheckel et al., 2013). Under acidic soil pH, protonation functional groups of the adsorptive sites increase the concentration of Pb^{+2} in soil solution. Thermodynamically, pyromorphite formation is favored when H3PO40 and H2PO41 are present, and these orthophosphate ions form in the soil solution under acidic soil pH (Porter et al., 2004).

When Fe, Al and Mn oxide concentrations in soil are high the concentration of phosphate in soil solution could reduce since above metal oxides have high affinity of phosphates, especially under acidic pH (Hingson et al., 1967; 1968). Further, adsorption of Pb onto clay minerals, metal oxides and soil organic C are the dominant Pb retention mechanism in soil. Formation of pyromorphite occurs when the concentration of Pb in the soil solution would exceed the Pb adsorption capacity of the soil. Therefore, when soils are contaminated with low to moderate levels of Pb (< 400 mg kg^{-1}), the pyromorphite formation might not be significant (Scheckel et al., 2013). In addition, high concentration of dissolved organic C can inhibit the precipitation of pyromorphite in soil by blocking the pyromorphite crystal seeds and by interfering the crystal growth (Lang and Kaupenjohann, 2003). There is limited research published showing the pyromorphite formation followed by P fertilizer application under field conditions. Scheckel et al. (2013) explain that the formation of pyromorphite under field condition is limited by several reasons. They are 1) high retention of soil Pb and phosphate by soil constituents limiting their availability to react with each other to form pyromorphite; 2) non-optimal soil pH resulting in rate-limiting release of Pb and phosphate to form pyromorphite; 3) high soil organic matter content; and 4) soil moisture content (soil moisture is important to mediate the solubility reactions of soil P and Pb). Care must be taken to control above limitations when using P fertilization as *in situ* Pb stabilization technique.

An increase of soluble inorganic P concentration in soil can negatively affect As immobilization. Arsenic exists as oxyanions in soil solution, predominantly with As(V) and As(III) oxidation states (e.g. H_3AsO_3, $H_2AsO_4^-$ and $HAsO_4^{-2}$) in natural soil Eh and pH conditions (Masscheleyn et al., 1991). These As oxyanions have a similar sorption characteristics as orthophosphate ligands; hence, they compete with orthophosphate ligands to metal oxide's

sorptive sites and also already sorbed As oxyanions can exchange with orthophosphates at high soluble P concentrations and become mobile in soil (Gao et al., 1991; Peryea, 1991). Therefore, P fertilizer application is not an effective *in situ* immobilization method for As-contaminated or Pb- and As-co-contaminated situations.

The addition of metal oxides (e.g. Fe and Mn oxides) is proven to be stabilizing both soil Pb and As *in situ*. These oxides have strong affinity to soil As and Pb (Martinez and McBride, 1999; Hettiarachchi et al., 2000; Redman et al., 2002; Beak et al., 2006). Strong inner-sphere adsorption of Pb^{+2} onto Fe and Mn oxide is reported (Gadde and Laitinen, 1974; McKenzie, 1980; Hettiarachchi et al., 2000). Strong adsorption of Pb via formation of ternary complexes (metal oxide-metal-ligand) has also observed (Weesner and Bleam, 1998). It has been found that inner-sphere adsorption mechanisms are involved, when binding As(V) onto metal oxide surfaces (Waychunaset al., 1996; Fendorf et al., 1997), whereas, Adsorption of As(III) on to metal oxide surfaces could happen via both inner-sphere and outer-sphere mechanisms (Arai et al., 2001). Regardless of all above promising observations on the use of metal oxide as a remediation technique for As- , Pb- and As-Pb co-contaminated situation, field level application of this technique is yet to be evaluated.

Organic matter amendment is becoming popular, as an *in situ* stabilization of potentially toxic elements in soils, especially with increasing interest in urban grading in potentially contaminated sites that could have low-moderate levels of contamination. Speciation and bioavailability of Pb and As in soils amended with organic matter depends on the composition of the organic matter and soil chemistry. Addition of biosolids containing high Fe concentrations (99,000 mg total Fe/kg) to Pb contaminated urban soils reduced bioavailability of soil Pb by 37% and 43% in the *in vivo* and the *in vitro* studies, respectively, while addition of compost with low

Fe concentrations (26,900-49,600 mg total Fe/kg) reduced Pb bioavailability more than 20 % (Brown et al., 2003). Amendment of barley straw and sludge greatly increases sorption of soil Pb as a collective result of increasing soil pH (approximately from 4.0 to 8.0), cation exchange capacity, soil organic matter content and Fe and Mn oxides content (Vega et al., 2009).

Not many promising results have been observed with reducing bioaccessibility of soil As upon high Fe biosolids application (Brown et al., 2003; Scheckel et al., 2005; Brown et al., 2012). Soluble inorganic P in biosolids and their competition with As oxyanions for the metal oxide sorptive sites may have partly been responsible for this observation. Further, soil organic matter, which have negatively charged functional groups under neutral soil pH also compete for sorptive sites of metal oxide and could contribute to increase As solubility (Redman et al., 2002). Increase of organic matter content and cation exchange capacity of the soils as a result of compost application increase Pb sorption capacity of soil (Martinez and McBride,1999; Vega et al., 2009).

Dissolved organic C can increase Pb and As solubility in soil upon compost addition. Application of leaf compost increased soil Pb solubility at soil pH range of 6.5 to 8 by promoting formation and dissolution of organic-Pb complexes (Sauve et al., 1998). Sauve et al. (1998) also found that soil Pb solubility in leaf compost-added soils was decreased, when pH increased from 3 to 6.5 and were independent from soil organic matter content. In the above context, soil and amendment specific studies are essential to evaluate soil Pb bioavailability.

Evaluating reduction of risk of soil contaminants

Bioavailability

Bioavailability means the portion of a substance or element in the soil that is available for absorption into living organisms, such as humans, animals, or plants. Bioavailability of soil Pb

cannot be explained solely by the total Pb concentration; rather, it largely depends on speciation of Pb in soil (Davis et al., 1992; Ge et al., 2000) and other site-specific soil chemistry (Hettiarachchi and Pierzynski, 2004). Soil pH, soil organic matter content (Sauve et al., 1998), total Pb concentration, and soluble P concentration (Hettiarachchi et al., 2000) collectively affect soil Pb speciation. Bioavailability of soil contaminants can be assessed by various methods: analyzing plant uptake of contaminants, determining solubility of contaminants by extraction methods, employing *in vivo* and *in vitro* methods to estimate oral bioavailability and gastrointestinal dissolution and absorption. Since contaminant's speciation governs its solubility and bioavailability in soil, determining contaminants speciation is important to test the risk of exposure, stability of contaminants and effectiveness of *in situ* remediation methods.

In vivo methods

Animal based models have been used to estimate the bioavailability of contaminants in soils. The animal species used for *in vivo* methods are juvenile swine, rats, rabbits and monkeys (Wragg and Cave, 2003). Data from *in vivo* studies are difficult to interpret with respect to their relevance to human health because of the physiological differences between humans and the experimental species being used (Ruby et al. 1999). Animal based models should be supported by additional studies to show how the animal bioavailability can be related to human bioavailability. *In vivo* methods on juvenile swine are preferred, because of juvenile swine's similar gastrointestinal tract characteristics to humans (Casteel et al. 1997; Dodds and Hsu, 1982). During *in vivo* studies a known amounts of contaminated soils are given to stimulate intermittent eating patterns and blood, urine samples are collected regularly and analyzed for contaminants concentrations (Wragg and Cave, 2003). After euthanization, brain, heart, liver,

lungs, gastrointestinal and urinary tracts are removed and analyzed for the contaminants concentrations.

In vitro Bioaccessibility methods

Although animal studies are superior to *in vitro* bioaccessibility tests in terms of reliability, the high cost, high labor involvement, and the negative public opinions associated with animal studies raise the importance of *in vitro* bioaccessibility tests. There are several methods developed and modified to estimate the bioaccessibility of soil Pb and As (Ruby et al., 1996; Medline, 1997; Oomen et al 2002; Juhasz et al., 2007a; 2007b Smith et al. 2011; USEPA, 2012a; Scheckel et al., 2013). In all these methods, soils are mixed with simulated gastric solution in an acidic pH (commonly used pH values are 1.5, 2.0, 2.3 and 2.5) at 37^{o}C to extract bioaccessible Pb and As. In addition to the pH variability, there are some variations in the composition of the enzyme mixture, soil: solution ratio, and the extraction time among these methods. Oomen et al. (2002) argued that the variable extraction pH is the reason for resulting different bioaccessibilities by these methods, regardless of all the other variabilities.

Further, Ruby et al. (1996) suggested that the pH variability of gastric phase does not affect that much for As solubility in the gastric solution, as it does for Pb. High bioaccessibility is often recorded with lower extraction pH values. Research shows that the bioaccessibility of soil Pb is over estimated when pH 1.5 was used as the extraction pH (Brown et al., 2003; Smith et al., 2011). Further, the reduction of Pb bioaccessibility in the amended soils was prominent at pH 2.5 or 2.2 (Brown et al., 2004; Ryan et al., 2004; Scheckel et al., 2013). Drexler and Brattin (2007) recommended using pH 1.5, because this pH limits the risk of underestimating.

It is important to note that these bioaccessibility methods have been developed using highly contaminated, non-amended mine impacted materials or soils. Their capability of

measuring reliable bioaccessibility values that correlate with animal based bioavailabilities in mildly-contaminated soil is questionable. Scheckel et al. (2013) explained that the correlation of Pb *in vitro* bioaccessibility (extractant: 0.4M glycine; extraction pH: 1.5, 2.0, and 2.5) with *in vivo* bioavailability is poor for P fertilizer amended mine waste materials. Therefore, performances of these *in vitro* bioaccessibility methods for low or moderately contaminated urban soil should be tested, especially because the soil Pb and As speciation of these soils could be deviated from that of highly contaminated mine impacted soils; and solubility and change of Pb and As in the gastrointestinal system could be largely influenced by their speciation in soil. Smith et al. (2011) provided some direct evidence of changing soil Pb speciation in an *in vitro* bioaccessibility extraction test performed at pH 1.5 using X-ray absorption near-edge structure (XANES) technique. Scheckel et al. (2005) provided indirect observation to formation of pyromorphite-like stable minerals during an *in vitro* bioaccessibility extraction method, in a study conducted with highly contaminated mine impacted soils.

Speciation methods

There are several methods available for speciation of metals in soil. Some of the common methods are described below. A detailed set of speciation methods are explained in D'Amore et al., 2005.

Sequential extraction procedures

These are not direct speciation methods, but divide elements in soils to operationally defined fractions. Various chemicals are used to sequentially-extract contaminants associated with different constituents in soil. For example, in the sequential extraction method suggested by Tessier et al. (1979) has five fractions: exchangeable; carbonates; reducible; oxidisable; and residual. Although this is a cost effective traditional method chemical reactions that occur during

the extraction can mislead the interpretations. For example Scheckel et al. (2005) reported that Pb can be precipitated as the pyromorphite during the sequential extraction proposed by Tessier et al. (1979). The applicability and the reliability of these methods can be improved by comparing the results of these methods with direct speciation techniques. Wenzel et al. (2001) proposed a sequential extraction method for soil As and that method had been tested using energy dispersive X-ray microanalysis (EDXMA). These methods are easily adaptable in routing soil analysis.

X-ray diffraction (XRD)

This is a rapid analytical technique that provides atomic structure of crystalline substances. Mostly pre-concentration of the analyte is needed via physical methods: particle size or density separation. The detection limits of this technique are approximately 1 to 5%, but this can be improved by synchrotron sources. High intensity, well-defined, and collimated synchrotron radiation allow a better resolution of diffraction peaks making synchrotron based-XRD a powerful tool to detect minor minerals in soils (Lombi and Susini, 2009). The main disadvantage of this method is the need of the presence of crystalline substances to identify the structure (D'Amore et al., 2005). Therefore, the usefulness of this method to determine speciation of trace elements in soils is low. Further, the adsorbed species cannot be detected.

Energy Dispersive X-ray Analysis (EDX)

The scanning electron microscopy part of this technique probes the surface morphology and the topography at nanometer scale. The EDX scans the surface by an electron beam and collects the X-ray fluorescence, emitted by the surface atoms (D'Amore et al., 2005). The X-rays are characteristic of the atoms emitting and give a semi-quantitative profile of the elemental composition (D'Amore et al., 2005). The speciation details are indirect as it shows only the co-

existence of atoms: co-existence may not necessarily guarantee a species (Cotter-Howells, 1996). Spectral overlap and sample preparation are two main disadvantages of this method (D'Amore et al., 2005).

X-ray absorption fine structure (XAFS) technique and direct speciation of soil Pb and As

The principle of XAFS is based on the details of how X-rays are absorbed by an atom at energies near and above the core-level binding energy of that atom (Newville, 2004). This technique provides information on the oxidation state and the coordination chemistry of the target atom: bond distances, coordination number and species of surrounding atoms (Lee et al., 1991; Newville, 2004). The XAFS spectra can be divided into two main regions: x-ray absorption near edge spectroscopy (XANES) and extended x-ray absorption fine-structure spectroscopy (EXAFS) (Newville, 2004). The former is focused on the much narrow range of energy which includes core-level binding energy, whereas the latter is focused on the energy range above the core-level energy. The XANES spectra features are dependent on the oxidation states and coordination chemistry. Therefore, XANES analysis is much more effective on speciation of elements with several possible oxidation states in soils like As. XANES has been used successfully for speciation of As and understanding As chemistry in soils (Takahashi et al., 2004; Arcon et al., 2005; Brown et al., 2012). The EXAFS spectra contain information on bond distances, coordination number, and species of the neighbors of the target atom (Newville, 2004) and provide more detailed insight into the local structure around the target atom (Arcon et al., 2005).

Minimal sample preparation requirement of this method is widely appreciated, and it makes XAFS techniques far superior to traditional speciation techniques like sequential extraction and nuclear magnetic resonance (NMR). Further, unlike some traditional techniques

like X-ray diffraction, XAFS spectroscopy has the advantage of determining the speciation of less abundant elements like Pb, regardless of the crystallinity of the species (Cotter-Howell et al., 1994; Scheckel et al., 2005) due to its high sensitivity. Furthermore, identification of metal sorption and surface precipitation on soil constituents is enhanced by XAFS (Fendorf et al., 1994; Manceau et al., 1996), which makes it much suitable for Pb and As speciation in moderately contaminated soils like many urban soils. The disadvantage of using XAFS spectroscopy in a heterogeneous systems like soils, where many species could be present, is that the XAFS spectral features provide an average picture of speciation in all the species that are present in the probing volume: the probing volume is a several cubic millimeter for bulk XFAS and it is several cubic micrometer for µ-XAFS (Manceau et al., 2002). Lead is identified as an element that is difficult to study by XAFS analysis because of several reasons (Manceau et al., 1996). They are 1) the outer shell electronic configuration of divalent lead has $6S^2$ electron pair which are stereochemically active and induce a strong deformation of divalent Pb polyhedral, 2) high variability of coordination number and interatomic distances, and 3) high thermal vibration of Pb atom in solids. These properties of Pb limit the quality XAFS to only 10-12$Å^{-1}$ and result mistakes of determining interatomic distances (Manceau et al., 1996). Therefore, care must be taken to collect quality Pb XAFS spectra with minimal interferences and when interpreting data.

Molecular information of As has been successfully retrieved by XANES and EXAFS (Takahashi et al., 2004; Arcon et al., 2005; Brown et al., 2012). Different crystalline and amorphous solid phase As; and surface-bound or adsorbed As on mineral particles can be identified using these techniques (Arcone et al., 2005) making them more useful in identifying As speciation in moderately contaminated soils. Since As can present as As(III) and As(V) in soil solution, identifying their different sorption mechanisms is important to understand the

chemistry of As in soil and develop successful remediation approaches for contaminated soils. Ability of XAFS techniques to generate molecular level information and to identify inner-sphere and outer-sphere sorption mechanisms (Wang and Mulligan, 2008) makes them useful for understanding chemistry of As under various environmental conditions.

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Chapter 3 - Field Evaluations on Soil Plant Transfer of Lead from an Urban Garden Soil

(Attanayake, C. P., G. M. Hettiarachchi, A. Harms, D. Presley, S. Martin and G. M. Pierzynski. 2014. Field evaluations on soil plant transfer of lead from an urban garden Soil. J. Environ. Qual. 43:475-487. doi:10.2134/jeq2013.07.0273)

Abstract

Lead (Pb) is one of the most common contaminants in urban soils. Gardening in contaminated soils can result in Pb transfer from soil to humans through vegetable consumption and unintentional direct soil ingestion. A field experiment was conducted in 2009 and 2010 in a community urban garden with soil total Pb concentration of 60 to 300 mg kg^{-1}. The objectives of this study were to evaluate soil-plant transfer of Pb, the effects of incorporation of a leaf compost as a means of reducing Pb concentrations in vegetables and the bioaccessibility of soil Pb, and the effects of vegetable cleaning techniques on the Pb concentrations in the edible portions of vegetables. The amount of compost added was 28 kg m^{-2}. The tested plants were Swiss chard, tomato, sweet potato, and carrots. The vegetable cleaning techniques were kitchen cleaning, laboratory cleaning, and peeling. Compost addition diluted soil total Pb concentration by 29–52%. Lead concentrations of the edible portions of vegetables, except carrot, were below the maximum allowable limits of Pb established by FAO and WHO. Swiss chard and tomatoes subjected to kitchen cleaning had higher Pb concentrations than laboratory-cleaned plants. Cleaning methods did not affect Pb concentrations in carrots. Bioaccessible Pb in the compost-added soils was 20–30% less than that of the no-compost soils; compost addition reduced the potential of transferring soil Pb to humans via vegetable consumption and direct soil ingestion. Thorough cleaning of vegetables further reduced the potential of transferring soil Pb to humans.

Introduction

Use of vacant lands in urban and suburban areas for vegetable production is growing. About 15% of the world's food is produced in urban areas (USDA, 2012). Urban agriculture has the ability to provide a substantial proportion of local food demand, because most of the produce is used for household consumption and the rest is sold in local markets. This helps alleviate the issue of food deserts in urban areas, at least seasonally. Many of these urban gardens are subdivided into small plots that are managed by individuals, families, or small groups. Community gardening improves social cohesion and the awareness of ecosystem (Hynes and Genevieve, 2004).

Urban lands often were previously used residential, industrial, and commercial activities. Using urban lands for food production is challenging, because the quality of urban soil is poor for growing plants; and urban soils generally have a high bulk density, low nutrient and organic C content, and low biological activity (Jim, 1998). Mielke et al. (1983) reported that urban soils may contain higher concentrations of potentially toxic trace elements based on a study done in metropolitan Baltimore. These trace elements can originate from lead-based paint; burning of fossil fuels, including leaded gasoline (before the 1970s); and lead-arsenate pesticides (Klein, 1972; Carey et al., 1980; Turer et al., 2001; Zhai et al; 2003). The possibility of urban soils containing potentially toxic trace elements is a perceived obstacle to developing urban gardens. Lead is one of the most common potentially toxic trace metals in urban soils. Elevated Pb in the environment can increase blood Pb levels in children (Lanphear and Roghmann, 1997). About 310,000 U.S. children between the ages of 1 and 5 years are believed to have blood Pb levels at or greater than 10 $\mu g\ dL^{-1}$ (ATSDR, 2007), which was the blood Pb level of concern

recommended by the United States Center for Disease Control and Prevention (CDC). If the blood Pb concentrations of many children are above the level of concern, community-wide primary lead poisoning prevention activities should be implemented. In 2012, CDC reduced the definition of elevated blood Pb in children from 10 µg dL^{-1} to 5 µg dL^{-1}based on CDC's Advisory Committee on Childhood Lead Poisoning Prevention recommendations (ACCLPP, 2012).

Soil Pb can transfer to humans through soil ingestion, consumption of Pb-contaminated foods, and inhalation of Pb-containing soil particles. The amount of Pb that can transfer through inhalation is minute compared with the other two pathways (Davies et al., 1990); of those, soil ingestion is the most significant (Lanphear and Roghmann, 1997). Plant absorption and accumulation of soil Pb is lower than other potentially toxic trace elements such as Cd and Se, mainly because of comparatively low concentrations of free Pb (Pb^{+2}) in the soil solution. When Pb is absorbed by plant roots, it can precipitate as Pb-phosphate forms in root cells and in xylem because of high P concentration and moderate pH in the root cells. In P-deficient soils, Pb precipitation in the root cells is limited and Pb is transferred to shoots efficiently. Under such conditions, plants may show high Pb absorption (Foy et al. 1978), so natural translocation limits may be more important than uptake limits from the acidified rhizosphere. Consumption of vegetables grown in Pb-contaminated soils may cause adverse health effects, but very little literature supports this idea. Especially in urban areas, plants may be superficially contaminated by the atmospheric deposition of Pb-containing particulates from contaminated soils or other atmospheric emissions. Combustion of leaded gasoline releases Pb-containing particulates into the atmosphere, and those particulates may deposit on plants that are grown close to major roads. Although leaded gasoline has been banned in many developed countries, it is still used in

developing countries. Nabulo et al. (2006) found higher Pb concentrations in vegetables grown along major highways in Kampala City, Uganda; furthermore, they observed a significant difference in Pb concentrations in unwashed and washed leafy vegetables grown in urban gardens (Nabulo et al., 2010). They analyzed Pb concentrations of 15 leafy vegetables in several urban gardens that had Pb concentrations ranging from 9 to 770 mg kg^{-1} near Kampala City, Uganda. Washed leafy vegetables had 35% lower Pb concentration than unwashed vegetables.

Bioavailability means the portion of a substance or element in a soil that is available for absorption into living organisms, such as humans, animals, or plants. Bioavailability of soil Pb cannot be explained solely by the total Pb concentration; rather, it largely depends on speciation of Pb in soil (Davis et al., 1992; Ge et al., 2000) and other site-specific soil chemistry (Hettiarachchi and Pierzynski, 2004). Soil pH, soil organic matter content (Sauve et al., 1998), total Pb concentration, and soluble P concentration (Hettiarachchi et al., 2000) collectively affect soil Pb speciation. Speciation of soil Pb can be altered by changing soil chemical properties through adding amendments to soils. Phosphorous fertilizers, Mn oxides, and organic matter (e.g., compost, straw, and manure) have been tested for their ability to reduce the bioavailability of Pb in soils. Adding P fertilizer increases soil-available P and results in the formation of Pb pyromorphite-like minerals, thereby immobilizing soil Pb (Ma et al., 1995; Hettiarachchi et al., 2000; Brown et al., 2003).

Speciation and bioavailability of Pb in soils amended with organic matter depends on the composition of the organic matter and soil chemistry. High Fe and Mn concentrations in biosolids reduce bioavailability of soil Pb (Brown et al., 2003). Increase of organic matter content and cation exchange capacity of soils as a result of compost application increase Pb sorption capacity of soil (Vega et al., 2009). In contrast, application of leaf compost increased

soil Pb solubility at soil pH range of 6.5 to 8 by promoting formation and dissolution of organic-Pb complexes (Sauve et al., 1998). In the above context, soil- and amendment-specific studies are essential to evaluate soil Pb bioavailability.

In addition to the direct effects of organic matter application on speciation of soil Pb, organic matter also provides plant nutrients to soil and improve soil physical properties such as bulk density and water holding capacity (Chaoui et al., 2003; Gallardo-Lara and Nogales, 1987; Khaleel et al., 1981). This improves plant growth and leads to high biomass content. High biomass content itself dilutes any absorbed potentially toxic elements in the plants (Ekvall and Greger, 2003). Increased plant nutrients (N, P, and K) in Pb-contaminated urban soil have been found to reduce Pb concentration in lettuce (Sterrett et al., 1996). Similarly, low concentrations of Pb, Cd, and Hg were found in water spinach grown in a high-nutrient medium compared with water spinach grown in a low-nutrient medium (Gothberg et al., 2004).

Regardless of the effects of organic matter amendment on Pb bioavailability, the addition of locally available compost is a common practice among community gardeners, because it improves the workability of soil and plant growth; therefore, study of the ability of locally available compost to reduce bioavailability of Pb in urban soils would provide useful and important information to urban gardening communities. Although the acceptable blood Pb concentration level in children continues to decrease due to the subtle health effects of elevated blood Pb concentrations, only a limited number of studies focused on uptake of soil Pb by plants (Sterrett et al., 1996; Cui et al., 2004) and how that uptake transfers Pb to humans, and very little attention has been given to the importance of cleaning of vegetables grown in urban areas as an effective way to minimize soil Pb transfer to humans through vegetable consumption.

We conducted a field experiment in an urban community garden located on a formerly residential brownfield site with soil contaminated by Pb to assess vegetable crop uptake of Pb, effectiveness of compost addition on Pb concentrations in vegetables, and the effects of vegetable-cleaning methods on Pb concentrations. The risk of Pb transfer via direct ingestion of garden soil also was evaluated using in-vitro bioaccessibility measurements.

Materials and Methods

A field study was performed in an urban community garden located in the Washington Wheatley neighborhood in Kansas City, Missouri (39^{o} 4' 55.8" N and 94^{o} 33' 4.3" W). The garden is 42 m × 37 m, and the site was formerly occupied by four houses. The houses were demolished in the late 1970s. We suspect the source of soil Pb concentration is past use of leaded paint on the houses, although the use of leaded gasoline until 1971 and burning coal for heat also may have contributed. First, the site soils were screened approximately every 3 m using a XL3T Niton handheld x-ray fluorescence (XRF) analyzer (Thermo Scientific, Billerica, MA) to detect the concentrations of potentially toxic trace elements. The initial XRF screening revealed that the site soils had elevated Pb concentrations; however, other potentially toxic trace element concentrations were not elevated. Spatial distribution of total Pb in the site soils was heterogeneous (Figure 3.1). Concentration of soil Pb ranged from 60 mg kg^{-1} to 300 mg kg^{-1}. Two field test studies were conducted in 2009 and 2010. In 2009, the test plots were located in an area closer to the average urban background concentrations of Pb (77 to 157 mg kg^{-1}) (USEPA, 2013), whereas in 2010, test plots were installed in an area with 186 to 388 mg kg^{-1} of Pb in the soils with the objective of gathering the maximum possible Pb uptake by the vegetables grown in the site soils.

Field study

Soil pH in urban garden soils that have elevated Pb should be maintained at a neutral level to minimize Pb solubility (Sterrett et al., 1996). Because soil pH was neutral (Table 3.1), this soil did not require pH adjustment or addition of lime. Mehlich-III P was high (Table 3.1), so no P fertilizer addition was recommended for this site. After excluding lime and P as our treatments, we selected locally available leaf compost (Missouri Organic, Kansas City, MO), which is commonly used by the gardeners in this area, as our field treatment. Some selected chemical properties of the compost are listed in Table 3.1.

In 2009, the experiment plot area was 2.4 m × 3.7 m, and in 2010 it was 5.2 m × 8.8 m. The plot area was demarcated from the rest of the garden using 20-cm-high plastic plot dividers such that 15 cm of the dividers remained aboveground. Compost was added at 28 kg m^{-2} and mixed with the top 15 cm of soil, representing a compost:soil ratio of approximately 1:3 (v/v). Control plots with no compost were maintained. A leafy vegetable, a fruiting vegetable, and a tuber/root vegetable were grown to assess Pb uptake of vegetables in the presence and absence of compost treatment. In 2009, Swiss chard (Beta vulgaris; variety: Gator Perpetual Spinach), tomatoes (Solanum lycopersicum; variety: Biltmore) and sweet potatoes (Ipomoea batata; variety: Beauregard) were grown. In 2010, Swiss chard (variety: Burpee's Rhubarb Chard), tomatoes (variety: San Marzano) and carrots (Daucus carota; variety: Danvers #126) were grown. Three blocks were prepared for each vegetable in 2009. The number of blocks was increased to four in 2010. Planting/sowing was done 16-days after incorporation of compost in both years. The recommended variety-specific spacing was maintained for all crops. At the end of the growing season, the edible portions of the plants were harvested as each plant type reached maturity. Representative soil samples were collected prior to adding compost, at planting, and at harvesting from each plot. When sampling soils, a composite soil sample was collected from

each plot by mixing soils collected from 4-5 random locations within a plot. The sampling depth was 0-15 cm.

Soil chemical properties (available N, P, and K; electrical conductivity; soil pH, soil organic matter content; total Pb and easily extractable Pb)

Soil samples were analyzed for available N, P, and K; pH; electrical conductivity (EC); and organic C concentration. Available N (ammonia and nitrate) of soils was extracted by 1 M KCl (Mulvaney, 1996). Available K was extracted by 1 M NH_4CH_3COOH (pH=7.0) (Helmke and Spark, 1996). Available P was extracted by Mehlich-III (Mehlich, 1984) and analyzed calorimetrically using a flow injection analyzer (Lachat Instruments, Loveland, CO). The Walkley-Black procedure was used to determine organic C content in soils (Nelson and Sommers, 1996). Soil pH was determined using a 1:10 soil:deionized water mixture and a pH meter with an 'Accumet' pH/ATC Electrode and Accumet AP 115 meter (Fisher Scientific, Pittsburgh, PA). Soil-soluble salts were extracted using a 1:2 (v/v) soil:water extraction (Sonneveld and Van den Ende, 1971). EC of the extraction was measured with a Seven Easy conductivity meter S30 and Inlab 731 electrode (Mettler-Toledo Inc., Columbus, OH). Dissolved organic carbon (DOC) concentrations were also determined in the 1:2 (v/v) soil:water extractions using a total organic carbon analyzer that purged inorganic carbon of the extractions using 1 M HCl (Shimadzu, Columbia, MD).

Soil total Pb and other trace element concentrations (Cr, As, Ni, Cd, Co, Cu, and Zn) were determined by EPA method SW846-3051A (USEPA, 2007b). To determine total trace element concentrations, 10 mL of trace metal–grade concentrated HNO_3 was added to 0.5 g of soil and digested in a microwave digestion unit (MARSXpress, CEM Corporation, Matthews,

NC). The temperature of the soil-acid mixture in the microwave digestion unit was increased to 165^{o}C in 5.5 min in the first stage of the temperature program. In the second stage, temperature was further increased to 175^{o}C in 4.5 min and the mixture was held at 175^{o}C for 5 min. A standard reference soil (NIST 2711a-Montana II) was digested along with every batch of test samples as a quality assurance/quality control (QA/QC) sample to evaluate digestion and analytical procedures. After filtering using the Whatman No. 42 filter papers, the solutions were analyzed for Pb using an inductively coupled plasma optical emission spectrophotometer (ICP-OES; Varian Inc., Foster City, CA).

Easily soluble, extractable Pb in the soils was extracted using 0.01M $Sr(NO_3)_2$. The soil:0.01M $Sr(NO_3)_2$ solution ratio was 20g:40mL. After shaking the suspension for 1 h, it was filtered using 0.45-µm syringe filters. The resulting solutions were digested using the EPA SW846-3015A method (USEPA, 2007a) in the microwave digestion unit. This digestion minimized the matrix suppression of Pb absorbance in the Graphite Furnace Atomic Absorption Spectrometry (GF-AAS; Varian Inc., Foster City, CA) and equalized the matrix effects in the extractions of soils with and without added compost. The matrix suppression for the Pb absorbance in the GF-AAS was high in the compost-added soils compared with the soils without added compost. To overcome this issue, 1 mL of concentrated nitric acid was added to 9 mL of the filtered solution, and the temperature was increased to 160^{o}C in 10 min, then to 170^{o}C in the next 10 min. The solution was again filtered using Whatman No. 42 filter paper and analyzed for Pb using GF-AAS.

Cleaning methods for vegetables

Produce in the 2010 study was divided into two portions and subjected to two cleaning procedures: laboratory cleaning and kitchen-style cleaning. Laboratory cleaning was done by rinsing the produce with tap water, deionized (DI) water, 5 g kg^{-1} sodium lauryl sulfate ($CH_3(CH_2)_{10}CH_2OSO_3Na$) solution, and again with DI water. This method was developed to remove all adhering soil dust particles from produce surfaces. Kitchen-style cleaning was done using only tap water to mimic the washing procedure of vegetables in a home kitchen. During kitchen cleaning, we removed all visible soil particles from the produce. In addition to these two methods, a portion of the lab-cleaned carrots was also peeled.

Plant digestion

Cleaned plant samples were chopped using a stainless steel knife, then dried at 70^{o}C for 4 to 5 days. Dried plant materials were ground using a Willey Mini Mill-Arthur Thomas–type grinder (Thomas Scientific, Swedesboro, NJ). The sieve size was 250 µm (60 mesh).

Plant samples were handled in a biosafety cabinet, class II type A2 (Esco Technologies Inc., St. Louis, MO), to avoid contamination by airborne dust in the laboratory. Ten milliliters of trace metal-grade concentrated HNO_3 were added to 0.5 g of ground plant material and digested in a microwave digestion unit. The temperature of plant-acid mixture in the microwave digestion unit was increased to 200^{o}C in 15 min, and the mixture was held at 200^{o}C for another 15 min. All the plant samples were digested in duplicates. In each batch of digestion, two samples of standard reference plant material (NIST 1515-Apple leaves) and two blanks (concentrated HNO_3 only) were included as QA/QC. Solutions were filtered using Whatman No. 42 filter papers and analyzed for Pb using GF-AAS. Different modifiers and temperature programs were used to

enhance the signals (absorbance) of Pb in GF-AAS. The modifier used for analyzing Pb in Swiss chard and sweet potatoes was 1mg mL^{-1} $NH_4H_2PO_4$ and 2% (w/v) H_3PO_4 for tomatoes and carrots. Recoveries of spiked digested solutions with known concentrations of Pb were used as a guide to method development in GF-AAS. The recoveries of spiked samples were 93% for Swiss chard, 98% for tomatoes and sweet potatoes, and 103% for carrots.

Simplified Physiologically Based Extraction Test (Simplified PBET)

Ruby et al. (1996) suggested that PBET can be used in site-specific studies to assess exposure of humans to Pb (and As) in soils. This in-vitro method extracts the bioaccessible Pb of Pb-contaminated soils in the human digestive system. Bioaccessible Pb levels were determined in the soil samples collected from tomato-growing plots at planting and at harvesting using the PBET procedure developed by Ruby et al. (1996) and simplified by Brown and Chaney (1997). We employed this method for two particle size fractions, < 250 µm and < 2 mm (whole soil). The < 250 µm size fraction represents the soil fraction that adheres to hand of a child and is recommended (USEPA, 2012); however, some scientists argue that larger soil particles can be ingested and may have a significant impact on Pb bioaccessibility (USEPA, 2007c). Furthermore, a complete characterization of soil fractions may be more consistent across sites (USEPA, 2007c). Based on these arguments, we also performed simplified PBET using < 2 mm soil fraction to estimate the bioaccessibility of the whole soil.

The gastric solution was prepared by mixing 1.25 g of pepsin, 500 mg of sodium L-malate, 500 mg of sodium citrate dihydrate, 500 µL of trace metal–grade acetic acid, and 420 µL of L(+)-lactic acid with 1 L of deionized water. The solution was acidified (pH was about 2.00) with trace metal–grade concentrated HCl prior to adding the solution to the soil. One gram of

soil and 100 mL of gastric solution were added to a 250-mL polypropylene bottle. The gastric solution was heated to 37^{o}C before adding it to the soil. The extraction was done at two initial pH values of the soil–gastric solution mixture, 1.5±0.02 and 2.5±0.02. pH 1.5 represents fasting gastric pH, and pH 2.5 represents the intermediate stomach state between fasting and fed conditions (Ruby et al., 1996). The pH adjustment of the soil–gastric solution mixture was done by adding varying volumes of trace metal–grade concentrated HCl. The soil–gastric solution mixture was shaken at 37^{o}C at 100 rpm for 1 h, then the samples were filtered using 0.45 µm syringe filters. A standard reference material (NIST 2711a-Montana II soil) was subjected to this test at both pH 1.5 and 2.5 with each batch of extraction (A batch consisted of 20 samples). Two blanks were also included in each extraction batch. Analysis of extractions for Pb was done by GF-AAS. The signal of the GF-AAS was enhanced by using 2% (w/v) H_3PO_4 as a modifier. Recovery of the spiked extraction with known concentration of Pb was 99%.

Statistical analysis

SAS 9.2 statistical software was used (SAS Institute, 2010). The Pb concentrations of vegetables and bioaccessible Pb concentrations were transformed to log base 10 to achieve normal distribution prior to statistical analysis. One-way analysis of variance (ANOVA) using PROC GLM was performed to analyze the effect of compost addition on Pb concentrations in vegetables. Separate analyses were done for each vegetable. The design was a split-plot design with completely randomized block arrangement with three (in 2009) or four (in 2010) blocks: the main plot factor was compost (compost-added and no compost), and the subplot factor was the cleaning techniques used. The effects of compost addition on bioaccessible Pb and % bioaccessible Pb (i.e., [bioaccessible Pb concentration in mg kg^{-1}/ total soil Pb in mg kg^{-1}]*100)

were analyzed using PROC GLM. Differences between bioaccessible Pb, determined at 16-days (at planting time) and 105-days (at harvest time of tomatoes) after compost treatment were analyzed using a paired t-test by PROC TTEST procedure.

Results and Discussion

Compost addition changed soil chemical properties

As expected concentrations of plant nutrients in compost-added soils were significantly higher than in soils that did not receive compost (Table 3.1). During the growing season, available N, Mehlich III-P, and available K concentrations reduced in compost-added soils. In soils that did not receive compost, concentrations of available N, Mehlich III-P, and available K did not decrease throughout the growing season. Soil pH in compost-added and no-compost-added soils was in the neutral range; furthermore, as one would expect, compost addition improved the concentration of soil organic carbon and cation exchange capacity (Table 3.1).

We observed high biomass production in compost-added soils. For example, in 2010 the average fresh Swiss chard harvest in compost-added plots weighed 1.61 kg m^{-2} (standard error 0.19), whereas in the plots that did not receive compost it was 0.58 kg m^{-2} (standard error 0.12). We also observed that plants in compost-added plots grew faster, were healthier, and produced higher biomass contents than the plants in plots that did not receive compost. It is not the intention of this paper to discuss soil fertility aspects in detail; however, due to possible effects of poor nutrient status on plant Pb uptake (this was discussed later in the paper), we were interested in the soil fertility status of the soil.

Dilution of soil matrix after compost addition significantly reduced total Pb concentrations in soils, and this effect was immediate. After adding compost, initial soil total Pb concentrations were reduced by 29–52% (data not shown). In contrast, easily soluble Pb

concentration, as estimated by 0.01M $Sr(NO_3)_2$ extraction, was high in compost-added soils compared with no-compost soils (Table 3.5). Increased extractable Pb concentration in compost-added soils related to increased DOC concentration in the soils upon compost addition (Table 3.1). Dissolved organic matter degrades over time and makes the dissolved Pb available for other reactions.

Lead concentrations in vegetables

All the vegetables had detectable amounts of Pb in their edible portions. From this point onward, the Pb concentration in plants refers to the Pb concentration in the edible portion: for tomatoes, it is the fruit; for Swiss chard, it is leaves; and for sweet potatoes and carrots, it is the root. No clear trend was observed for the relationship of Pb concentrations of vegetables to soil total Pb concentration (Figure 3.5). Compost addition increased the extractable P concentration in soils, as indicated by Mehlich III-P (Table 3.4 and 3.5). Although increased soluble P concentration can reduce the solubility of Pb in soils, there was no clear relationship between Mehlich III-P concentrations with the bioconcentration factors of the vegetables.

Effects of compost addition

The effects of compost addition on Pb concentrations in vegetable crops were determined after cleaning with the laboratory procedure, which aims to remove all adhering soil particles from the surface of the produce. In the 2009 study, compost addition did not significantly reduce Pb concentrations in any of the three vegetables (Table 3.4). The reason could be low concentrations of total Pb in soil (77–157 mg kg^{-1}) that resulted in only mild elevation of Pb concentrations in vegetables. In 2010, test plots were established in comparatively high total-Pb

soils (186 to 388 mg kg^{-1}) and, addition of compost significantly reduced ($P<0.05$) Pb concentrations in Swiss chard and carrots (Table 3.5). In compost-added plots, Pb concentrations were 59% lower in Swiss chard and 20% lower in carrots compared with Swiss chard and carrots grown in soils that did not receive compost. Lead concentrations of tomatoes were not significantly different in compost-added and no compost-added soils. Concentrations of Pb in tomatoes (average of ~0.07 mg kg^{-1}-dry weight) may be too low to show the effect of compost addition. In general, Pb bioconcentration factors express the proportion of soil total Pb concentrations absorbed by plants. Carrots and sweet potatoes had the highest bioconcentration factor, followed by Swiss chard, followed by tomatoes, supporting the fact that accumulation of Pb was highest in roots, followed by leaves, then fruits. This could be a result of translocation of Pb within the plant. Finster et al. (2004) found Pb concentration of tomato roots to be 33 times higher than that of the shoot and >72 times higher than that of the fruits in tomatoes grown in Pb-contaminated (3,740 mg Pb/kg) residential soils. Soil P concentration may affect translocation of Pb within a plant (Foy et al., 1978). When plants are grown in soils that have high P concentration, Pb phosphates precipitate in the root organelles. This limits the translocation of Pb from root to shoot. The moderate pH of the root cells and the xylem sap may assist Pb phosphate formation in roots.

The concentration of Pb in vegetables does not necessarily reflect total Pb uptake by that vegetable. Although compost addition reduced Pb concentrations in Swiss chard, it did not reduce its Pb uptake (total Pb) (Table 3.6), demonstrating that increased plant biomass as a result of compost addition diluted Pb concentrations in Swiss chard. Similar results were observed by Ekvall and Greger (2003). They showed that increasing plant total biomass of seedlings of *Pinus sylvestris* as a response to physiological conditions in the environment diluted Cd concentrations

in the plant, especially in the root. In our experiment, one no-compost plot for carrot produced 87% less root biomass than other no-compost plots because the soil was unusually compacted due to high clay content. This plot had the highest concentration of total soil Pb (387.9 mg kg^{-1}). The bioconcentration factor of carrots in this plot was 0.0206, which was 1.8 times higher than the average bioconcentration factor of carrots harvested from the other three no- compost soils (i.e., 0.0112, Table 3.5). This result indicates that an increase of total biomass of vegetables could be an effective means of reducing potential Pb transfer to humans.

Effects of vegetable cleaning methods

Lead concentrations of vegetables determined after cleaning with different techniques were significantly different for Swiss chard and tomatoes, but not for carrots (Figure 3.4). Swiss chard cleaned with the kitchen cleaning method contained 2.6 to 4.6 times greater Pb concentrations than that cleaned with the lab cleaning method. Similarly, kitchen-cleaned tomatoes had 3.0 times greater Pb concentrations than lab-cleaned tomatoes.

Transfer of Pb from plants to humans could occur not only because of uptake of Pb by plants, but also because of contaminated soil particles that adhere to the plant surface or are embedded in the waxy outer layer of plants. Fruits, leaves, and non-woody stems such as aerial parts of higher plants have an extra cellular membrane called the cuticle that consists of soluble and polymerized lipid covers (Heredia and Dominguez, 2009). Sodium lauryl sulfate, used in the lab cleaning method, is an anionic surfactant. Anionic (and nonionic) surfactants have the ability to solubilize water-insoluble materials such as cutin and dissolve a larger portion of the cuticle barrier (Furmidge, 1959). By using the lab cleaning method, we may have effectively removed particles embedded in the plant surface by solubilizing the cutin lipid cover; therefore, lab-

cleaned tomatoes and Swiss chard showed lower Pb concentrations. In contrast, cleaning methods did not significantly affect Pb concentrations in carrots. This can be explained by the absence of cuticle lipid layer on the roots. Peeling also did not statistically change Pb concentrations in carrots. When peeling, we removed a very thin outer layer of the carrots. Synchrotron-based x-ray fluorescence mapping has shown that the concentrations of Pb in the peel and the phloem of the carrot are low compared with the concentration of Pb in the inner xylem tissues of the carrots (Codling et al., 2007), which could explain the lack of difference in Pb concentrations of peeled carrots in this study.

Comparison of plant Pb with maximum allowable levels

When human health is concerned, it is important to interpret contaminant concentrations with respect to standard values of maximum allowable levels (MLs). The Codex Alimentarius Commission (CODEX), established by the Food and Agriculture Organization (FAO) and World Health Organization (WHO), develops international food standards, guidelines, and codes of practice to protect the health of the consumers and ensure fair practices in the food trade (JECFA, 1993). The CODEX committee on contaminants in food established or endorsed permitted maximum levels (MLs) or guidelines levels for contaminants and naturally occurring toxicants in food and feed and includes maximum levels for Pb concentrations in vegetables. We used these MLs as a guideline to compare concentrations of Pb in vegetables. It is important to note here that these limits are not developed based on bioavailability of Pb in food. Research has shown that Pb ingestion along with food reduces bioavailability of Pb in the digestive system (James et al., 1985: USEPA, 2003). Calcium and phosphates in the food may contribute to this reduction, but the exact mechanism is not understood (USEPA, 2003). CODEX MLs might have

been developed considering the upper limit or the maximum potential health risk of consuming Pb contaminated vegetables.

According to the CODEX guidelines, the ML of Pb is 0.3 mg kg^{-1} of fresh matter for leafy vegetables and 0.1 mg kg^{-1} of fresh matter for fruiting vegetables and root/tuber vegetables (FAO/WHO-CODEX, 1995; 2010 amendment). Because we analyzed dried plant materials to determine Pb concentrations in the vegetables, for the convenience of interpreting results, we converted the above MLs to a dry weight basis. For Swiss chard, which is a leafy vegetable, the ML of Pb is 5.0 mg kg^{-1} of dry matter (assuming moisture content of Swiss chard was 94%). The ML of Pb for tomatoesis 1.6 mg kg^{-1} dry matter (assuming moisture content of 94%; Pennington et al., 1998), and for carrots and sweet potatoes, the ML is 1.5 mg kg^{-1} of dry matter (assuming the moisture contents of 93%; Pennington et al., 1998).

Average concentrations of Pb in Swiss chard and tomatoes in both compost-added and no-compost soils were lower than the ML for Pb in leafy vegetables and fruiting vegetables both in 2009 and 2010 test plots (Figure 3.4). Concentrations of Pb in sweet potatoes and carrots were close to the ML for Pb in tuber and root crops. Consumption of leafy and fruiting vegetables grown at this site does not carry any health risk, but consumption of root and tuber crops grown at this site potentially carries the risk of ingesting harmful levels of Pb.

Bioaccessibility of soil Pb

Simplified PBET estimates the bioaccessibility of Pb in the event of direct ingestion of soil. Brown et al. (2003) showed that bioaccessible Pb determined by simplified PBET, which is also known as rapid PBET, correlates well with Pb concentrations in rat bones (at pH 1.5,

R2=0.84; at pH 2.3, R2=0.90). Furthermore, this correlation is stronger than the correlation between bioaccessible Pb recovered by the original PBET procedure developed by Ruby et al. (1996) and Pb concentrations in rat bones (at pH 2.0, R2=0.66).

Ingestion of food dilutes and buffers the pH of the gastric solution, making it less acidic at fed states (Davenport, 1984). Bioaccessible Pb as determined at pH 2.5 was 81% (compost-added soils) and 79% (no-compost soils) lower than the bioaccessible Pb determined at pH 1.5 (Tables 3.2 and 3.4). This was expected, because dissolution of Pb is highly pH-dependent. Similarly, a 65% reduction in dissolution of soil Pb was observed when pH of the soil-gastric solution increased from 1.3 to 2.5 (Ruby et al., 1996). Soil Pb bioaccessibility determined at pH 2.3 correlate well with bioavailability determined using *in vivo* methods (Medlin, 1997; Brown et al., 2003). Researchers have shown that the reduction of Pb bioaccessibility in the amended soils was prominent at pH 2.5 or 2.2 (Brown et al., 2004; Ryan et al., 2004) and that pH 1.5 overestimates the bioavailability of soil Pb (Brown et al., 2003; Smith et al., 2011). Drexler and Brattin (2007) recommended using pH 1.5, because this pH provides greatest improvement in predicting relative bioavailability and limits the risk of underestimating.

In this study, regardless of the extraction pH, the majority of soil Pb did not dissolve in the gastric solution. Only 33–44% of total Pb in the soils that did not receive compost and 21–32% of total Pb in the soils that received compost were dissolved in the gastric solution at pH 1.5, whereas 3.5 to 6.0% of total Pb was dissolved in the gastric solution at pH 2.5. This result indicated that the majority of soil Pb at this site is not bioaccessible. Past research also observed that a considerable portion of Pb in urban soils was not bioaccessible (Yang et al., 2001; Brown et al., 2003; Farfel et al., 2005). The fraction of bioaccessible Pb can be different from one site to another, depending on Pb speciation and other soil chemical properties. Unpublished x-ray

absorption data from our laboratory shows most Pb in this soil was in either ferrihydrite adsorbed- or humic acid adsorbed forms. Smith et al. (2011) showed that soil Pb was strongly associated with Fe oxyhydroxide minerals or the soil organic fraction in a study conducted with urban soils. Researchers have found that outer-sphere and inner-sphere adsorption are two major processes of Pb immobilization in soils (Zimdahl and Skogerbo, 1977; Strawn and Sparks, 2000). In urban soils, the concentration of Pb generally may not reach high enough levels to expect significant formation of Pb precipitates; however, we cannot rule out the possibility of localized Pb precipitation when concentration of Pb and other constituents of the common Pb minerals in soils (e.g., carbonates and phosphates) are high (Zimdahl and Skogerbo, 1977; Cotter-Howells, 1996).

Effects of compost addition on bioaccessible Pb after 16-days of compost addition as determined at pH 1.5

Compost addition reduced bioaccessible Pb in the < 2 mm fraction (Table 3.2). In the < 250 µm fraction, bioaccessible Pb was not statistically different in soils with compost and without compost. Dilution of soil total Pb and increase in soil-available P upon compost addition may have caused the reduction of soil bioaccessible Pb (< 2 mm fraction) in the compost-added soils. Figure 3.2 shows a comparatively high relationship ($R^2 = 0.71$) between soil total Pb and bioaccessible Pb at 1.5 pH. This dilution of soil total Pb in compost-added soils was not intense in the < 250 µm fraction, because most of the compost materials were removed with larger particles during sieving. The percentage of bioaccessible Pb is a measure of bioaccessibility independent from soil total Pb; therefore, percentage bioaccessibility values are not influenced by the dilution effect of soil total Pb upon compost addition. The percentage of bioaccessible Pb in both < 2 mm and < 250 µm fractions was significantly lower in the compost-added soils than

in the soils that did not receive compost (Table 3.2). This result suggests that in addition to the dilution effect, compost reduced the bioaccessibility of soil Pb. Increased soil-available P upon compost addition (average available P concentrations were 68 mg kg^{-1} in the soils that did not receive compost and 438 mg kg^{-1} in the soils that receive compost) might partially contribute to the decreased percentage of bioaccessible soil Pb in compost-added soils. Figure 3.3 shows the relationship of bioaccessible Pb to available P concentrations. Reductions in Pb bioaccessibility with increases in available P concentrations have been observed by past researchers (Hettiarachchi et al., 2000; Yang et al., 2001; Hettiarachchi et al., 2003). Available P reacts with Pb in soil and forms relatively stable Pb species such as hydroxypyromorphite (or hydroxypyromorphite-like minerals) and chloropyromorphites (or chloropyromorphite-like minerals) (Hettiarachchi et al., 2000).

In addition to total soil Pb and soil-available P concentrations, soil total organic C (Ruby et al., 1996) also affect bioaccessibility of soil Pb. Ruby et al. (1996) argued that total organic C in soil increases the bioaccessibility of Pb as estimated by the stomach phase of the original PBET procedure (Ruby et al. [1996] consisted of two phases, stomach and intestinal). The authors explain that organic C provides additional sorptive surfaces that may readily desorb Pb in the gastric environment. Although total organic C concentrations were higher in the compost-added soils (3.2%) than in soils without added compost (2.1%) in our study, the effects of soil organic C on Pb bioaccessibility might have been counteracted by the effects of increased P concentrations in the compost-added soils. Further, Fe and Mn oxides in compost reduce bioaccessibility of soil Pb (Hettiarachchi et al., 2000; Brown et al., 2003). In a recent study, Brown et al. (2012) demonstrated that not only the Fe concentration but also the reactivity of Fe in the biosolids affected the bioaccessibility of Pb in soils. The compost we used in our

experiment was a leaf-based compost, which had only 3.8 g kg^{-1} total Fe and 0.2 g kg^{-1} of total Mn, which were fairly low concentrations compared with the composted biosolids used by Brown et al. (2003) and Brown et al. (2012). Therefore, the higher concentration of soil-available P was the predominant factor that reduced the percentage of Pb bioaccessibility in compost-added soils compared with the soils without added compost.

Effects of compost addition on bioaccessible Pb after 16 days of compost addition as determined at pH 2.5

Compost addition did not reduce bioaccessible Pb at pH 2.5 in both < 2 mm and < 250 µm fractions (Table 3.3). To improve the statistics, we did PBET extraction at pH 2.5 in soil samples collected from all 12 blocks (4 blocks per each vegetable) after 16-days of compost addition, but we still did not see a significant reduction in bioaccessible Pb upon compost addition (Table 3.3 shows only the results of 4 blocks). Unlike at pH 1.5, the dilution of soil total Pb as a result of compost addition seemed to have a minimal effect on reducing bioaccessible Pb at pH 2.5. This result was supported by the poor relationship between bioaccessible Pb and soil total Pb at pH 2.5 (Figure 3.2).

Percentage of bioaccessible Pb was significantly lower in compost-added soils than in soils that did not receive compost in the < 250 µm fraction ($P<0.15$; $p = 0.12$). This was not significant in < 2 mm fraction (Table 3.3). As previously explained, lowering the percentage of bioaccessible Pb in compost-added soils can be attributed to enhanced available P in soil through compost addition. Figure 3.3 also shows a poor relationship of available P and percentage of bioaccessible Pb in the < 2 mm fraction. In this size fraction, the effects of enhanced P on percentage of bioaccessible Pb could have been masked by increased Pb solubility in the compost-added soils as a result of elevated concentrations of DOC. The effects of DOC on

percentage of bioaccessible Pb could be minimal in the < 250 µm fraction and not high enough to mask the effects of enhanced available P because the proportion of compost material in this fraction was relatively low. Concentration of DOC at pH 1.5 was expected to be lower than that at pH 2.5. Past research found that at lower pH, the dissolution of soil organic carbon was less than that at high pH (You et al., 1999). Therefore, the increase in Pb solubility by DOC might not have been high enough to override the effects of elevated available P concentration on the percentage of bioaccessible Pb at pH 1.5.

Change of bioaccessibility of soil Pb over the growing period

We used the percentage of bioaccessible Pb instead of bioaccessible Pb to evaluate this time effect because bioaccessible Pb depends on the total Pb concentration, and the total Pb concentrations in two soil samples (16-days and 105-days after adding compost) collected from the same plot can be different depending on the variability of the total Pb concentration at the site: Average difference and standard error of difference in total Pb concentrations of the samples collected 16-days after compost addition (at planting) and 105-days after compost addition (at harvesting tomatoes) were 24 mg kg^{-1} and 5.4 mg kg^{-1}, respectively. These are low and acceptable for a field experiment. The change in bioaccessible Pb concentration throughout the growing period was analyzed only in the compost-added soils 16-days and 105-days after compost addition to evaluate the effects of compost addition on the percentage of bioaccessible Pb through time. Percentage of bioaccessible Pb in compost-added soils did not decrease significantly over time when measured at pH 1.5 in both < 2 mm and < 250 µm fractions (Table 3.2). As discussed above, high available P concentration reduced the percentage of bioaccessible Pb in the compost-added soils. A small reduction in the percentage of bioaccessible Pb in phosphate-applied soils over time was observed in previous research (Hettiarachchi et al., 2000;

2001), which could be because either the majority of the bioaccessible Pb reduction happened within the first 16-days of compost addition or the Pb-P reaction happened under the gastric phase of PBET and was not affected by the contact time of Pb and P in the field, as in Hettiarachchi et al. (2001).

When bioaccessibility was measured at pH 2.5, the percentage of bioaccessible Pb seemed to decrease over time in compost-added soils in the < 2 mm fraction, but at a low significance level ($p < 0.15$; $p = 0.11$; Table 3.3). This was not significant in the < 250 µm fraction. At pH 2.5, solubility of Pb due to DOC was a prominent factor that affected the percentage of bioaccessible soil Pb, as discussed earlier. Dissolved organic carbon introduced by compost addition decreased over time; therefore, we expect lower Pb solubility in the soils collected 105-days after compost addition compared with 16-days after compost addition. This could explain the significant reduction in the percentage of bioaccessible of Pb in the < 2 mm fraction over time at pH 2.5. The representation of compost material in the < 250 µm fraction would be low, as mentioned before. Therefore, the DOC effect on Pb bioaccessibility could be minimal in this fraction and could explain the lack of significant difference in percentage of bioaccessible of Pb in this fraction of compost-added soils over time.

A significant decrease in percentage of bioaccessible Pb in soils with compost added was observed compared with soils without added compost after 105-days of compost addition in the < 250 µm fraction. This result indicated that the effects of enhanced available P in compost-added soils lasted > 105-days. This difference was not significant in the < 2 mm fraction, which can be attributed to the higher variability of the bioaccessibility values observed in this size fraction compared with the < 250 µm fraction.

Conclusions

The extent of Pb contamination was highly variable within a small area. Compost addition diluted initial total soil Pb concentrations, indicating that the continuous addition of compost would lower total Pb concentration in soils significantly. Compost addition reduced the potential risk of soil Pb transfer to humans indirectly through consumption of vegetables grown at this site and directly through soil ingestion by decreasing plant Pb and bioaccessible Pb concentrations. Dilution of soil total Pb concentration and increase in soil-available P followed by compost addition helps reduce soil Pb transfer to humans through direct ingestion of soils. In addition, compost addition helps maintain good soil nutrient status in soils. Maintaining good soil fertility and thereby increasing biomass production diluted Pb concentrations in the vegetables. The highest concentrations of Pb in edible portions were found in root/tuber crops, followed by leafy and fruiting vegetables. Thorough cleaning and removal of soil/dust particles deposited on edible portions of vegetables, especially leafy and fruiting vegetables, further reduces food chain transfer of soil Pb to humans.

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Tables and Figures

Table 3.1 Selected nutrients and fertility parameters in preliminary soil samples (soil), compost, and plot soils at planting and at harvest of vegetables (in 2010)

	Soil	Compost	No-compost plot soils				Compost-added plot soils†			
			At planting	At harvest			At planting	At harvest		
				Swiss chard	Tomato	Carrot		Swiss chard	Tomato	Carrot
			Days after compost addition							
			16	69	105	121	16	69	105	121
Total Pb, mg kg^{-1}	68-305	24	208±14‡	-	-	-	146±13‡	-	-	-
pH (1:10 soil: water)	6.80	8.5	6.9	7.1	7.1	6.7	7.60\	7.5	7.6	7.0
CEC ¶, $cmol_+$ kg^{-1}	-	-	20.4	-	-	-	33.8	-	-	-
Sand, silt and clay, %	4, 75, 21	-		-	-	-	-	-	-	-
Organic C, %	-	21	2.1	0.9±0.1§	0.9±0.1§	1.0±0.1§	3.2	3.1±0.2§	2.7±0.1§	2.9±0.1§
DOC#, mg L^{-1}	-	-	51	-	18	18	158	45	47	44
Electrical conductivity, dS m^{-1}	0.19	5.6	-	-	-	-	-	-	-	-
Total N, mg kg^{-1}	1907	13470	-	-	-	-	-	-	-	-
Available N, mg kg^{-1}	13-127	555	18±0	28±1	12±1	16±2	279±18	46±4	29±3	30±2
Mehlich III-P, mg kg^{-1}	57-154	-	68±06	57±8	63±11	58±10	438±25	355±23	264±28	279±14
Available K, mg kg^{-1}	225-624	-	328±20	285±19	310±20	336±18	1978±97	1091±137	893±15	862±26
C:N ratio	-	16	-	-	-	-	-	-	-	-
Total P, mg kg^{-1}	-	3150	-	-	-	-	-	-	-	-
Total Fe, mg kg^{-1}	-	6280	-	-	-	-	-	-	-	-
Total Mn, mg kg^{-1}	-	397	-	-	-	-	-	-	-	-

†Rate of compost-28 kg m^{-2}.

‡Average and standard error of 12 blocks: 4 blocks for each of 3 vegetable crops.

§Average and standard error of 4 blocks.

¶Cation Exchange Capacity.

Dissolved organic C in 1:2 soil:water (v/v) extract

Table 3.2 Soil bioaccessible Pb in tomato-growing plots as determined at pH 1.5

	16-days after adding compost (at planting)		105-days after adding compost (at harvesting)		Standard soil
	Bioaccessible Pb	% bioaccessible Pb†	Bioaccessible Pb	% bioaccessible Pb	% bioaccessible Pb
< 2 mm fraction (Whole soil)	mg kg^{-1}		mg kg^{-1}		
No compost	57.4±5.0*‡	37.5±2.5**	55.6±7.0	33.6±3.5	-
Compost	44.1±6.0*	28.7±1.7**	46.2±11.0	26.1±3.3	-
< 250µm fraction					
No compost	78.7±19.5	32.9±2.3*	84.4±27.5	35.4±1.5**	-
Compost	53.9±6.8	29.0±1.0*	56.7±5.7	26.7±0.8**	-
NIST 2711 a	-	-	-	-	78.9§

* Two values in the same column within a size fraction were significantly different at 0.1 probability level.

** Two values in the same column within a size fraction were significantly different at 0.05 prbability level.

† Bioaccessible Pb as a percentage of soil total Pb. Soil total Pb in the < 2mm or < 250 µm fraction was used to calculate percentage of bioaccessible Pb in the corresponding fraction.

‡Standard error of 4 blocks.

§Acceptable range is 75.2-96.2 % when 0.4M glycine is used as the extractant (USEPA, 2012)

Table 3.3 Soil bioaccessible Pb in tomato-growing plots as determined at pH 2.5

	16-days after adding compost (at planting)		105-days after adding compost (at harvesting)		Standard soil
	Bioaccessible Pb	% bioaccessible Pb†	Bioaccessible Pb	% bioaccessible Pb	% bioaccessible Pb
	mg kg^{-1}		mg kg^{-1}		
< 2 mm fraction (Whole soil)					
No compost	13.4±6.4‡	6.3±2.0	12.1±3.4	5.2±1.3	-
Compost	9.2±1.3	6.0±0.4*	6.4±1.8	3.6±0.6*	-
< 250µm fraction					
No compost	14.1±4.8	5.6±0.9**	12.8±5.1	5.1±0.5***	-
Compost	7.4±1.4	3.9±0.4**	8.5±1.8	3.9±0.5***	-
NIST 2711 a	-	-	-	-	35.2

* Two values in the same row within a size fraction were significantly different at 0.15 probability level.

** Two values in the same column within a size fraction were significantly different at 0.15 probability level.

***Two values in the same column within a size fraction were significantly different at 0.1 probability level.

†Bioaccessible Pb as a percentage of soil total Pb. Soil total Pb in the < 2mm or < 250 µm fraction was used to calculate percentage of bioaccessible Pb in the corresponding fraction.

‡ Standard error of 4 blocks.

Table 3.4 Selected chemical properties of soils† and Pb concentrations in vegetables (in 2009)

	Soil total Pb	Soil pH	Mehlich III-P	Vegetable Pb‡	BCF§
	mg kg^{-1}		mg kg^{-1}	mg kg^{-1}, dry weight	
Experiment plots					
No compost					
Swiss chard	95±13	6.92	99±6	0.39±0.12	0.0052
Tomato	123±21	7.03	103±2	0.07±0.00	0.0006
Sweet potato	105±8	7.05	99±7	0.83±0.12	0.0081
Compost					
Swiss chard	81±4	7.88	215±10	0.26±0.03	0.0032
Tomato	97±11	8.06	260±22	0.07±0.01	0.0008
Sweet potato	102±16	7.99	240±29	1.32±0.11	0.0132
Community samples ¶					
Swiss chard A	-	-	-	0.89	-
Swiss chard B	-	-	-	0.98	-
Mustard A	-	-	-	0.17	-
Mustard B	-	-	-	0.27	-
Carrot	-	-	-	1.03	

† Soils collected immediately after adding compost to the experiment plots.

‡ Pb concentrations of edible portion of vegetables in dry weight basis determined after washing with the lab cleaning technique.

§ Bioconcentration factor: ratio of Pb concentration in the edible portion of plant and total Pb concentration in soil.

¶ Randomly collected plant samples from the rest of the garden. Varieties of plants were not known; different varieties are indicated by A and B. Gardeners added compost prior to planting.

Table 3.5 Selected chemical properties of soils† and Pb concentrations in vegetables (in 2010)

	Soil total Pb	$Sr(NO_3)_2$-extractable soil Pb	Soil pH	Mehlich P	Vegetable Pb ‡	BCF §
	mg kg^{-1}	mg kg^{-1}		mg kg^{-1}	mg kg^{-1}, dry weight	
Experiment plots						
No compost						
Swiss chard	221± 47	<0.005	6.93	62±10	0.71 ±0.084 **	0.0037
Tomato	189±28	<0.005	6.88	75±09	0.09 ±0.029	0.0005
Carrot	224±55	<0.005	6.97	68±12	1.37±0.179**	0.0112
Compost						
Swiss chard	154±35	0.021±0.007	7.70	456±44	0.29 ±0.04 **	0.0020
Tomato	153±15	0.020±0.006	7.57	450±16	0.06 ±0.02	0.0004
Carrot	129±11	0.020±0.005	7.65	409±64	1.41±0.23**	0.0110
Community samples ¶						
Tomato (Red cherry)	66		7.66		0.20	0.0030
Tomato (Yellow cherry)	102		7.77		0.06	0.0006
Sweet pepper	73		7.85		0.08	0.0011
Lettuce	92		7.87		0.32	0.0034
Okra	95		7.47		0.08	0.0009

† Soils collected 16-days after adding compost (at planting) to the experiment plots.

‡ Pb concentrations of edible portion of vegetables in dry weight basis after washing them with the lab cleaning technique.

** Two values of the same vegetable across the compost treatments were statistically significant at 0.05 probability level.

§ Bioconcentration factor: ratio of Pb concentration in the edible portion of plant and total Pb concentration in soil.

¶ Randomly collected soil and respective plant samples from the rest of garden. Samples were collected at harvest. Gardeners added compost prior to planting.

Table 3.6 Effects of compost addition on Pb concentration and total Pb in Swiss chard leaves (in 2010)

	Pb concentration	Aboveground biomass	Total Pb
	μg kg^{-1}, fresh matter	Kg, fresh mater	μg, fresh matter
No compost	42.3±5.1**	0.92±0.19	44.8±9.3
Compost	17.2±2.6**	2.54±0.30	40.7±12.9

Average±standard error of 4 blocks.

** Two values were statistically significant at 0.05 probability level.

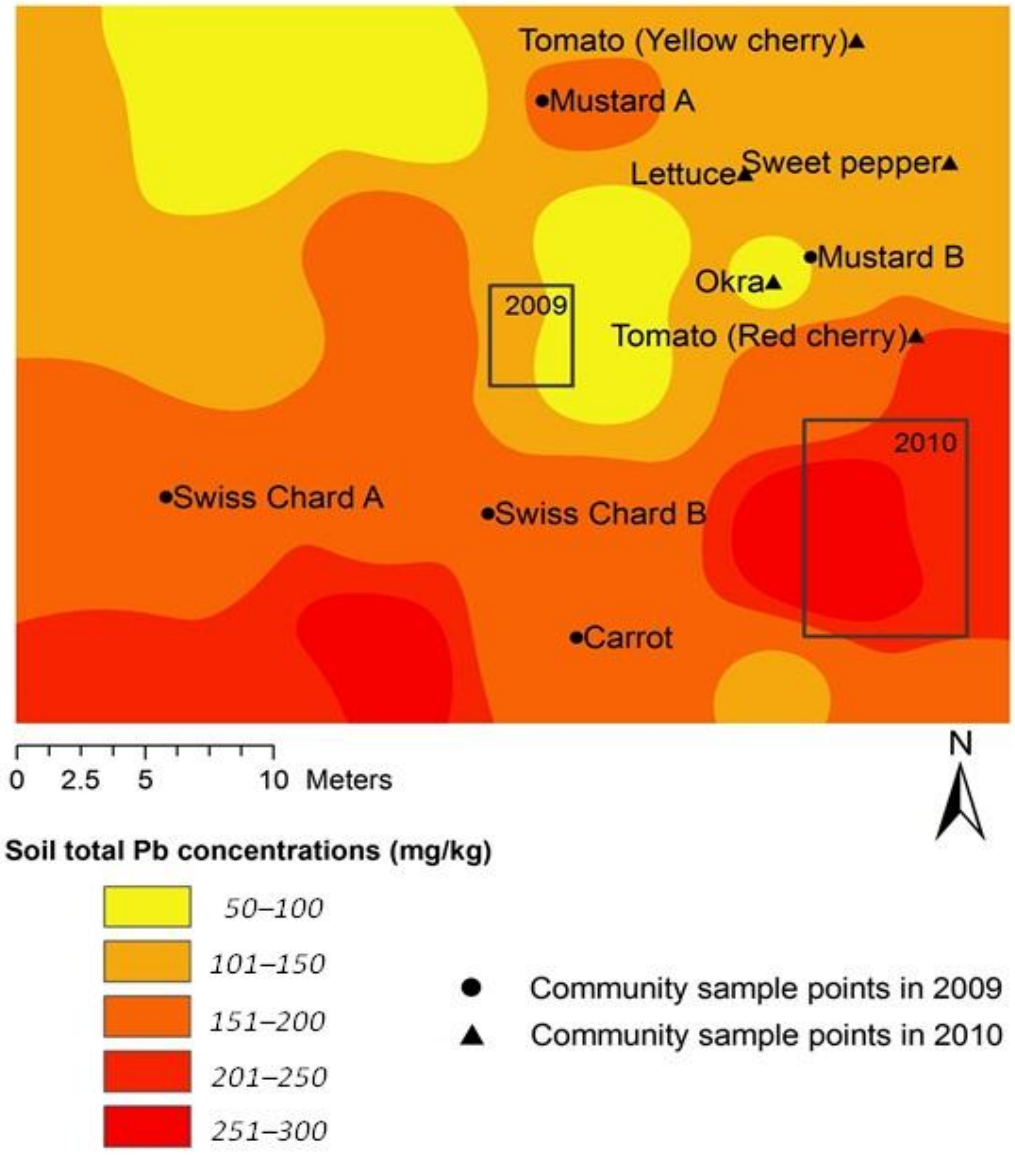

Figure 3.1 Distribution of soil total Pb concentrations in the site and locations of test plots in 2009 and 2010 and community sample points

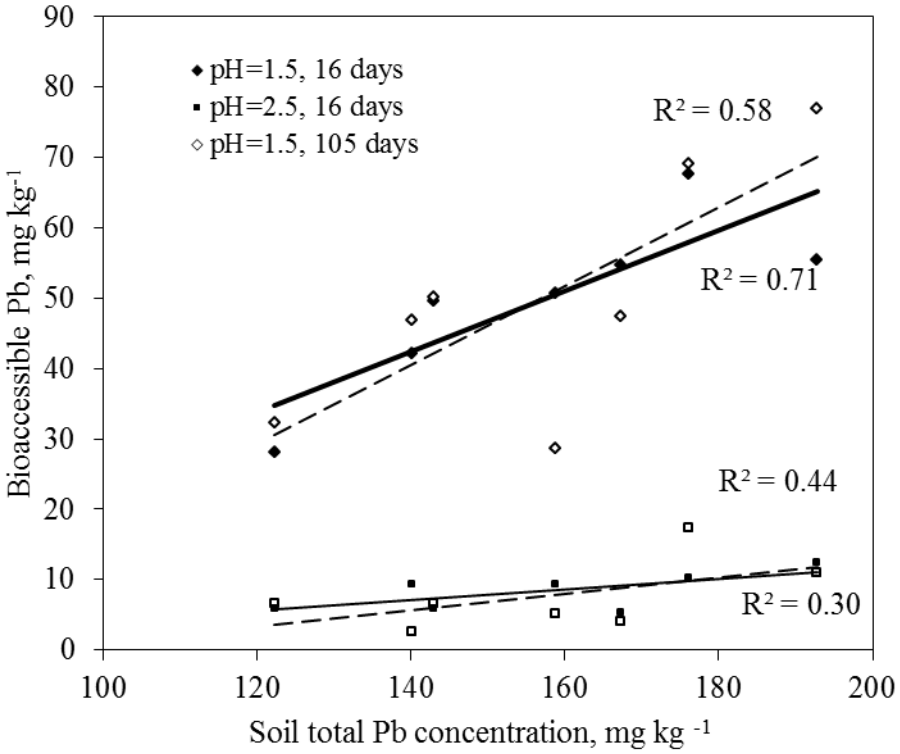

Figure 3.2 Relationship of soil bioaccessible Pb in the < 2 mm size soil fraction to soil total Pb concentration in the same fraction

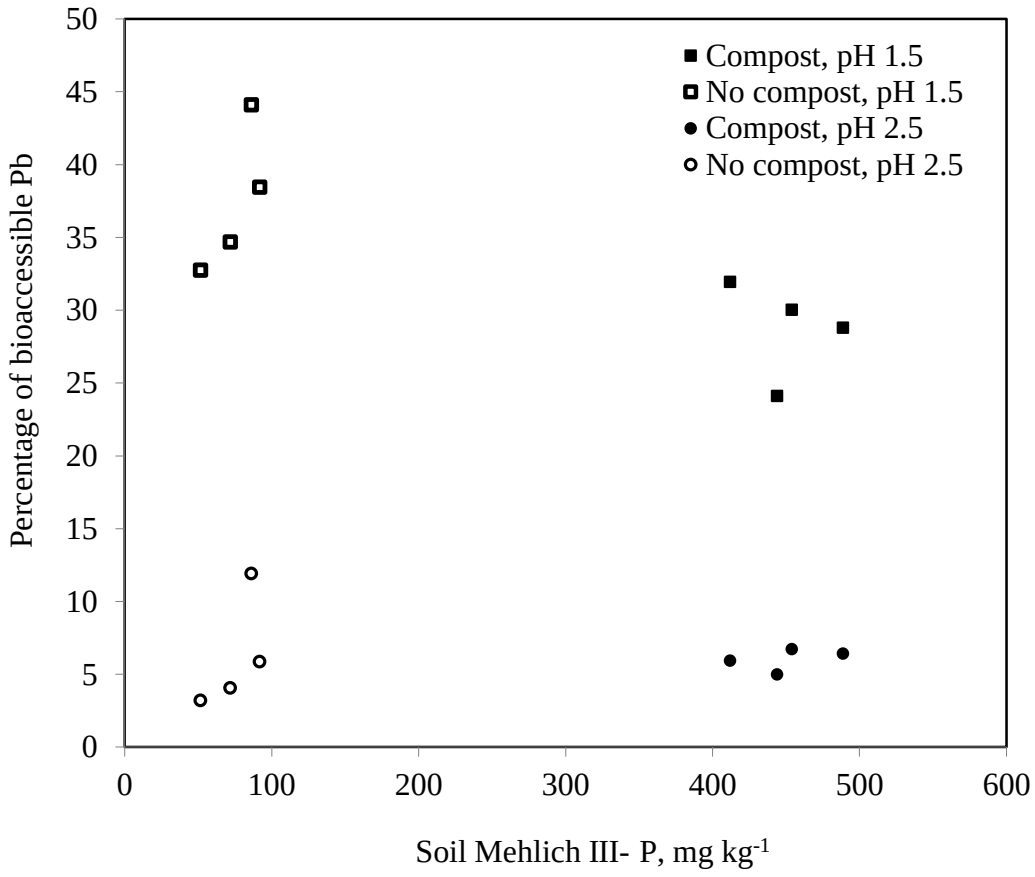

Figure 3.3 Relationship between percentage of bioaccessible Pb and soil Mehlich III-P in the < 2 mm size soil fraction 16-days after compost addition

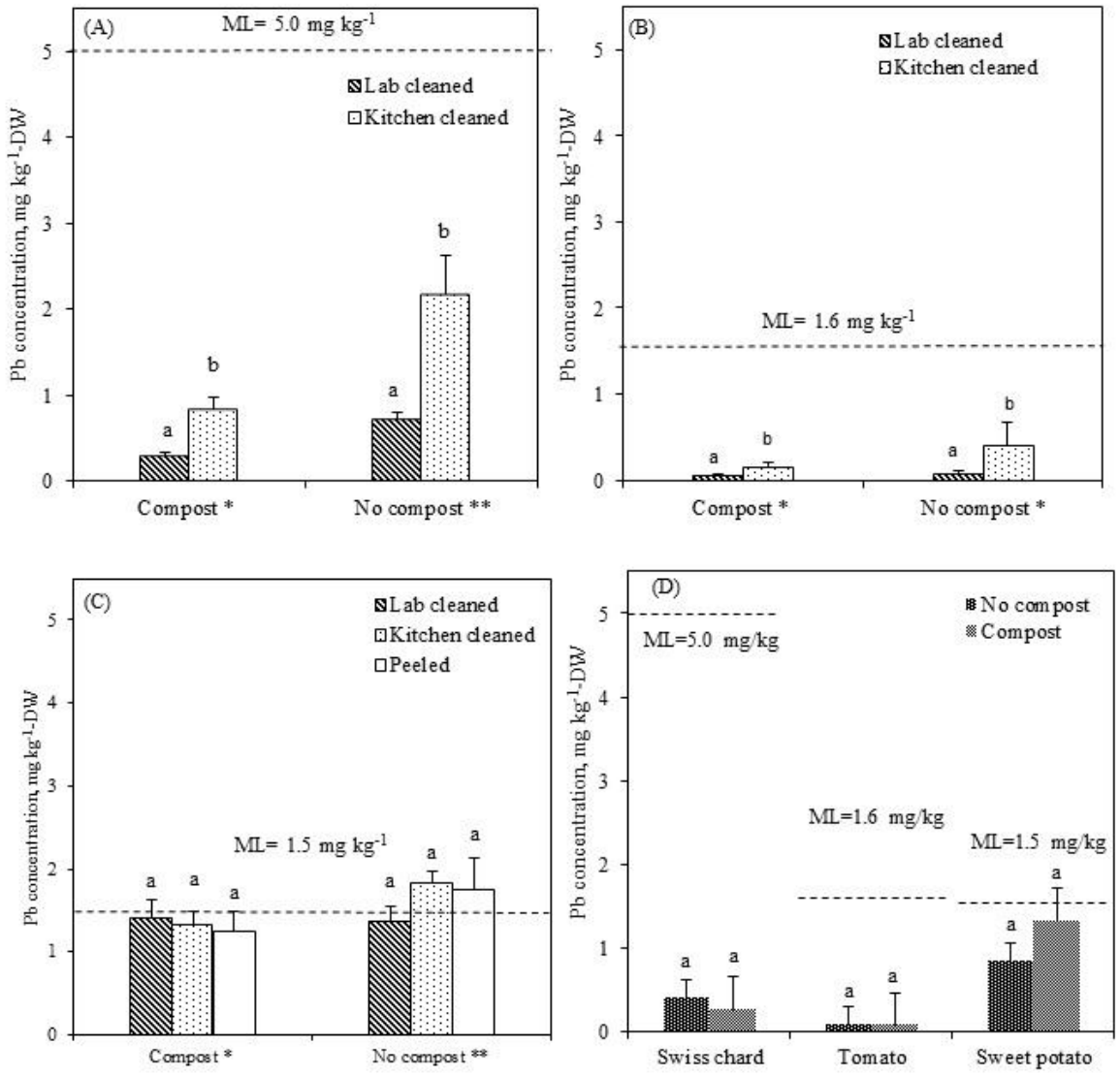

Figure 3.4 Comparison of vegetable Pb concentrations with maximum allowable levels† of Pb in vegetables. †Maximum allowable levels: leafy vegetables, 0.3 mg kg^{-1}-fresh weight; fruiting vegetables, root and tuber crops, 0.1 mg kg^{-1}-fresh weight (FAO/WHO-CODEX, 1995; 2010 amendment). (A) Swiss chard in 2010; ML=5.0 mg kg^{-1}-dry weight, moisture content 94%. (B) Tomato in 2010; ML=1.6 mg kg^{-1}-dry weight, moisture content 94%. (C) Carrot in 2010; ML=1.5 mg kg^{-1}-dry weight, moisture content 93%. (D) Vegetables in 2009; ML 5.0, 1.6 and 1.5 mg kg^{-1} as in (A), (B), and (C) respectively.

***, ** Two categories are significantly different at 0.05 probability level. Different letters indicates the significance difference within the category at 0.05 probability level and similar letters indicates no significant difference within the category.**

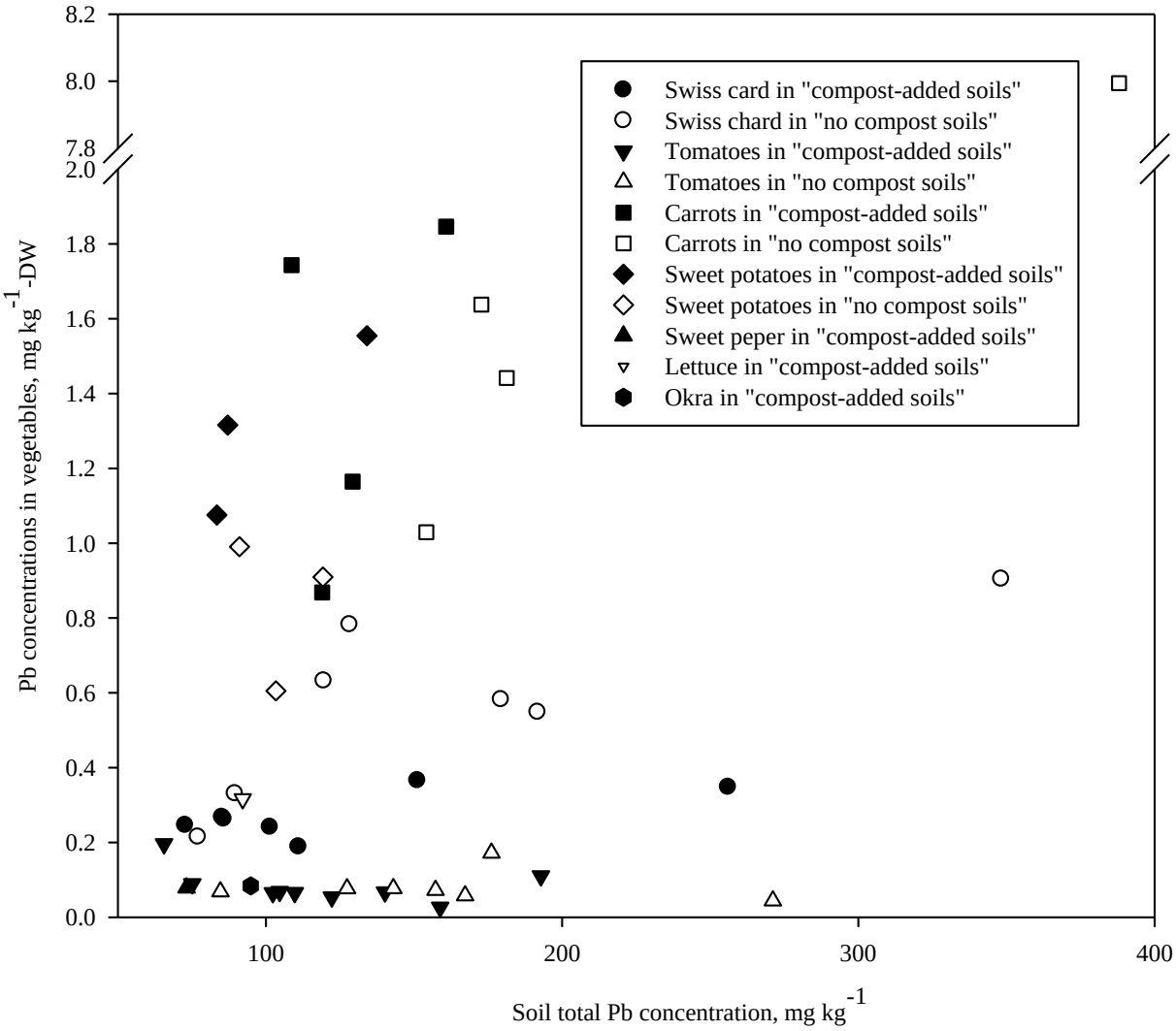

Figure 3.5 Relationship of Pb concentrations of vegetables to soil total Pb concentrations in 2009 and 2010 test plots and 2010 community samples

Chapter 4 - Lead Speciation in Urban Garden Soils with and without Compost and Its' Changes during an *In-vitro* Bioaccessibility Extraction Test

Abstract

Lead (Pb) is a toxic element that is commonly found at elevated concentrations in urban soils. The main pathway of human exposure to soil Pb is direct soil ingestion. The bioavailability of soil Pb depends on Pb speciation and other site specific soil properties. *In situ*-soil amendments can change soil Pb speciation and other soil properties modifying the bioavailability of soil Pb. From previous field experiments, we found that the bioaccessibility of soil Pb in urban soils is low, and the effect of adding *in situ* organic amendments on bioaccessibility (as determined by *in vitro* methods that mimic gastrointestinal dissolution process) of soil Pb is insignificant. Lead speciation and bioaceessibility in three urban garden soils contaminated with different sources of Pb and containing different total Pb concentrations, were investigated using X-ray absorption fine structure (XAFS) spectroscopy and an *in vitro* bioaccessibility test. The change of soil Pb speciation upon *in situ* organic amendments over time and the chemical changes that occurred during the *in vitro* extraction method were also assessed to evaluate the *in vitro* method response for amended urban soils. Lead speciation of urban soils was dominated by Pb sorbed to Fe oxy(hydr)oxide (Pb-Fh) and to soil organic C (SOC) (Pb-Org). The fraction of Pb-Org was high in soils with high organic C and increased with time after application of compost in the field. The Pb-Fh fraction dissolved and redistributed in to Pb-Org during the *in vitro* extraction method as a result of reductive Fe dissolution under extremely low pH. This reductive Fe dissolution was limited in soils with field matured compost. Hydroxypyromorphite was formed during the *in vitro* extraction method. Speciation of urban soils and its' changes

during the *in vitro* method suggested the necessity of validating the *in vitro* methods for amended urban soils *in vivo* animal model studies.

Introduction

Lead (Pb) is one of the most common contaminants in urban soils. Lead is toxic for humans and exposure to Pb can damage the nervous system and affect kidney performance (ATSDR, 2007). In an urban gardening scenario people can be exposed to soil Pb via two main pathways: direct soil ingestion (soil-human transfer) and food chain transfer (soil-plant-human transfer). Direct soil ingestion is the most significant exposure pathway for Pb (Lanphear and Roghmann, 1997) and, hence bioavailability of soil Pb has been widely studied. Bioavailability explains the portion of a substance or element in a soil that is available for absorption into living organisms, such as humans, animals, or plants. The bioavailability of soil Pb largely depends on speciation of Pb in soils (Davis et al., 1992; Ge et al., 2000). Lead carbonates, Pb_3O_4, PbO and $Pb(OH)_2$ are more soluble and potentially have high bioavailability. Lead phosphates, PbS, Fe-Pb oxides, Fe-Pb sulfates, Mn-Pb oxides, $PbCrO_4$ and $PbSO_4$ are less soluble and, hence have low bioavailability (Lindsay 1979; Hettiarachchi and Pierzynski, 2004; Barrett et al., 2010). The majority of Pb in urban soils originated from leaded gasoline and leaded paint. The original form of Pb in gasoline is tetraethyl lead (Kovarik, 2005). Pigments in paints utilized $2PbCO_3.Pb(OH)_2$, a lead hydroxycarbonate (Welcomme et al., 2007). In the soil, these original species of Pb undergo different biochemical reactions with soil constituents (SOC, clay, P etc.), alter with time, and convert to other more stable Pb species. These reactions and the stability of Pb species depend on properties of soils such as soil pH, SOC concentration, soluble inorganic P concentration, amorphous Fe and Mn concentration and clay content.

Various *in situ* soil amendments have been shown to alter soil properties and stabilize soil Pb. It has been shown that P amendment is a promising *in situ* remediation approach for Pb-

contaminated soil (Ryan et al., 2004; Baker et al., 2014). The formation of Pb phosphates, and in particular pyromorphite, which is one of the most stable forms of Pb in soils under a wide range of environmental conditions (Nriagu, 1974; Lindsay, 1979), upon addition of apatite or soluble inorganic P amendments was observed in Pb contaminated soil materials (Ma et al., 1993; Laperche et al., 1996; Cotter-Howells and Caporn, 1996; Hettiarachchi et al., 2001). Reduced plant Pb uptake was also observed upon apatite or triple superphosphate (TSP) addition to Pb-contaminated soils (Laperche et al., 1997; Brown et al., 1999; Hettiarachchi and Pierzynski, 2002). Changes in soil Pb speciation upon amendments with biosoilds (Scheckel et al., 2005: Brown et al., 2012) or various inorganic P sources (Baker et al., 2014) has also been addressed. Increases in the concentrations of organic C, metal oxy(hydr)oxides, and soluble inorganic P via different types of composted material addition may stabilize soil Pb by providing additional sorptive sites and formation of pyromorphite like stable Pb minerals. Both SOC and metal oxy(hydr)oxides are proven to have a strong affinity to soil Pb (Martinez and McBride,1999).

Our science-based knowledge on handling/evaluating soil Pb impact on health and environment and *in situ* remediation of soil Pb is mostly limited to mine impacted materials or soils with unusually high Pb concentrations. Considering increasing interest in urban gardening and the recent lowering of the blood lead level of concern to 5 µg dL^{-1} (ACCLPP, 2012), there is a great need for science based knowledge on managing risk associated with moderately contaminated urban soils. The concentration of Pb in urban soils is fairly low compared to the Pb concentrations in mine waste materials and soils near mine tailings. Total Pb concentration can influence the Pb speciation. At low to moderate concentrations Pb sorbed to inorganic and organic constituents could be the dominant Pb species controlling Pb solubility via adsorption/desorption reactions, whereas in highly contaminated soils Pb precipitation as Pb-

phosphates and carbonates could be dominant. It should be noted, however, even at low to moderate total Pb concentrations, Pb can be precipitated in microenvironments/localized sites where the Pb concentration and the other constituents of the Pb precipitate are high (Zimdahl and Skogerbo, 1977). Published literature on Pb speciation in amended urban soils contaminated with residential and industrial activities are lacking. Further knowing the speciation of Pb in amended soil provides a better understanding of bioavailability and mobility of Pb in soil and the effectiveness of the amendments to stabilize soil Pb.

We have investigated the effect of different soil organic amendment addition on bioaccessibility (potential for soil to human direct exposure via ingestion) and food chain transfer of Pb in few different urban soils (Hettiarachchi et al., 2013; Attanayake, et al., 2014; Defoe et al. 2014). The plant Pb concentrations and the bioaccessibility of soil Pb, as measured using a physiological based extraction test (PBET, a modified Ruby et al., 1996 as modified according to Medlin, 1997), were low in these soils, indirectly suggesting that the greater portion of Pb in these urban soils could be in sparingly soluble forms. Research efforts focus on cross-correlating direct soil Pb speciation studies with bioaccessibility studies would provide opportunities to clearly link the binding mechanism of Pb in soil to plant and human bioavailability.

The risk of exposure for soil Pb via direct soil ingestion can be assessed directly using animal feeding studies or indirectly using preciously validated *in vitro* bioaccessibility tests developed to mimic the gastrointestinal dissolution process. Although animal studies are superior to *in vitro* bioaccessibility tests in terms of reliability, the high cost, high labor involvement, and the negative public opinions associated with animal studies raise the importance of development of *in vitro* bioaccessibility tests that correlate with animal studies. Researchers use the term

'bioaccessibility' for the estimated amount of Pb that could potentially dissolve and available for absorption by human digestive system. There are several *in vitro* methods developed to estimate bioaccessibility of soil Pb (Ruby et al., 1996; Oomen et al 2002; Smith et al. 2011; Scheckel et al., 2013). These methods have been developed using highly contaminated, non-amended mine impacted materials or soils. Given the significance of urban gardening in the past decade, the necessity to investigate the applicability of existing *in vitro* bioaccessibility extraction methods to estimate risk of direct soil ingestion in moderately contaminated urban soils is becoming critical. Therefore, more insight should be given towards how these methods operate for urban soils. Smith et al. (2011) provides some direct evidence of changing soil Pb speciation in an *in vitro* bioaccessibility extraction test performed at pH 1.5 using X-ray absorption near-edge structure (XANES) technique. Scheckel et al. (2005) provided indirect observation to formation of pyromorphite-like stable minerals during an *in vitro* bioaccessibility extraction method in a study conducted with highly contaminated mine impacted soils.

The application of P fertilizer and compost has shown promising results for reducing bioaccessibility when they were tested against non-amended soils. However, the bioaccessibility of soil Pb has not changed or changed very little when the contact time of soil to amendment is increased (Hettiarachchi et al., 2000; 2001). We observed the same from our field studies. This suggests that the reduction of bioavailability of soil Pb happened because of the reactions that occur in the short run and/or because of the reactions that occur during the *in vitro* bioaccessibility extraction methods. Here, we attempted to understand the Pb speciation before and after subjecting the soil to an *in vitro* bioaccessibility method via a direct speciation technique, x-ray absorption fine structure (XAFS) spectroscopy. Knowing the changes of Pb speciation during the bioaccessibility methods and cross-linking those to bioaccessible Pb and

soil properties provides useful information to develop effective remediation techniques that target minimizing Pb exposure through direct soil ingestion. Unlike some traditional techniques like X-ray diffraction, XAFS spectroscopy has the advantage of determining the speciation of less abundant elements like Pb, regardless of the crystallinity of the species (Cotter-Howell et al., 1994; Scheckel et al., 2005). Further, identification of metal sorption and surface precipitation on soil constituents is enhanced by XAFS (Fendorf et al., 1994; Manceau et al., 1996).

The specific objective of this research was to assess the change of Pb speciation upon addition of organic amendments *in situ* and how it related to the bioaccessibility of Pb in three urban soils. In addition, change of Pb speciation and bioaccessibility over time and the change of Pb speciation during the bioaccessibility extraction test were studied.

Material and Methods

Soil Samples

The soil samples for this experiment were collected from three field studies conducted in three urban garden sites located in Kansas City, MO (39^{o} 4' 55.8" N and 94^{o} 33' 4.3" W); Philadelphia, PA (39^{o} 58' 43.2" N and 75^{o} 07' 14.1" W; and Indianapolis, IN (39^{o} 46' 09.8" N and 86^{o} 09' 31.7" W). The Kansas City site had low to moderate soil total Pb concentration (60 to 380 mg kg^{-1}) whereas the Philadelphia site high Pb concentration (1200 to 2100 mg kg^{-1}). The soil total Pb concentration in the Indianapolis site ranged from 430 to 520 mg kg^{-1}. The main objective of these field experiments was to evaluate how to minimize soil Pb transfer to humans via direct soil ingestion and food chain transfer. All three sites had sufficient to very high available P concentrations in the soils. Therefore, we did not use P fertilizers as an *in situ* field treatment. Further, the soil pH was neutral in all three sites, so no pH adjustment or lime was needed for these sites. Locally available composts were used as field treatments to mimic the

gardening scenario. Two different leafy composts were used at the Kansas City, MO and Philadelphia, PA sites. A biosolids (composted, class A) was used at the Indianapolis, IN site. The basic properties of these composts are provided in Table 4.4 in supporting information. Test plots were in a balanced split plot design with four replicates arranged in a randomized complete block design; the main plot factor was compost treatments (compost-added and control) and the sub plot factor was the vegetable type (tomatoes, carrots, and a leafy vegetable that was either Swiss chard, collard greens or lettuce). More details about general field experiment arrangement can be found in (Attanayake et al., 2014).

Several soil sampling scenarios from the field test sites were chosen for this experiment. The sampling depth was 0-15 cm. At the Kansas City site, several composite soil samples (prepared by thoroughly mixing samples collected from 4-5 random locations within a plot) were collected from three compost-added plots and two control plots 16-days after adding compost (compost-added soils: KC-C1-16d, KC-C2-16d and KC-C3-16d; control soils: KC-4-16d, KC-5-16d) in 2010. Soil samples were again collected at the time of harvest for those plots (KC-C1-121d, KC-C2-69d, KC-C3-105d, KC-4-121d and KC-5-69d). Furthermore, a composite soil sample that was prepared using soils collected from the three compost-added plots prior to adding compost was also included for obtaining soil properties and Pb speciation prior to adding compost. At the Philadelphia site, five soil samples were collected from random locations across the field plot area in 2011 to be used as control soils (P-1, P-2, P-3, P-4, and P-5). Later in 2012 test plots were established, and a composite soil sample was collected from a compost-added plot 115-days after adding compost (P-C-115d). From the Indianapolis site, composite samples from a compost-added and a control plots were collected at 7-, 349-, and 642-days after adding compost. The first two soil samples were collected at the beginning of the first growing season in

2011 (compost-added soil: I-C-7d, control soil: I-2-7d). The other two sets of samples were taken at the beginning of the second and the third growing seasons in 2012 and 2013 (compost-added soils: I-C-369d, I-C-642d; control soils: I-2-369d, I-2-642d). The soils were air-dried and sieved to 2 mm size using a stainless-steel sieve. For the *in vitro* bioaccessibility extractions, a subsample of the < 2mm size fraction was further sieved to obtain the < 250 µm size fraction.

Basic soil properties

Total Pb in < 250 µm and < 2mm fractions of soils was analyzed using EPA 3051A method (USEPA. 2007). Soil samples were digested in a microwave digestion unit (MARSXpress, CEM Corporation, Matthews, NC) and the filtered solutions were analyzed for Pb using inductively coupled plasma optical emission spectrophotometer (ICP-OES; Varian Inc., Foster City, CA). The detailed digestion and analysis procedure is in Attanayake et al. (2014). The < 2 mm soil fraction was used for analyzing basic soil properties. Soil pH was measured in a soil-deionized water mixture (1:10) using a pH meter with an 'Accumet' pH/ATC Electrode and Accumet AP 115 meter (Fisher Scientific, Pittsburgh, PA). Mehlich-III extractable P was analyzed following Mehlich (1984) and the P was analyzed calorimetrically using a flow injection analyzer (Lachat Instruments, Loveland, CO). The soil organic C concentration was determined using the Walkley-Black procedure (Nelson and Sommers, 1996). Soil humic acid fraction was extracted as described in Swift (1996). Amorphous Fe and $Na_2C_2O_4$ extractable Al were extracted as described in Loeppert and Inskeep (1996) and analyzed using ICP-OES. Soil texture was analyzed using the Pipette method (Gee and Bauder, 1986).

Physiologically based extraction technique

The method developed by Ruby et al. (1996) with some modifications according to Medlin (1997) was used. The extraction solution was the gastric solution which was prepared by

mixing 1.25 g of pepsin, 500 mg of sodium citrate dihydrate, 500 mg of sodium L-malate, 500 µL of trace metal–grade acetic acid, and 420 µL of L(+)-lactic acid with 1 L of deionized water. The pH of the gastric solution was adjusted to 2.5 by adding concentrated trace metal grade HCl. The <250 µm is the fraction that potentially adheres to the children's hands (USEPA, 2012); however, larger particles can be also ingested and may have a significant impact of soil Pb bioaccessibility (USEPA, 2007b). We employed this method for two size fractions of soils: <250 µm and <2 mm. Another reason for using <2mm size fraction was that the addition of compost could cause some aggregation of small soil particles. So including larger the particle size to estimate Pb bioaccessibility of compost-added soils may be helpful in evaluating the effect of compost on bioaccessibility compared to the untreated soils. One g of soil was added to 100 mL of gastric solution. The gastric solution was heated to 37^{o}C prior to adding to the soil. The pH of the soil-gastric solution mixture was adjusted to 2.50±0.02 using trace metal grade HCl. The soil-gastric solution mixture was shaken in an incubated orbital shaker at 37^{o}C for 1 h. Then 20 mL the solution was filtered using a 0.45 µm syringe filter. The filtered solution was analyzed for Pb using graphite furnace atomic absorption spectrometry (GF-AAS; Varian Inc., Foster City, CA) using the standard addition calibration method. The balance of extract: soil mixture was centrifuged to collect the residue (PBET residue) for Pb XAFS analysis. First, the solution was centrifuged at 3000 rpm for 5 min and followed by decantation. Then, the residue was washed 3 times with 15 mL of deionized water. The supernatant was decanted at each washing step. The residue was stored at -20^{o}C until Pb XAFS analysis was performed.

X-ray Absorption Fine Structure Spectroscopy

This was done for air dried soils (<2 mm size fraction) and for wet PBET residues (< 2 mm size fraction). Before collecting the XAFS data, the soils were ground into a fine powder

using an agate mortar and pestle. Soils and PBET residues were packed in sample holders made out of 2 mm thick Plexiglas. The both sides of the sample holders were sealed with Kapton® tape, so that the X-ray beam passed through the sample that is sandwiched in between the two Kapton tapes. Fluorescence emission at LIII-edge (13055eV) was collected at 5-BMD beamline of DND-CAT at the Advanced Photon Source (APS), Argonne National Laboratory in Argonne, IL. This beamline has an energy range of 4.5-25 keV and a Si (III) double crystal monochromator that has a focused beam size of 15000 x 500μm and unfocused beam size of 15 x 5mm. Canberra 13-element Ge solid state detector was used. Two Al foil layers were placed on the detector to reduce the background fluorescence emissions. The angle of the incident X-ray beam was ~45^{o} with respect to the sample surface. The energy was calibrated using Pb-metal foil standard. The XAFS of Pb-metal foil was collected simultaneously with every sample spectrum. This standard spectrum was used to identify any energy drifts caused by the monochromator drifts during the sample run. Lead standards were scanned in transmission mode. All together 21 standards were used in the data analysis. They were anglesite, chlropyromorphite, hydroxypyromorphite, galena, leadhillite, magnetoplumbite, lead hydroxide, hydrocerrusite, cerussite, hinsdallite, $Pb(NO_3)_2$ (aq), vauquelinite, lead chloride, lead oxide (PbO), plattnerite, plumboferrite, plumbogumite, plumbonacrite, plumboyarosite, Pb sorbed to humic acid (Pb-Org) and Pb sorbed to ferrihydrite (Pb-Fh). The sorption standards (Pb-Org and Pb-Fh) were prepared as in Scheckel et al. (2004). XAFS of the standards were plotted in Figure 4.4 in supporting information. The number of spectra collected per standard/samples was 2 to 9.

In the process of data analysis, first the spectra were merged, then normalized and the background was removed. Then, the sample and standard spectra were aligned for any energy drifts using the first derivative spectra. Then, the spectra were converted to k space, and further

analysis was done for the range of 2 to 9 $Å^{-1}$. Next principal component analysis (PCA) followed by target transformation (TT) were performed. Principal component analysis and TT for the spectra of each site were done separately. The objective of PCA is to get the minimum number of significant components (also known as principle components, PCs), required to satisfactorily regenerate the data matrix, using a reduced space (Beauchemin et al., 2002). Therefore, PCA identifies the number of linearly independent components derived mathematically from a mixture of sample spectra. The numbers of PCs, given by the lowest IND values were three for Kansas City soil spectra, one for Philadelphia soil spectra and two for Indianapolis soil spectra. Target transformation gives a spoil value for individual standard. The spoil value explains the degree that the standard should be changed to fit the PCs. The standards that gave < 6 spoil value were used in Liner Combination Fitting (LCF) to identify the major Pb species and their relative proportion in the samples. If the spoil value is > 6, that standard does not represent the PCs (Beauchemin et al., 2002). The spoil values obtained were mentioned in the Table 4.5 in supporting information. Data processing and LCF of XAFS was done using Athena software, version 0.8.061 (Ravel, 2001-2009). The PCA and TT was done using the LabView software package from beamline 10.3.2 at the Advanced Light Source (Marcus et al., 2004).

Results and Discussion

Basic soil properties

Physical and chemical properties of the soils are given in Table 4.1. The Kansas City soil exhibited a silt loam texture with 21% clay, whereas the other two site's soils were coarse textured (sandy loam) with very low clay percentages. The pH of all the soils were between 6.4 to 7.9. The organic C concentration was the lowest in the Kansas City soil, while the remaining

two sites had similar and high organic C concentrations. Compost-added soils had higher humic acid percentages than the control soils in all three sites. Mehlich–III extractable P was classified as medium in the Indianapolis and the Kansas City control soils whereas it was high in the compost-added soils (Marr et al., 2010). The highest Mehlich–III extractable P was found for the Philadelphia site, regardless of the compost addition. . Continuous addition of compost for many years prior to this could be the reason for high Mehlich–III extractable P observed in this soil. The highest amorphous Fe concentration was observed in the Indianapolis soils followed by the Philadelphia and Kansas City sites. Amorphous Fe concentrations were not much different when comparing compost-added and control soils from all three sites. Compost-added soils had lower Pb concentrations than the control soils in all three sites, due to dilution of the soil matrix as a result of compost addition (Table 4.2).

Soil Pb bioaccessibility and speciation

Soils from all three sites had low Pb bioaccessibility, ranging from ~2-10 % of total Pb. Compost addition did not seem to change bioaccessibility of soil Pb significantly. Inherently low bioaccessibility of soil Pb in these soils might have not supported any further changes to Pb upon compost addition. The neutral pH range of these soils may have also contributed to this low Pb bioaccessibility. Under acidic soil pH conditions, the metal sorption capacity of soils is reduced, due to protonation of functional groups in inorganic and organic sorptive surfaces. When bioaccessibility of soil Pb is measured under extremely low pH (i.e., gastric pH: 2.5), survival of sorbed Pb in the solid phase indirectly suggests strong inner-sphere Pb sorption. In general, under alkaline soil pH conditions, Pb could potentially precipitates as Pb-carbonates. The bioaccessibility of Pb-carbonates is high as it dissolves in extremely acidic stomach pH. The soil pH of the compost-added soils was also in the neutral range, although slight pH increments was

observed upon compost addition in the Kanas City soils, later it became comparable to the control soils. These negligible pH changes may also have partially contributed to the similar bioaccessibility in compost-added and control soils. Generally, the soils with high sand contents have low metal sorption capacity and, hence they are expected to have high bioaccessibility of potentially toxic trace elements like Pb. However, in this experiment, soils from the Indianapolis and Philadelphia sites had the lower or similar Pb bioaccessibilities as compared to soils from the Kansas City site despite having greater sand contents. High amorphous Fe and SOC concentrations in Indianapolis and Philadelphia soils may have contributed to this low bioaccessibility despite their low clay fraction. Strong affinity of Pb for soil Fe oxides and organic C is widely recognized (Martinez and McBride 1999; Brown et al., 2003; 2012).

Studies that report bioaccessible Pb (determined at pH 2.5) in soils that are contaminated by industrial or residential activities is very limited. The bioaccessible Pb reported for highly contaminated mine impacted urban soils are greater than the values that we observed here for urban soils contaminated with industrial and residential activities (Smith et al., 2011). Smith et al. (2011) reported that the Pb bioavailability in soils contaminated with incinerator wastes and from landfill activities ranged from 10 to 28% whereas it was 30 to 89% for mine impacted and shooting range soils as determined by an *in vivo* mouse model. This low soil Pb bioaccessibility that we observed could be mainly because of the specific sorption of Pb onto Fe oxides and organic surfaces. The mine impacted soils may contain significant proportions of Pb minerals like PbS, $PbSO_4$ and $PbCO_3$ (Ryan et al., 2004; Brown et al., 2012), some of which would be soluble in the mimicked GI tract systems. To form precipitates of Pb minerals, the concentration of Pb^{+2} in the soil solution should be high and exceed the sorption capacity of the sorptive sites in soil like Fe-oxides, clay minerals and organic molecules. These sorptive sites have high

affinity to Pb^{+2} ion which is a soft Lewis acid with a large atomic size. Soft Lewis acids prefer to bind with soft Lewis bases like those occurring in Fe-oxides and organic molecules over hard ligands such as PO_4^{-3}, CO_3^{-2} (Essington, 2004).

According to the results of the Pb XAFS study, the predominant Pb species at all three sites were Pb sorbed to ferrihydrite (Pb-Fh), and to humic acid (Pb-Org) (Table 4.3). In this experiment, Pb-Fh represents the Pb sorbed to Fe oxy(hydr)oxide and Pb-Org represents the Pb sorbed to SOC. The association of Pb primarily with amorphous mineral and organic phases in urban soils has been reported previously (Smith et al., 2011). In the Kansas City soils, 82 to 92% of Pb was in Pb-Fh form in the control and the compost-added soils, 16-days after compost addition. The Pb-Org fraction was only about ~10-20% which is like likely because of the low SOC concentration in control soils compared to the other two sites and that 16-days may not have been long enough to sorb Pb on the SOC introduced by the compost addition. As indicated by the speciation of KC-C1-121 and KC-C2-69 soils, the percentage of Pb-Org increased when the compost matured in the field. In contrast, the Pb-Org percentage was low in the KC-C3-105 sample which was collected 105-days after compost addition. This difference of Pb-Org fraction in compost-added soils could be attributed to rhizosphere modification of Pb speciation which depends on plant species (Hashimoto et al., 2011). Sample KC-C3-105 was collected from an area with tomatoes growing. Tomato has an extended root system compared to the plants grown in KC-C1 (carrot) and KC-C2 (Swiss chard) soils. The representation of rhizosphere soil in the bulk soil samples we collected could be high in the KC-C3-105. The acidic nature of the rhizosphere soil may enhance the protonation of functional groups of SOC and maintain low Pb-Org (Hashimoto et al., 2011). The SOC concentration of this soil was also low (Table 4.1). Enhanced decomposition of SOC in this soil may also have affected the Pb speciation. Schroth et

al. (2008) et al. found that Pb sorbed to SOC may redistribute to other phases such as Fe oxy(hydr)oxide and birnessite, upon decomposition of SOC. In addition to Pb-Fh and Pb-Org, small amounts of hydroxypyromorphite and plumboferrite were found in KC-C1-16 and KC-5-16, respectively.

Soil samples from the Philadelphia site had 29 to 47% of Pb as Pb-Org while those from Indianapolis had about 30% of Pb as Pb-Org. The greater proportion of Pb-Org species from these two sites compared to the Kansas City site soil can be explained by greater organic C concentrations at these two sites. Similar to the compost-added soil in the Kansas City site, the Pb-Org species increased as compost matured in the field in the Indianapolis site (I-C-349 and I-C-642). Although the Pb-Org species in the compost-added soils had increased, their soil bioaccessibility had not changed much, when compared with control soils (Tables 4.2 and 4.3). A set of field and incubation experiments conducted by Brown et al. (2003; 2012) using biosolids-added Pb-contaminated urban soils showed that compost reduced bioaccessible Pb when reactive Fe-oxides were added along with the compost and the increase of organic C upon compost addition did not contribute to a reduction of bioaccessible Pb. In our experiment, none of the composts increased the amorphous Fe concentration (Table 4.1). Therefore, this could be a major reason for not seeing any Pb bioaccessibility difference between compost-added and the control soils in this study. The Pb speciation in control soils did not change much during the study period.

Compost introduced additional amounts of inorganic soluble P into the soil as indicated by Mehlich-III extractable P concentration in the Kansas City and Indianapolis soils (Table 4.1). Some soluble inorganic P, more specifically orthrophosphate ions such as HPO_4^{-2} and $H_2PO_4^-$, can react with Pb to form stable Pb phosphate minerals such as pyromorphite (Scheckel et al.,

2013). However, very limited research shows formation of pyromorphite with increasing soluble P concentration under field conditions. There is one field research conducted in a smelter contaminated site, in Joplin, MO, where pyromorphite was formed with increasing soluble P concentration in soil (Ryan et al 2004). Scheckel et al. (2005) had biosolids and biosolids with added soluble P treatments as field treatments in the same site and observed 1-16% of pyromorphite formation in plots with those treatments. However, Joplin, MO soils were highly contaminated with Pb (1100 to 5300 mg Pb kg^{-1}) unlike the soils used in this experiment. In this study, Pb XAFS did not reveal the formation of pyromorphite in the compost-added soils. This may be because these soils had only low to moderate Pb concentrations and soil solution Pb was likely controlled by the sorption capacity of the soils, as also supported by the low bioaccessibility of soil Pb. In contrast to the Kansas City and Indianapolis sites, the Philadelphia soil had higher Pb concentration (~1100-2300 mg kg^{-1}); however no pyromorphite or any other Pb mineral was found in these soils either. This could be because of high sorption capacity of these soils as suggested by the relatively high SOC and moderate amorphous Fe concentrations, or due to a different Pb source (industrial) compared to mine impacted Joplin soils.

Change of soil Pb speciation during PBET

The acidic environment during the PBET affected the Pb speciation of the soil solid phase left after the extraction (PBET residue) (Table 4.3). The fraction of Pb-Fh in the PBET residue was low or undetected compared to the soil prior to PBET in all the tested soils, except P-C-115 and I-C-642. The reduction of the Fe oxide sorbed Pb fraction in some urban soils during the acidic (1.5 pH) gastric phase of an *in vitro* bioaccessibility method was previously explained by Smith et al. (2011). Smith et al (2011) had observed an increase of Fe concentration in the gastric solution during the gastric phase. Dissolution of Fe oxy(hydr)oxide under acidic

soil conditions has been widely reported (Suter et al., 1991; Roden, 2003; Johnson et al., 2012). This can happen in two ways: Protons or specifically adsorbed chelate ligands replace Fe (III) from Fe oxy(hydr)oxide and increase Fe solubility, and organic ligands (or metal complex) act as electron donors to reduce Fe (III) in Fe oxy(hydr)oxide via microbial or chemical reactions, hence increasing Fe solubility via reductive dissolution. This reductive dissolution of Fe oxy(hydr)oxides is much faster than the release of Fe (III) from Fe oxy(hydr)oxides (Suter et al., 1991)

In KC-C1-16 and KC-C2-16 compost-added soils the reduction of Pb-Fh during PBET was lower than the two control soils (KC-4-16 and KC-5-16). This could be a result of a slight pH increase in the extraction solution for compost-added soils during the PBET. Although the pH of the soil-gastric solution mixture was adjusted to 2.50±0.02 at the beginning of the extraction, during 1h extraction time this pH slightly increased. The allowable pH change during PBET extraction is ± 0.5 units (USEPA, 2012). We followed this recommendation, but the pH increment during the extraction was 0.30-0.39 units higher in the compost-added soils than the control soils in samples from the Kansas City site. This slight pH increment may encourage readsorption of some dissolved Pb on to the sorptive sites by increasing the surface negative charges in compost-added soils. Although reprecipitation of amorphous Fe is a possibility with increasing pH (pH 6 to 7) at high soluble Fe concentration (Smith et al., 2011), this may not happen during the gastric phase of the PBET extraction since the pH is maintained to be < 3. This pH increase during the PBET extraction was similar for compost-added and control soils in Indianapolis site.

Further, when the compost was mixed with soils the total dissolved organic C concentration in soil may rise temporally. This dissolved organic C could enhance reductive Fe

dissolution acting as an effective electron donor for Fe (III) reduction (Petruzzell et al., 2005). Therefore, when compost is fresh in the soils or soon after compost was added to the soil the reduction of Pb-Fh fraction during the PBET extraction could be high, as evident with the Indianapolis soil sample collected from 7-days after compost addition. This effect was counteracted in compost-added soils in Kansas city site's soils because pH of those soils were slightly increased during 1 h-extraction time of PBET, which could reduce Fe oxy(hydr)oxide dissolution. On the other hand the compost that was used in the Indianapolis site was a composted biosolids that may have reactive Fe oxides originated from the microbial mediated composting process (Huang et al., 2005). Although reactive these less crystalline newly formed Fe oxides may have been more prone to reductive dissolution. This might be the reason that Pb-Fh in compost-added soil, from the Indianapolis site became negligible after PBET extraction.

With time the concentration of dissolved organic C goes down and other organic C forms such as fulvic acid and humic acid may tend to act as electron donors for the Fe reductive dissolution process. However, the efficiency of humic acid as an electron donor is low because of its' structural complexity (Petruzzell et al., 2005). So as compost matured in the field the reductive dissolution of Fe oxy(hydr)oxides becomes less significant. Further, the contact of microorganisms with Fe oxy(hydr)oxides surface is a critical factor for Fe (III) reduction (Petruzzell et al., 2005). Humic acid like recalcitrant organic C in the compost-added soils could alter the contact of Fe oxy(hydr)oxide mineral surfaces with microbial biofilms and block the reductive dissolution reactions in the compost-added soils. Adsorption of humic acid on to Fe oxides is greater under low pH conditions (Avena and Koopal, 1998). As the compost matured in the soil masking of Fe oxy(hydr)oxides by humic acid can become more and more effective on

blocking the Fe oxide solubilization reactions. This could be the reason for not reducing the Pb-Fh fraction in KC-C3-105d, P-C-115d and, I-C-642d soils during the PBET extraction.

While the Pb-Fh reduced the Pb-Org species increased during the PBET indicating that some of the Pb released by the Fe oxy(hydr)oxides have partitioned into SOC. Although the metal sorption capacity of organic matter favors high pH as Fe oxy(hydr)oxides do, organic molecules can maintain a better metal sorption capacity during acidic pH as the rate of metal desorption from organic matter is low (Strawn and Sparks, 2000). Further, the concentration of dissolved organic C would be low under low pH due to adsorption process (Jardine et al., 1989) indicating high retention of dissolved organic C at low pH. This means Pb that was sorbed to dissolved organic C could partition into solid phases during the acidic gastric phase extraction and contribute to the increase of Pb-Org fraction in the PBET residue.

Lead hydroxypyromorphite (HP) was formed during PBET extraction in both compost-added and control soils from the Kansas City site (KC-C1-16d, KC-C2-16d, KC-C3-105d, KC-4-16d and, KC-5-16d) and the soil sample collected at 7-days after compost addition in the Indianapolis site (I-C-7d). When the concentration of Pb^{+2} in the acidic gastric solution increases during the PBET extraction, these Pb^{+2} react with soluble inorganic P in the gastric solution and form HP. Scheckel et al. (2005) had provided some indirect evidence indicated by an increase of Pb in residual fraction when comparing the percentage of pyromorphite in original soil as measured by XAFS and the percentage of Pb remaining in PBET residue. The soil mentioned above with added compost had higher soluble inorganic P compared to their counterpart control soils as indicated by Mehlich III extractable P concentrations (Table 4.1). Therefore, the formation of HP during PBET would be more likely in compost-added soils than in the control soils. Further, reductive dissolution of Fe oxy(hyr)oxide during the PBET extraction could

increase the soluble inorganic P that was sorbed on to Fe oxy(hyr)oxide increasing soluble P available for this reaction. The PBET residues of the control soils in Kansas City site also had similar percentage of HP, whereas the control soil in the Indianapolis site did not have HP in the PBET residue. This difference in the two sites may be explained by the difference of the sorption capacities of the soils most likely determined by the concentrations of amorphous Fe and organic C. Based on amorphous Fe and OC contents we could expect soil from the Indianapolis to have higher sorption capacity compared to that from the Kansas City site. Therefore, the Pb^{+2} in the gastric solution that is available to react with phosphates during the PBET extraction could be lower in Indianapolis soils compared to Kansas City soils.

The above changes to Pb speciation during the PBET extraction happened under the acidic pH environment in the gastric phase. In the human digestive system, this gastric phase is followed by an intestinal phase where the pH is about 6.5. The Pb speciation can again be altered within this phase. Smith et al. (2011) showed that Fe that dissolved during the gastric phase precipitates as ferrihydrite under the high pH in the intestinal phase, and then this newly precipitated ferrihydrite would bind Pb reducing Pb bioaccessibility.

Although research found that the amount of Pb that is yielded by the gastric phase of the *in vitro* method correlates well with the bioavailability of Pb in animals/humans (Pb that dissolves and available for absorption in the digestive system), these comparisons were made mostly using non-treated mine waste materials with the exception of a few studies (Hettiarachchi et al, 2003; Ryan et al., 2004). The speciation of Pb in mine waste materials is different from that of urban soils. Mostly the soils in mine waste materials have low adsorption capacity and Pb solubility is mainly governed by Pb minerals such as PbS, $Pb(CO_3)_2$ and Pb-phosphates. Phosphate amendments immobilize Pb and do not completely re-dissolve in the stomach

following ingestion and therefore, P additions have been shown to reduce *in vitro* Pb bioaccessibility as well as *in vivo* bioavailability (Scheckel et al., 2013). The fate of these Pb minerals in the gastric phase pH and intestinal phase pH could be different from that of Pb-Fh and Pb-Org. The bioaccessible Pb in soils as measured by *in vitro* tests may not accurately portray true Pb bioavailability in compost treated Pb-contaminated urban soils. Therefore, it is important to cross-relate the *in vitro* bioaccessibility methods against *in vivo* animal models using urban soils in order to find reliable in-situ remediation techniques and safe guidelines for Pb contaminated urban soils.

Conclusions

The bioaccessibility of soil Pb was low in all three tested urban soils containing medium to very high soil Pb concentrations, most likely due to inherently high sorption capacity of these soils due to high concentrations of active Fe species, or SOC. The Pb sorbed to Fe oxy(hydr)oxides and to SOC dominated the Pb speciation in all three soils. Application of compost increased Pb sorbed to SOC significantly and this became even more pronounced as the compost matured in the field. The fraction of Pb sorbed to SOC survived and/or increased during the acidic environment created during the *in vitro* bioaccessibility extraction test. In addition, enhanced SOC tended to stabilize the Pb sorbed to Fe oxy(hydr)oxides through restricting/limiting the reductive dissolution of Fe oxy(hydr)oxides. The additional available P provided via compost application induced the formation of Pb hydroxypyromorphite during the PBET extraction. It is important to compare the *in vitro* bioaccessibility data with animal feeding studies conducted on treated-urban soils considering the Pb speciation changes that undergo under extreme pH levels of the extraction/digestive system.

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Tables and Figures

Table 4.1 Selected basic soil properties and the crops grown in the tested soils

Sample	Treatment	Days after compost addition	Crop	pH	Organic C	Mehlich-III P	Amorphous Fe	$Na_2C_2O_4$ extractable Al	Humic Acid	CEC	Texture
					g kg^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}	%	$cmol_+$ kg^{-1}	
Kansas City site; At the beginning of the growing season											
KC-C1-16d	Compost	16	Carrot	7.53	26	427	4.1				
KC-C2-16d	Compost	16	Swiss chard	7.70	27	464	3.5	0.99	4.4	33.8	4% sand, 75% silt, and, 21% clay
KC-C3-16d	Compost	16	Tomato	7.62	27	489	3.6				
KC-4-16d	Control	16	Carrot	7.25	17	103	3.6	1.03	0.8	20.4	
KC-5-16d	Control	16	Swiss chard	7.20	16	79	3.4				
Kansas City site; At harvest											
KC-C1-121d	Compost	121	Carrot	6.90	25	315	3.9	1.28	3.9	-	-
KC-C2-69d	Compost	69	Swiss chard	7.90	28	411	-			-	-
KC-C3-105d	Compost	105	Tomato	7.64	20	205	3.3		2.6	-	-
KC-4-121d	Control	121	Carrot	6.69	10	87	3.5	0.99	0.8	-	-
KC-5-69d	Control	69	Swiss chard	7.60	8	75	3.3			-	-
Philadelphia site											
P-C-115d	Compost	115	Carrot	7.39	54	738	6.4	1.41	6.2	31.3	
P-1	Control ‡	-	Lettuce	7.08	36	745	8.5	-			
P-2	Control	-	Lettuce	6.66	30	746	9.4	-			56% sand, 36% silt, and, 8% clay
P-3	Control	-	Lettuce	6.42	30	746	10.7	-	2.2	31.9	
P-4	Control	-	Mustard	7.19	32	666	11.5	-			
P-5	Control	-	Mustard	6.88	37	758	9.4	-			
Indianapolis site											
I-C†	-	-	-	7.65	44	241	37.9	1.33	-	-	
I-C-7d	Biosolids	7	Collard greens	7.34	54	450	22.2	1.81	2.5	30.9	
I-C-349d	Biosolids	349	Tomato	7.64	49	539	21.9	1.82	-	-	72% sand, 27% silt and, 1% clay
I-C-642d	Biosolids	642	-	7.86	48	562	25.3	1.84	2.9	-	
I-2-7d	Control	7	Carrot	7.42	41	152	31.4	1.51	1.0	13.9	
I-2-349	Control	349	Carrot	6.82	46	233	26.2	1.45	-	-	
I-2-642d	Control	642	-	7.89	48	95	27.3	1.08	0.9	-	

‡ Control soils in the Philadelphia site were collected as preliminary samples for characterizing site soils one year before the test plot establishment.

†Sample collected from the compost-added soil prior to compost addition.

Table 4.2 Soil total Pb concentrations and their bioaccessible Pb concentrations

Plot	Treatment	Days after compost addition	Total Pb		Bioaccessible Pb		% bioaccessible Pb	
			<2 mm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm
			mg kg^{-1}					
Kansas City site; At the beginning of the growing season								
KC-C1,C2, C3†	-	-	338	311	19.9	19.0	5.9	6.1
KC-C1-16d	Compost	16	161	186	7.2	17.1	4.5	9.2
KC-C2-16d	Compost	16	256	156	5.7	5.4	2.2	3.5
KC-C3-16d	Compost	16	193	199	12.4	7.3	6.4	3.7
KC-4-16d	Control	16	388	358	27.8	32.7	7.2	9.1
KC-5-16d	Control	16	417	421	29.8	28.6	7.1	6.8
Kansas City site; At harvest								
KC-C1-121d	Compost	121	184	234	13.8	17.2	7.5	7.4
KC-C2-69d	Compost	69	182	217	6.6	7.0	3.6	3.2
KC-C3-105d	Compost	105	238	263	11.0	13.2	4.6	5.0
KC-4-121d	Control	121	395	394	29.3	29.6	7.4	7.5
KC-5-69d	Control	69	383	372	29.3	29.4	7.6	7.9
Philadelphia site								
P-C-115d	Compost	115	958	1281	50.3	81.4	5.3	6.4
P-1	Control ‡	-	1627	2232	105.9	138.3	6.5	6.2
P-2	Control	-	1906	2383	133.5	135.3	7.0	5.7
P-3	Control	-	2117	2832	155.1	185.1	7.3	6.5
P-4	Control	-	1618	1124	109.8	111.0	6.8	9.9
P-5	Control	-	1758	2179	124.0	153.8	7.1	7.1
Indianapolis site								
I-C[£]	-	-	331	647	14.4	17.7	4.4	2.7
I-C-7d	Biosolids	7	143	320	8.0	9.6	5.6	3.0
I-C-349d	Biosolids	349	-	256	6.0	6.3	-	2.5
I-C-642d	Biosolids	642	-	409	7.7	8.2	-	2.0
I-2-7d	Control	0	551	877	32.8	42.9	5.7	4.9
I-2-349	Control	349	-	1054	33.0	32.9	-	2.5
I-2-642d	Control	642	-	736	12.0	14.7	-	2.0

† Composite soil sample of compost-added soils prior to compost addition.

‡ Control soils in the Philadelphia site were collected as preliminary samples for characterizing site soils one year before the test plot establishment.

[£]Sample collected from the compost-added soil prior to compost addition.

Table 4.3 Relative proportions of Pb species in soils and PBET residues as estimated by linear combination fitting on XAFS spectra

Plot	Treatment	Days after compost addition	Pb-Fh [#]	Pb-Org[#]	HP[#]	PlF[#]	R-factor [§]
			%				
Kansas City site; Soil: At the beginning of the growing season							
KC-C1,C2, C3†	-	-	82	18	-	-	0.06
KC-C1-16d	Compost	16	84	-	-	16	0.19
KC-C2-16d	Compost	16	86	14	-	-	0.08
KC-C3-16d	Compost	16	92	8	-	-	0.08
KC-4-16d	Control	16	82	18	-	-	0.07
KC-5-16d	Control	16	92	-	8	-	0.06
Kansas City site; Soil: At harvest							
KC-C1-121d	Compost	121	74	26	-	-	0.32
KC-C2-69d	Compost	69	64	36	-	-	0.06
KC-C3-105d	Compost	105	96	4	-	-	0.14
KC-4-121d	Control	121	96	8	-	-	0.05
KC-5-69d	Control	69	95	5	-	-	0.08
Kansas City; PBET residues							
KC-C1-16d	Compost	16	24	67	9	-	0.10
KC-C2-16d	Compost	16	37	53	10	-	0.09
KC-C3-105d	Compost	105	90	-	10	-	0.13
KC-4-16d	Control	16	-	83	17	-	0.05
KC-5-16d	Control	16	-	93	7	-	0.03
Philadelphia site; Soil							
P-C-115d	Compost	115	55	44	-	-	0.22
P-1	Control ‡	-	64	36	-	-	0.19
P-2	Control	-	53	47	-	-	0.19
P-3	Control	-	59	41	-	-	0.22
P-4	Control	-	71	29	-	-	0.25
P-5	Control	-	66	34	-	-	0.24
Philadelphia site; PBET residues							
P-C-115d	Compost	115	65	35	-	-	0.26
P-2	Control	-	22	78	-	-	0.18
P-4	Control	-	6	94	-	-	0.15
Indianapolis site; Soil							
I-C[£]	-	-	79	21	-	-	0.15
I-C-7d	Biosolids	7	75	24	-	-	0.12
I-C-349d	Biosolids	349	68	32	-	-	0.16
I-C-642d	Biosolids	642	38	62	-	-	0.15
I-2-7d	Control	0	71	29	-	-	0.11
I-2-349	Control	349	68	32	-	-	0.07
I-2-642d	Control	642	74	26	-	-	0.15
Indianapolis site; PBET residues							
I-C-7d	Biosolids	7	-	68	32	-	0.17
I-C-642d	Biosolids	642	41	59	-	-	0.20
I-2-7d	Control	0	35	65	-	-	0.11
I-2-642d	Control	642	55	45	-	-	0.22

† Composite soil sample of compost-added soils prior to compost addition.

‡ Control soils in the Philadelphia site were collected as preliminary samples for characterizing site soils one year before the test plot establishment.

[£]Sample collected from the compost-added soil prior to compost addition.

[#] Pb-Fh: Pb sorbed to Fe oxy(hydr)oxide; Pb-org: Pb sorbed to organic C; HP: hydroxypyromorphite; PlF: Plumboferrite

[§] R-factor: normalized sum of the squared residuals of the linear combination fit.

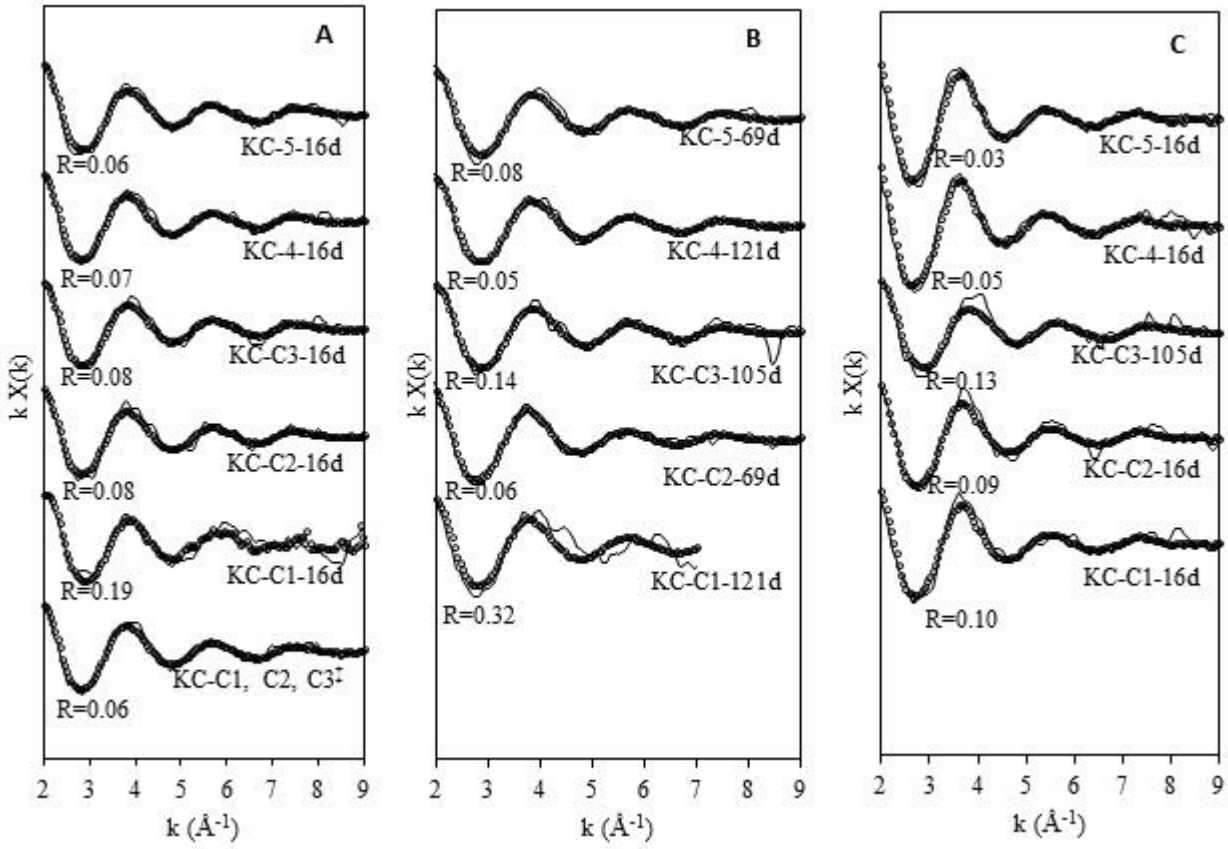

Figure 4.1 Pb XAFS spectra of Kansas City site soils and PBET residues (solid lines) and their Linear combination fits (LCF: dotted lines); compost-added soils: KC-C1-16d, KC-C2-16d, KC-C3-16d, KC-C1-121d, KC-C2-69d and, KC-C3-105d; control soils: KC-4-16d, KC-5-16d, KC-4-121d and KC-5-69d. The number followed by "d" in the sample names denotes the days after compost addition. A) Soil at the beginning of the growing season;‡Composite soil sample of compost-added soils prior to compost addition. B) Soil at harvest. C) PBET residues. R: normalized sum of the squared residuals of the linear combination fit.

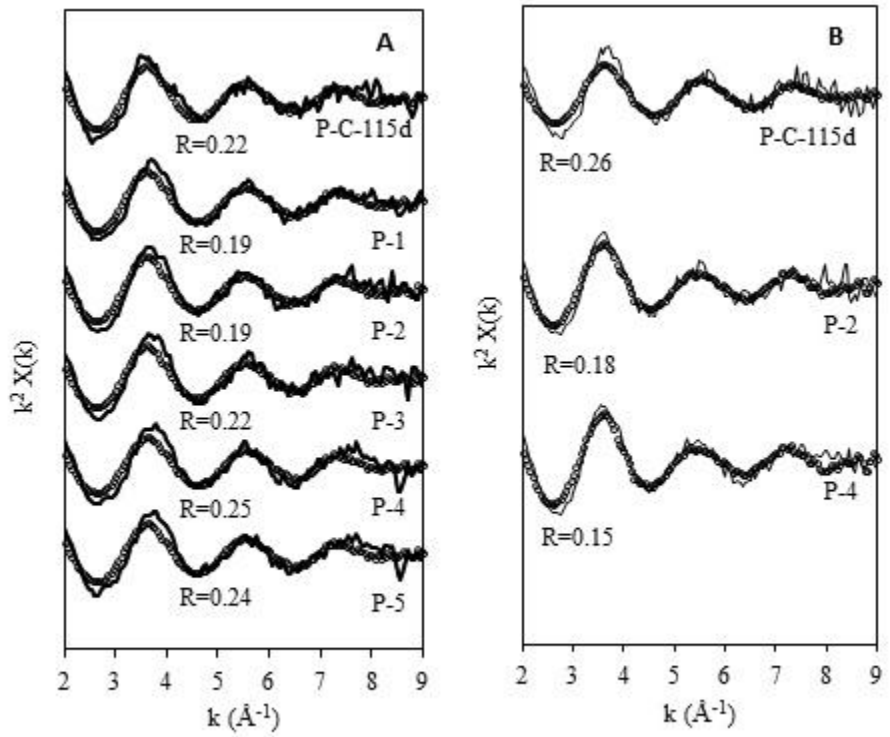

Figure 4.2 Pb XAFS spectra of Philadelphia site soils and PBET residues (solid lines) and their Linear combination fits (LCF: dotted lines). A) Soil; P-C-115d: Compost-added soil collected 115-days after compost addition; P-1, P-2, P-3, P-4, P-4: Control soils that were collected as preliminary samples for characterizing site soils one year before the test plot establishment. B) PBET residues. R: normalized sum of the squared residuals of the linear combination fit.

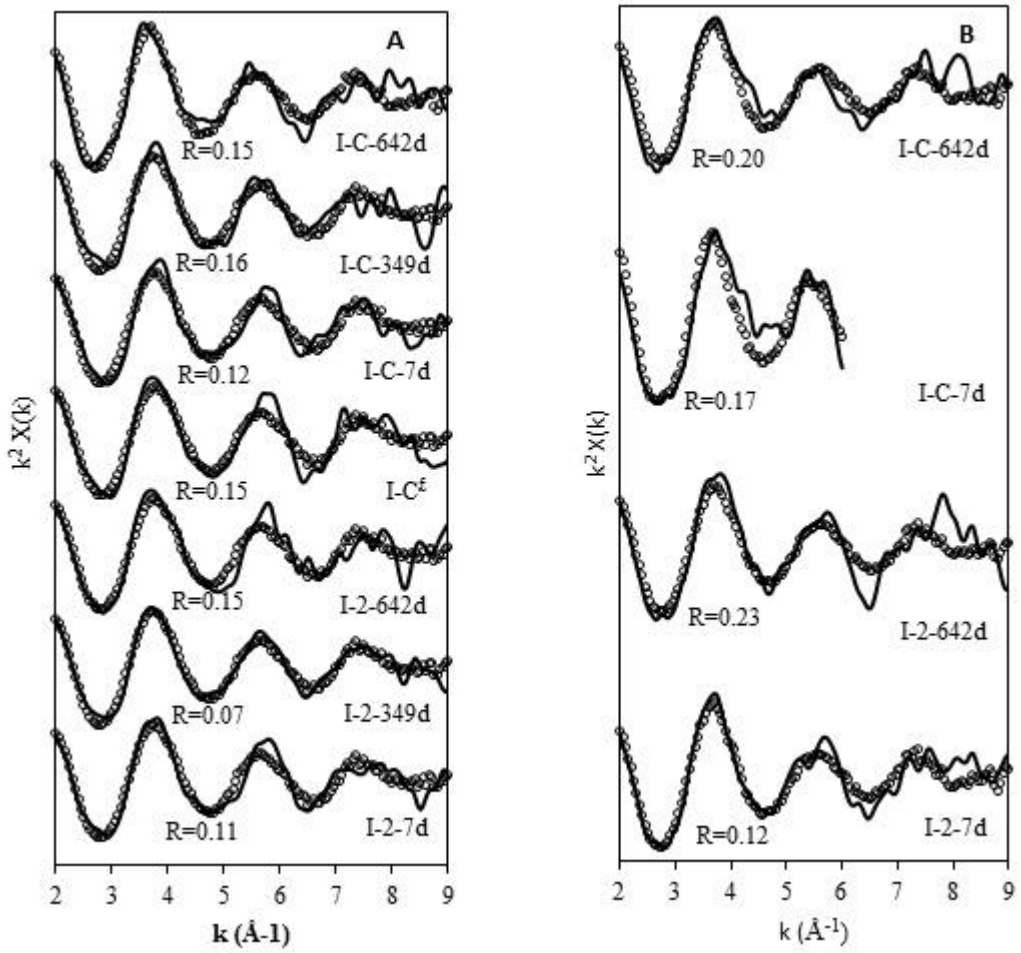

Figure 4.3 Pb XAFS spectra of Indianapolis site soils and PBET residues (solid lines) and their Linear combination fits (LCF: dotted lines). I-C£: Sample collected from the compost-added soil prior to compost addition. Compost-added soils: I-C-7d, I-C-349d, and I-C-642. Control soils: I-2-7d, I-2-349d, and I-2-642d. The number followed by "d" in the sample names denotes the days after compost addition. A) Soil B) PBET residues. R: normalized sum of the squared residuals of the linear combination fit.

Supporting Information

Table 4.4 Properties of compost-types

Property	Kansas City site	Philadelphia site	Indianapolis site
Type of compost	Leaf based compost	Leaf based compost	Biosolids (Type A, composted)
C:N ratio	16.0	12.2	13.8
pH	8.46	-	6.18
Pb, mg/kg	24	-	51
Total Fe, mg/kg	6280	19000	26500
Total Mn, mg/kg	397	1370	581
Total N, %	1.347	1.76	0.890
Organic N, %	1.291	1.74	0.848
NH4+-N, %	0.053	<0.003	<0.003
NO3—N, %	0.0025	0.02	0.04
Total P, %	0.315	0.760	0.716
Total K, %	0.746	1.54	0.224
Total S, %	0.497	1.28	0.357
Total Ca, %	7.54	6.54	4.82
Total Mg, %	0.431	1.02	1.16

Table 4.5 Spoil values of the standards resulted by Principle Component Analysis (PCA) followed by Target Transformation (TT)

Standards	Spoil values		
	Kansas City site	Philadelphia Site	Indianapolis Site
Anglesite	2.53*	29.35	12.31
Chloropyromorphite	3.41*	9.47	7.74
Galena	12.44	26.41	32.63
Hydroxypyromorphite	5.88*	3.38	6.47
Leadhillite	6.34	11.88	16.51
Magnetoplumbite	3.60*	41.66	14.57
Lead hydroxide	2.69*	1171.95	37.13
Hydrocerrusite	3.88*	16.93	4.88*
Cerussite	3.20*	199.51	8.54
Hinsdalite	6.38	30.61	-
Lead sorbed to humic acid	1.73*	5.45*	6.36*
$Pb(NO_3)_{2\,(aq)}$	1.41*	11.00	8.17
Vanquelinite	6.39	68.25	18.68
Lead chloride	6.92	25.71	17.10
Lead oxide	3.19*	125.14	9.07
Plattnerite	3.07*	22.87	20.20
Plumboferrite	5.66*	29.80	10.33
Plumbogumite	5.78*	33.15	8.32
Plumbonacrite	14.95	22.75	20.65
Plumboyarosite	5.72*	156.69	11.02
Lead sorbed to ferrihydrite	1.98*	4.15*	4.12*

* Standards that were selected for Linear Combination Fitting (LCF). Lowest IND value for the Kansas City site was 3, for the Philadelphia site was 1 and for the Indianapolis site was 2.

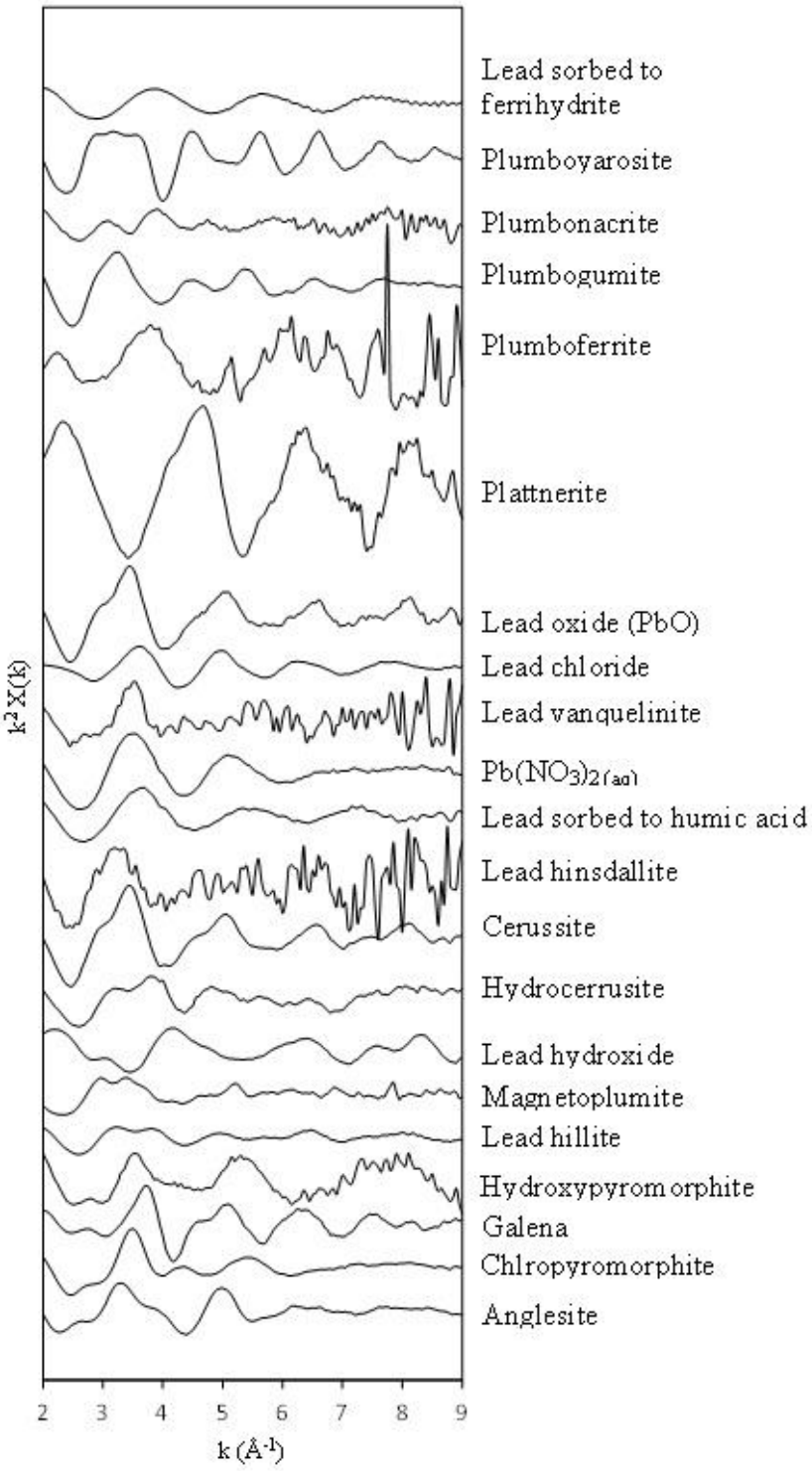

Figure 4.4 XAFS of the Pb standards used in the experiment

Chapter 5 - Potential for Transfer of Lead, Arsenic and Polycyclic Aromatic Hydrocarbons from Amended Urban Soils to Humans and Vegetables

Abstract

Urban soils that have the potential for gardening may contain harmful concentrations of toxic contaminants originated from past and nearby industrial and residential activities. Lead (Pb), arsenic (As) and polycyclic aromatic hydrocarbons (PAHs) are examples for such common contaminants. These contaminants can transfer from soil to humans via direct soil ingestion and consumption of food crops grown in contaminated soils. The bioavailability of these contaminants can be reduced by stabilizing them with *in situ* amendments. The effectiveness of the amendments depends on site specific soil characteristics and amendment properties. The objectives of this research were to assess the effectiveness of adding different compost-types on reducing both direct (soil-human) and indirect (soil-plant-human) exposure of Pb, As, and PAHs to humans. A field experiment was conducted in a potential urban garden site that had elevated concentrations of Pb (200-750 mg kg^{-1}), As (23-95 mg kg^{-1}), and PAHs (total of 16 priority PAHs: 23-50 mg kg^{-1}). Four compost-types were used as treatments: composted Class A biosolids, non-composted Class A biosolids, mushroom compost, and leaf compost. The rate of compost added was 44 kg m^{-2}, and the composts were mixed with top 15 cm of soil. A control treatment with no-compost was maintained. The vegetables grown were collard greens, tomatoes, and carrots. The compost-types and vegetable-type were arranged in split plot design in completely randomized four blocks (main-plot factor: compost-type; and sub-plot factor: vegetable-type). The vegetables were grown in 2011 and 2012 summers on the composts added in 2011 summer. Inorganic fertilizers (N, P and K) were added at the beginning for 2012 season.

The potential for direct exposure was evaluated using an *in vitro* bioaccessible test also known as physiologically based extraction procedure (PBET) while indirect exposure was evaluated by measuring concentrations of contaminants in plants. The bioaccessibility of soil Pb and As were low, regardless of the treatments. The soil Pb bioaccessibility ranged from 1 to 4.3%, and As bioaccessibility ranged from 7.3 to 12.3%, as percentages of total concentrations in soil. Composted biosolids, which may have relatively high active Fe, reduced the bioaccessibility of soil Pb by ~17% compared to control. Further, composted biosolids temporarily increased bioaccessibility of As by ~ 69% compared to control, when soil inorganic P concentration was elevated with P fertilizer application. The bioaccessibility of soil Pb was reduced by ~38% in all the treatments, when inorganic P concentration was elevated with P fertilizer most likely due to Pb-P reactions during *in vitro* bioaccessibility extraction. The concentrations of Pb, As and PAHs were low (Pb and As) or non-detectable (PAHs), except for Pb in carrots. Composted biosolids reduced As concentrations in collard greens whereas none of the other treatments were effective on reducing Pb and As in vegetables. Compost addition induced biodegradation of PAHs in soils, and it reduced about 15 to 72% of PAHs in soils over a year. The risk of exposure to soil Pb and As via direct soil ingestion is minimal in this site. Consumption of vegetables grown in this site may cause insignificant transfer of soil contaminants to humans, except for Pb through carrots.

Introduction

Use of vacant lots in cities for community gardening has become popular over the past decade. Since there is a potential for having harmful levels of toxic contaminants that are originated from past anthropogenic activities, the United States Environmental Protection Agency (USEPA) has given attention for the safeness of gardening and consumption of produce grown in these sites. Lead (Pb), arsenic (As) and polycyclic aromatic hydrocarbons (PAHs) are some of the common potentially toxic contaminants in urban soils (USEPA, 2011). Lead is mostly originated from the use of leaded gasoline and Pb based paint. Common sources of soil As are As based pesticides and wood preservatives. PAHs are by-products of partial burning and are significant components in creosote which has been used to treat railroad ties to protect those from fungi and insects (USEPA, 2011).

Elevated Pb in the environment increase blood Pb concentrations in children (Lanphear and Roghmann, 1997). About 310,000 U.S. children between the ages of 1 and 5 years are believed to have blood Pb concentrations at or greater than 10 μg dL^{-1} (ATSDR, 2007), which was the blood Pb level of concern recommended by the United States Centers for Disease Control and Prevention (CDC) until 2012. In 2012, CDC reduced this to 5 μg dL^{-1} based on their Advisory Committee on Childhood Lead Poisoning Prevention recommendations (ACCLPP, 2012). Arsenic is a potential carcinogen (USEPA, 2014). Elevated concentrations of As in urine of children was found in a communities living near a former smelter site (Hwang et al., 1997). In their study, the concentration of As in the interior dust was significantly related to the proximity to the smelter site and wind direction. The dietary intake of inorganic arsenic in the U. S. has been estimated to range from 1 to 20 μg day^{-1} with grains and produce (ATSDR, 2007).

Direct ingestion of soils has been identified as the most significant exposure pathway of humans for soil Pb and As (Lanphear and Roghmann, 1997; Beak et al., 2006). The term bioaccessibility is used to describe the amount of soil contaminant that could potentially dissolve and available for absorption in the human digestive system (i.e., predicted solubility in the gastrointestinal tract). *In vitro* methods that mimic gastrointestinal dissolution process have been developed (Ruby et al., 1996; Oomen et al., 2002) and are widely being used to estimate bioaccessibility of soil Pb (Brown et at, 2003; Brown et al., 2004; Ryan et al., 2004; Scheckel et al., 2005) and As (Pouschat and Zagury, 2006; Juhasz et al., 2007; Juhasz et al., 2009). Food chain transfer of soil Pb and As to humans via consumption of produce grown in contaminated soils and inhalation of contaminated dust are considered as minor exposure pathways (Davies et al., 1990; Zhao et al., 2010). However, it is an obvious potential exposure pathway with regards to gardening activities. Food chain transfer could have a certain impact in the gardening context and needs to be incorporated in the risk evaluation studies. In addition, superficial contamination of produce with contaminated dust cannot be ignored. Significant amounts of dust that carry Pb deposited on leafy vegetables and fruits (aerial parts of the plants) grown in gardens closer to urban settings had been reported (Nabulo et al., 2010; Attanayake et al., 2014). Limited literature is available on accumulation of these potentially toxic contaminants in food crops grown in contaminated urban soils and the need of filling such data gap has become important with the increasing number of urban gardens.

Lead and As can be stabilized *in situ* by adding amendments to soils. For soil Pb, P fertilizer is one such promising amendment which contributes to form stable Pb-P minerals like pyromorphite, by reacting orthophosphate ligands (e.g. $H_3PO_4^{o}$ and $H_2PO_4^{-1}$) with Pb^{+2} (Ma et al., 1993; Laperche et al., 1996; Cotter-Howells and Caporn, 1996; Hettiarachchi et al., 2001;

Scheckel et al., 2013). Solubility of pyromorphite under natural soil conditions and human stomach conditions is very low or negligible (Nriagu, 1974; Lindsay, 1979; Scheckel et al., 2005; Scheckel et l., 2013). Arsenic in soil solution exists as oxyanions predominantly with As(V) and As(III) oxidation states (e.g. H_3AsO_3, $H_2AsO_4^-$ and $HAsO_4^{-2}$) in typical soil Eh and pH conditions (Masscheley et al., 1991). These As oxyanions have similar sorption characteristics as orthophosphate ligands; hence, they compete with orthophosphate ligands to metal oxide sorptive sites and also already sorbed As oxyanions can exchange with orthophosphates at high soluble P concentrations and become mobile in soil (Gao et al., 1991; Peryea, 1991). Therefore, increases of soluble inorganic P concentrations in soil can negatively affect As immobilization as and P fertilizer application alone may not be effective *in situ* immobilization method for Pb- and As-co-contaminated situations.

Iron, Al and Mn oxides have a strong affinity for soil As and Pb (Martinez and McBride, 1999; Redman et al., 2002; Beak et al., 2006). The application of active Fe oxides had been identified as an effective method to immobilize soil As *in situ* (Lombi et al., 2004). Biosolids with high Fe and Mn effectively reduces bioaccessibility of soil Pb; however no such promising results have been observed for soil As (Brown et al., 2003; Brown et al., 2012). Soluble inorganic P in biosolids and their competition with As oxyanions for the metal oxide sorptive sites may have partly been responsible for this observation. Further, soil organic matter, which have negatively charged functional groups under neutral soil pH also compete for sorptive sites of metal oxide and increase As solubility (Redman et al., 2002; Grafe et al., 2001). Although dissolved organic C (DOC) can increase Pb solubility in soil (Sauve et al., 1998), the solid phase organic C provides effective sorption sites for soil Pb (Vega et al., 2009). The effectiveness of applying compost on reducing bioavailability of Pb and As in soils depends on composition and

properties (e.g. pH) of the compost and soil chemistry. Therefore, soil- and amendment-specific studies are essential to evaluate soil Pb and As bioavailability in soils.

Much less attention has been given to bioavailability of organic contaminants like PAHs in urban soils although some of the PAHs are categorized as potential human carcinogens. The exposure pathways identified for soil PAHs are: food chain transfer (soil-plant-humans), direct soil ingestion, dermal absorption, and inhalation. Most of the research has been conducted with artificially contaminated soils or pure standards (no soil) (ATSDR, 1995). There is a research gap that addresses PAHs transfer to humans from naturally contaminated soils. Further, organic matter application can affect the fate of PAHs in soils. High molecular weight PAHs have high affinity to soil organic matter and can possess low bioavailability and mobility. On the other hand, increases of microbial activity upon organic matter application can increase biotic degradation of PAHs.

In this research, we conducted a field based evaluation in an urban garden contaminated with Pb, As, and PAHs to test the effectiveness of adding four different compost-types on reducing bioaccessibility of soil Pb and As; and concentrations of Pb, As and PAHs in three vegetable types.

Materials and Methods

A field experiment was conducted at a site known as Monon Acres, Indianapolis, IN, in an area adjacent to a lot that was being considered for use for urban agriculture. The site location is $39^{o}46'09.8"$ N and $86^{o}09'31.7"$ W. This soil at the site had elevated concentrations of Pb, As, and PAHs (Table 5.1). The possible sources of the contaminants are: the Monon railroad ran about 5m of the north side of the garden; an old railroad service area located approximately on or

adjacent to the tested area; coal burning for railway activities; industrial wastes; previous use of leaded gasoline; and contaminated soil transported from various unknown sources. The site was initially covered with grasses, and several preliminary soil samples (0-15cm depth) were collected randomly from the site and analyzed for potentially toxic trace elements such as Pb, As, Cd, Ni, Cu, Zn, Co, Se, and Cr. The area closer to the Monon railroad was highly contaminated with Pb and As, and that area was selected for establishing the test plots.

Preliminary soil analysis showed that the soil pH was neutral and no pH adjustment was needed. Soil analysis also showed that Mehlich III extractable P and available K concentrations were in medium ranges, whereas the concentration of available N was low (Marr et al., 2010). Soil texture was sandy loam. The test plots were established in May 2011. Four types of compost treatments and a non-amended control were used as main plot treatments. Compost treatments were composted biosolids, non-composted biosolids, mushroom compost and leafy compost. Both biosolids based amendments were class-A biosolids, which is categorized as exceptional quality (EQ) biosolids with regards to the pollutants levels (USEPA, 2002). Composted biosolids were obtained from Soil Solutions Co., Roanoke, IN. This product was made of using City of Fort Wayne's biosolids. Non-composted biosolids, which can be mixed with top soils, was collected from a nearby water treatment facility in Carmel, IN. Both water treatment facilities use aerobic and/or anaerobic digestion as well as disinfection treatment process for the removal of harmful pathogens in biosolids. Further, non-composted biosolids were dried and mixed for four to six weeks to reduce the moisture content whereas, the composted biosolids undergo much long (two to three year) composting and drying process. Leafy compost and mushroom composts were purchased from Home Depot, Indianapolis, IN. Mushroom compost is horse manure and straw based compost used as a substrate to grow mushrooms. The basic properties of the

compost materials were mentioned in Table 5.1. The amount of compost added was 44 kg m^{-2}. Each main plot was divided into three subplots, and three types of vegetables were planted in the test plots in May 2011 and May 2012. They were a leafy vegetable (collard greens: *Brassica oleracea*, cultivar: Georgia), a fruiting vegetable (Tomato: *Solanum lycopersicum*, cultivar: Better boy) and a root crop (carrot: *Daucus carota*, cultivar: Long Imperator #58 in 2011 and Chantenay Red cored in 2012; two types of cultivars were grown because of the unavailability of the same cultivar). The compost- and plant-types were in split plot arrangement in a randomized complete block design (RCBD) containing four blocks. Urea was added to control plots to raise soil available N concentration to a level adequate for plant growth. The edible portions of the each vegetable type were harvested as they reached the maturity. In 2011, the age of maturity was 55, 84 and 105 days for collard green, tomatoes and carrots, respectively. The tomatoes were kept in the field little longer in 2012 (i.e., >105 days), since the fruits matured late in that year due to dry weather. After harvesting in 2011, debris was removed and a cover crop (Ryegrass: *Lolium perenne*) was planted until April 2012 to protect the treatments from cross mixing and eroding. After uprooting ryegrass and mixing the soil in main plots, N:P:K fertilizer was applied at recommended rate to all the plots prior to planting in 2012. Plant-types were again randomized in sub plots. No plants were grown in 2013, but a set of soil samples from all the main plots were collected. The description of soil samples collected in all three years is mentioned in Table 5.2. Soil samples were collected from 0-15 cm depth from randomly chosen 4-6 locations within a main-plot or sub-plot, and those samples were composited and well-mixed at sample preparation before analysis.

Soil analysis for basic properties, total Pb, As and PAHs

Available N ($NH4^+$ and NO_3^-) of soils was extracted by 1 M KCl (Mulvaney, 1996). Available K was extracted by 1 M NH_4CH_3COOH (pH=7.0) (Helmke and Spark, 1996). Available P was extracted using Mehlich-III (Mehlich, 1984) and analyzed calorimetrically using a flow injection analyzer (Lachat Instruments, Loveland, CO). The Walkley-Black procedure was used to determine organic C concentration in soils (Nelson and Sommers, 1996). Soil pH was determined using a 1:10 soil:deionized water mixture by a pH meter with an 'Accumet' pH/ATC Electrode and Accumet AP 115 meter (Fisher Scientific, Pittsburgh, PA). Soil total Pb, As and other trace element concentrations (Cd, Cu, Cr, Zn, Ni, Se and Co) were determined by EPA method SW846-3051A (USEPA, 2007a). To determine total trace element concentrations, 10 mL of trace metal-grade concentrated HNO_3 was added to 0.500±0.005 g of soil and digested in a microwave digestion unit (MARSXpress, CEM Corporation, Matthews, NC). The temperature of the soil-acid mixture in the microwave digestion unit was increased to 165^oC in 5.5 min, and then it was further increased to 175^oC in 4.5 min and held at 175^oC for 5 min. A standard reference soil (NIST 2711a-Montana II) was digested along with every batch of test samples as a quality assurance/quality control (QA/QC) sample to evaluate digestion and analytical procedures. After filtering using the Whatman No. 42 filter papers, the solutions were analyzed for Pb, As and other elements using an inductively coupled plasma optical emission spectrophotometer (ICP-OES; Varian Inc., Foster City, CA).

The extraction of PAHs in soil samples collected soon after compost addition in 2011 was done using the Pressurized Fluid Extraction (PFE) method as described in SW846-EPA 3545 A method (USEPA, 2007b) in USEPA Region 5, Chicago Regional Laboratory. In 2012, a set of soil samples were collected from all the main plots, 377-days after compost addition and analyzed for PAHs using SW846-EPA 3541: automated soxhlet extraction method (USEPA,

1994) by ALS Environmental Laboratory (Houston, TX). The extracted solutions were analyzed in Gas chromatography-Mass Spectrometry (GC-MS) following SW846-EPA 8270D (USEPA, 2007c).

Cleaning and digestion of the plants for total Pb and As

Edible parts of the plants were harvested at their maturity and then cleaned using three cleaning methods to assess the effect of surface cleaning of vegetables on reducing soil Pb and As transfer to humans. The cleaning methods were lab cleaning (with deionized water and sodium lauryl sulphate), kitchen cleaning (with tap water only) and peeling (used only for carrots). A descriptive explanation on how these methods were employed can be found in Attanayake et al. (2014). The lab cleaning was expected to remove all adhered soil particles from the surface of the produce, whereas kitchen cleaning removed all visible soil particles from the produce mimicking the cleaning process that is performed in household level. The cleaned plant materials were chopped using a stainless steel knife and dried at 70^{o}C for 3-5 days. Then, they were ground and passed through 40 mesh size (420 µm) sieve (Willey Mini Mill-Arthur Thomas–type grinder (Thomas Scientific, Swedesboro, NJ). Plant samples were handled in a class II, type A2 biosafety cabinet to avoid dust contamination from laboratory air (Esco Technologies Inc., St. Louis, MO). The plants were digested in a microwave digestion unit using trace metal grade concentrated nitric (HNO_3) acid. Ten mL of HNO_3 acid was added to 0.500±0.003 g of plant materials. In the digestion unit, the temperature of the plant-acid mixture was increased to 200^{o}C in 15 min, and the mixture was held at 200^{o}C for another 15 min. All the samples were digested in duplicates. Digested samples were filtered using Whatman No 42 filter papers and analyzed for Pb and As using a GF-AAS. The absorbance for Pb and As in GF-AAS was enhanced by modifiers; 2 % (w/v) H3PO4 for Pb and 0.2 % (v/v) Pd in nitrate for As.

Standard reference materials (NIST 1515 and NIST 1573a) were digested with every digestion batch and analyzed as QA/QC. The recoveries of spikes samples for Pb and As were 93% and 94% for collard green, 88% and 96% for tomatoes and103% and 102% for carrots, respectively.

In vitro soil Pb and As bioaccessibility test

The physiologically based extraction procedure (PBET) described by Ruby et al. (1996), modified according to Medlin (1997), was used to access the bioaccessibility of soil Pb and As. The <250 µm fraction of soils collected from carrot plots was used for this analysis. The extraction solution, representing the gastric phase, was prepared by mixing 1.25 g of pepsin, 500 mg of sodium L-malate, 500 mg of sodium citrate dihydrate, 500 µL of trace metal–grade acetic acid, and 420 µL of L(+)-lactic acid with 1 L of deionized water. The solution was acidified to ~2.0 with trace metal–grade concentrated HCl prior to use. One g of soil and 100 mL of gastric solution were added to a 250-mL polypropylene bottle. The gastric solution was heated to 37^{o}C before adding it to the soil. The pH of the soil-solution was adjusted to 2.50±0.02 by adding varying volumes of trace metal–grade concentrated HCl. The soil–gastric solution mixture was shaken at 37^{o}C at 100 rpm for 1 h, and then the samples were filtered using 0.45 µm syringe filters. A standard reference material (NIST 2711a-Montana II soil) was subjected to this test with each batch of extraction (A batch consisted of 20 samples) as a QA/QC. Two blanks were also included in each extraction batch. Analysis of extractions for Pb and As was done by GF-AAS.

Extraction and Analysis of PAHs in plants

Fresh samples of lab cleaned carrots and tomatoes from the 2011 test plots and lab cleaned collard greens from the 2012 test plots were used for this analysis. The QuEChERS ("Quick, Easy, Cheap, Effective, Rugged, and Safe") method proposed by Anastassiades et al.

(2003) was used as modified by Slizovskiy et al. (2010). Samples were chopped finely, crushed, and homogenized. Fifteen grams of sample was weighed into 50 mL Teflon centrifuge tubes (Thermo Scientific). Then 6 g of $MgSO_4$, 1.5 g of $NaCH_3COO$, and 15 mL of acetonitrile was added into the centrifuge tubes. Sixty µL of deuterated phenanthrene from a 10 mg L^{-1} stock solution was added to all the samples as an internal standard. Then, the mixture was shaken by hand until the major clumps were broken up and in a wrist action shaker for 30 minutes followed by centrifugation at 3000g for 10 minutes. Ten mL of the aliquot was pipetted and transferred into another Teflon centrifuge tube that contained 1.5 g of $MgSO_4$ and 500 mg PSA (primary secondary aluminum) bonded silica, and 2 mL of toluene. The mixture was shaken thoroughly by hand and centrifuged at 3000g for 10 minutes. Six mL of aliquot was transferred into a glass concentrator tubes and concentrated to less than 1.0 mL using N_2 gas flow, while the sample was heated at 50^o C in a concentrator (Brinkmann Instruments Inc, Westbury, NY). Then the samples were carefully transferred into GC-MS vials and the final volume was brought to 1.0 mL using toluene. Blank and samples spiked with known amounts of PAHs were included in each batch of extraction as QA/QC. Then the solutions were analyzed in GC-MS (Varian Inc., Foster City, CA) for all the 16 priority PAHs.

Statistical analysis

Statistical analysis was performed using SAS 9.2 software (SAS Institute, 2010) using PROC GLM procedure. Percent bioaccessible Pb and As concentrations were analyzed using split plot design in RCB arrangement: the main plot factor was compost-type and the sub plot factor was days after compost addition. Plant Pb and As concentrations were analyzed using a split plot design: the main plot factor was compost-type and the sub plot factor was cleaning method or RCBD with compost-type as the treatment (when only one cleaning method is

available). Separate analyses were done for each vegetable in each year. A Tukey test was used for multiple mean comparisons when the treatment effect was significant.

Results and Discussion

Soil chemical properties

Compost application increased soil organic C, Mehlich III extractable P (Table 5.3) and available K concentrations. Although we expected to have higher available N concentrations in compost-added soils than the control soil (prior to urea application), a significant rise of available N was only observed in the biosolids-added soils. The majority of available N was in NO_3^- form in composted biosolids-added soils, whereas NH_4^+ was the major available source of N in non-composted biosolids-added soils. Mushroom compost-added soils had slightly elevated available N concentrations than in the control soils. The available N concentrations in leaf compost-added soils were lower than the control soils. This could be a result of immobilization of available N originally present in the soil upon leaf compost application. Mehlich III extractable P concentrations were higher in all the compost-added soils than the control soil. The highest Mehlich III extractable P concentration was observed in composted biosolids-added soils, followed by non-composted biosolids-added soils. Although non-composted biosolids had a significantly higher total P concentration than the other compost-types (Table 5.1), it seems that the majority of P in non-composted biosolids was in insoluble forms, indicating that most of the P are likely in association with Fe oxides. Initially, soil pH was slightly low in composted biosolids-, non-composted biosolids- and leafy compost-added soils. In contrast, there was a slight pH increase with mushroom compost addition. It is not the objective of this paper to discuss the soil fertility parameters in detail; however, we were interested on this aspect since it could affect the contaminant uptake and their concentrations in vegetables. When soil/growth

medium is rich with plant essential nutrients, the potentially toxic trace elements have to compete with those nutrient ions to be absorbed into plants (Gothberg et al., 2004), and this leads to lower absorption of non-essential potentially toxic trace elements. On the other hand, the plant total biomass could be high with sufficient nutrients, and that could dilute the already absorbed/accumulated contaminants in plant tissues (Attanayake et al., 2014; Ekvall and Greger, 2003). Since all the treatment plots, including control for which urea was added, had adequate available N, P and K, except N in leaf compost-added soils, we believe that soil nutrient concentration difference may not have influenced Pb and As concentrations in the vegetables. Also, no significant biomass differences were observed among the treatments.

The addition of compost addition diluted total Pb and As concentrations in soil. Dilution of contaminants upon compost addition was previously observed (Attanayake et al., 2014; Brown et al., 2012). This dilution level was different from one compost-type to another. Soil total Pb dilution ranged from 19 to 56% and soil total As dilution ranged from 5 to 58%. The highest dilution was observed for the composted biosolids-added soils, and the lowest was for non-composted biosolids-added soils. This was most likely due to differences in moisture contents, particle sizes of different compost types and heterogeneity of contaminant concentrations in soils. This dilution of total contaminant concentrations upon compost addition is an immediate beneficial effect that could lower the transfer of contaminant from soil to humans, especially via direct soil ingestion.

Bioaccessibility of soil Pb and As

The absolute bioaccessibility immediately after compost addition (7-days after compost addition) ranged from 9.9 to 23.7 mg kg^{-1} for soil Pb and from 3.0 to 5.3 mg kg^{-1} for soil As. The absolute bioaccessibilities of soil Pb and As were significantly lower in composted biosolids-

added soils than that of the other treatments, including control, initially (data are not shown). This is most likely due to the significant dilution of total Pb and As concentrations (56-58%) occurred with the composted biosolids application. Although other compost-types diluted the total Pb and As concentrations in soil that the dilution might not have been sufficient enough to lower the absolute bioaccessibility significantly. The true change of bioaccessibility of soil Pb and As occurred due to chemical changes induced by compost addition should be evaluated using percent bioaccessibility value (Percent bioaccessibility=Absolute bioaccessibility [mg kg^{-1}]/Total Pb or As [mg kg^{-1}] ×100), which is independent of the total concentration. In general, the percent bioaccessibility of soil Pb and As was low in this soil. Percent bioaccessibility ranged from 1.0 to 4.3% for Pb and from 7.3% to 12.3% for As (Figure 5.1 and Figure 5.2). This low percent bioaccessibility could be a result of inherently high amorphous Fe and organic C concentrations in soil (Table 5.1). Further, the neutral pH of soil supports low bioaccessibility of Pb and As. Under acidic soil pH conditions, the functional groups in inorganic and organic sorptive surfaces protonate. This reduces cationic metal (e.g. Pb^{+2}) sorption capacity and increases ligand (e.g. As oxyanions) binding capacity. In contrast, alkaline pH promotes cationic metal binding capacity and suppress ligand binding capacity by deprotonation of sorptive sites. Further, alkaline pH could precipitate Pb as Pb-carbonates, under adequate CO2 pressure. Bioaccessibility of Pb-carbonates is high as it dissolves readily in the extremely acidic stomach pH. Therefore, neutral soil pH would help maintain low Pb and As solubility under field and acidic stomach conditions.

Due to the inherent low Pb bioaccessibility in this soil, observing significant differences upon addition of treatments or among the treatments was difficult. The interaction of compost-type and days after compost addition was not significant for soil Pb bioaccessibility. Only

composted biosolids was effective on reducing bioaccessibility of soil Pb ($p<0.1$), when bioaccessibilities were averaged across the days after compost addition (Figure 5.1). When comparing composted biosolids with other compost-types, it may have had higher proportion of newly precipitated active Fe oxides, since the composting process would oxidize Fe (Cornell and Schwertmann, 2003; Brown et al., 2012). If this were the case, Fe oxides in composted biosolids would sorb more Pb compared to other treatments, and this could have resulted a significant reduction in soil Pb bioaccessibility in soil receiving composted biosolids. Although non-composted biosolids had a high Fe concentration, this Fe may not be as reactive as the Fe in composted biosolids. Further, additional soluble P in the compost-added soils could have an impact on reducing the Pb bioaccessibility, though the effect of P seems to be minimal in this experiment. Non-composted biosolids had the highest Mehlich-III extractable P throughout the experiment (Table 5.3) but showed no significant reduction of Pb bioaccessibility (Figure 5.1). The reason for this observation could be the inherently low solubility of Pb in these soils. Lead cations prefer to bind with Fe oxides and organic C functional groups over phosphate ligands, since Pb^{+2} is a soft Lewis acid that prefers soft Lewis bases like Fe oxides and organic C functional groups over phosphates that are hard Lewis bases. Soils with high amorphous Fe oxide and organic C concentrations; and moderately contaminated with Pb most likely maintain Pb solubility via adsorption/desorption reactions.

There were some changes of Pb bioaccessibility over the tested period. Although it was not statistically significant, at 7-days after compost application, the soil Pb bioaccessibility in the mushroom compost-added soil was higher than that of the other soils. Mushroom compost-added soils had a slightly higher pH which was a result of the slightly alkaline pH of the compost. At high pH the concentration of DOC increases (You et al., 1999). So mushroom compost-added

soils may have had a higher DOC concentration than the other soils, and this may have contributed to the initial higher soil Pb bioaccessibility in those soils. As mentioned previously, at the beginning of the second season (in 2012, 351-days after compost addition), N, P and K fertilizer was added to test plots to be able to grow plants. The soil Pb bioaccessibility was significantly low in all soils including the control soils collected after adding P fertilizer and in the following set of soil samples which were collected 484-days after compost addition. This reduction of Pb bioaccessibility did not appear in the next set of soils sample that was collected 664-days after compost addition. This suggests that the reduction of soil Pb bioaccessibility in soil samples collected after 351- and 484- days after compost addition might not have happened due to Pb-P reaction that occurred in the field, but because of the Pb-P reaction that occurred during the *in vitro* bioaccessibility extraction test. The unpublished Pb EXAFS data from our lab showed that the hydroxypyromorphite was formed during the *in vitro* bioaccessibility extraction test (Attanayake et al., 2014). To form Pb pyromorphite, the soluble P should present as $H_3PO_4^o$ and $H_2PO_4^{-1}$ forms and these orthophosphates' formation is favored under acidic soil pH (<2.12 to 7.21) (Scheckel et al., 2013). Also, acidic soil pH increases the Pb solubility by releasing sorbed Pb from the sorptive sites, as a result of protonation of their functional groups. Hence, the extreme pH in the gastric solution may facilitate the formation of Pb pyromorphite and reduces Pb bioaccessibility in soil. The moderate pH of field soils may not support Pb pyromorphite formation as much as the gastric environment. Nevertheless there are reported cases that Pb pyromorphite was formed under field conditions or conditions similar to field upon increasing soluble P concentration in mine impacted, highly contaminated soils (Hettiarachchi et al., 2000; 2001; Scheckel et al., 2005). Scheckel et al. (2005) explained that the increase of soil P via biosolids application would also form pyromorphite, but to a lesser extent compared to soluble P.

None of the compost treatments reduced bioaccessibility of As (Figure 5.2). The interaction between compost-type and days after compost addition was significant for bioaccessible As. Significantly high bioaccessibility of soil As was observed 7-days after adding compost treatments in the mushroom compost-added soils (Figure 5.2). Mushroom compost initially increased percent bioaccessible As. At 7-day mushroom compost had the highest pH, so, mushroom compost-added soils may have high DOC concentration. An increase of DOC concentration enhances desorption of As from Fe oxides (Grafe et al., 2001). In fact, all the compost treatments seem to maintain slightly higher As bioaccessibility than the control, although it was not statistically significant. This could be due to the competition between negatively charged functional groups of organic matter introduced by compost and As oxyanions to sorb to metal oxides (Redman et al., 2002; Grafe et al., 2001). Although compost addition introduced additional metal oxides (Fe and Mn), which could increase As adsorption, this effect of additional metal seemed to be negated by the effect of increased soil organic C. A similar observation was reported by Brown et al. (2012).

In composted biosolids-added soils, the As bioaccessibility was significantly high 351-days after compost addition (Figure 5.2). As mentioned before, composted biosolids could have a higher proportion of newly precipitated active Fe oxides, and that might have resulted significant amount of As in soils to be redistributed and adsorbed to Fe oxides provided by the composted biosolids. When the inorganic P concentration was raised by P fertilizer application, some of the As oxyanions that were adsorbed onto Fe oxides in composted biosoilds may have been exchanged with and replaced by phosphate ligands. The presence of phosphate ligands limits As sorption on metal oxides, and this limitation increases as soluble P concentration increases (Gao et al., 1991). This competition of phosphates and arsenate to be adsorbed to Fe

oxides could happen in a wide range of pH (Violante and Pigna, 2002); hence it could potentially occur in both field and gastric environments. It is possible that the bioaccessible As concentrations were also affected by the reactions happening during acidic PBET environment in addition to the in-field transformations that might have occurred. Further, the majority of adsorbed As in composted biosolids-added soils may have been via outer-sphere mechanisms, therefore, allowing As to desorb easily upon enhanced ortho-P (such as $H_2PO_4^-$) concentration in soil solution due to P fertilizer application. The two samples collected at 484- and 664- days after compost addition did not show this increase of As bioaccessibility in the composted biosolids-added soils. The soluble P concentrations of these two samples were not as high as the soluble P concentration in the previous sample and P concentrations may not be high enough to desorb significant amounts of As from Fe oxides.

The highest amount of soluble P was observed in the non-composted biosolids-added soils, but this treatment did not show a significant increase of As bioaccessibility. Again, this suggests that differences in As adsorption mechanisms could result in different outcomes during the *in vitro* bioaccessibility extraction test. When composting, Fe can be precipitated as oxides with higher reactivity through biological reactions (Brown et al., 2012). So, it is possible that the Fe oxides in non-composted biosolids may not be as reactive as Fe oxides in composted biosolids.

Vegetable Pb concentrations

None of the compost treatments was effective on reducing the Pb concentrations in vegetables in either year. In 2011, the highest Pb concentration was observed for carrots, followed by collard greens and tomatoes. High accumulation of Pb in roots was previously reported (Finster et al., 2004; Attanayake et al., 2014). It has been documented that roots

accumulate Pb in xylems in Pb-phosphate form (Codling et al., 2007). When soil has high P, the formation of Pb-phosphates in roots limits the upward translocation of Pb in plants. When plants were grown in P deficient soils, upward movement of Pb is enhanced, since Pb-phosphate formation in root cells is limited (Foy et al., 1978). In this experiment, adequate P was provided for plant growth, including control plots, based on the Mehlich III-P concentrations (Table 5.3).

In 2012, the concentrations of Pb in carrots were significantly lower than in 2011, although different cultivars were grown in these two years. Cultivars may differ in uptake and accumulation of potentially toxic trace elements because of physiological differences (Alexander et al., 2006; Liu et al., 2010; Price and Hettiarachchi, 2012). Selection of cultivars with low Pb accumulation potential is gaining attention as a means to reduce food chain transfer of those trace elements from soil to humans (Liu et al., 2010; Price and Hettiarachchi, 2012).

The concentration of Pb in tomatoes and collard greens was higher in 2012 compared to 2011. Note that the same cultivars of tomatoes and collard greens were grown in these two years. High Pb concentrations in tomatoes and collard greens in 2012 could be because of the higher temperature of that year compared to 2011. When plants are grown under high temperature, they tend to accumulate more potentially toxic trace elements. Merry et al. (1986) postulated that higher temperatures increase the uptake of Pb, Cu and As in vegetable crops by increasing the permeability of root cells to absorb more ions and by increasing the diffusion rate on ions in soils. Further, the tomato fruits were split in 2012 because of the moisture and heat stress; and the cracks may have allowed dust to enter the tomato fruit that would not be removed by washing. We believe that some superficial contamination had occurred in 2012 tomatoes and the Pb concentration in tomatoes in that year may not reflect only the accumulation of Pb in the tomato fruits through Pb uptake from the soil. Maintaining healthy plant growth through

providing adequate nutrients and water seems to be important for lowering the soil Pb transfer via food chain.

Since all the vegetables had detectable concentrations of Pb in their edible portions, it is important to compare the vegetable Pb concentrations with maximum allowable safe limits to assess the risk of Pb transfer to humans via food chain. Codex Alimentarius Commission (CODEX) develops international food standards, guidelines, and codes of practice to protect the health of the consumers and ensure fair practices in food trade (JECFA, 1993). The CODEX committee on contaminants in food established or endorsed Maximum levels (MLs) or guideline levels for contaminants and naturally occurring toxicants in food and feed, including MLs for Pb concentrations in vegetables. It is important to note that these limits were not developed based on the bioavailability of Pb in food. The bioavailability of Pb in food can be low when Pb is ingested along with food (James et al., 1985; USEPA, 2003). So CODEX MLs might have been reflecting the upper limit or the maximum potential health risk of consuming Pb-contaminated vegetables. More details on these standards are presented in Attanayake et al. (2014). The concentrations of Pb in collard greens in 2011 and 2012, and tomatoes in 2011, were below the ML. The concentration of Pb in carrots was at or above the ML in both years. Attanayake et al. (2014) reported similar results with carrots grown in urban soil contaminated with lower Pb concentrations than this soil. Leafy and fruiting vegetables accumulate low Pb and do not possess the risk of Pb contamination via food chain transfer. As mentioned above the tomatoes in 2012 had elevated Pb and these concentrations were above the MLs, but the values may not have been reflecting the true uptake and accumulation of Pb by tomatoes due to surficial contamination; and therefore, we do not attempt to make any generalized conclusions based on our 2012 tomato Pb concentrations.

Vegetable As concentrations

Detectable levels of As were found only in collard greens in both years. Tomato and carrot As concentrations were below the detection limit of the method used (<0.12 mg kg^{-1}). Uptake, translocation, and accumulation of As in plants seems to be much variable among species and genotypes (Zhao et al., 2009; Zhao et al., 2010). Therefore, As concentrations in collard greens, tomatoes and carrots may not reflect the translocation pattern of As in plants, unlike what was found for Pb. Arsenic is absorbed by plants via phosphate transporters and the Si pathway. The latter is believed to be limited to As uptake by rice (Zhao et al., 2009). The absorbed As (V) is rapidly reduced into As (III) in plant root cells. These As (III) can 1) complex with thiol-peptides and stored in vacuoles, 2) efflux to the external medium, and 3) translocate to shoot via xylem (Zhao et al., 2009). Composted biosolids seem to be effective in reducing As concentration in collard greens. This could be due to the sorption of As on active Fe oxides provided by composted biosolids.

Safe limits for As in vegetables have not been published by CODEX. The joint FAO/WHO expert committee on food additives published that provisional tolerable weekly intake (PTWI) of inorganic As is 0.015 mg of inorganic As per kg of body weight (i.e.,130 µg day^{-1} for a 60 kg person) (WHO, 1989); however this limit was withdrawn recently (WHO, 2011) and has yet to be revised. Considering the above PTWI of inorganic As and the maximum As concentration in collard greens (i.e., 889 µg kg^{-1} in dry weight), it is clear that the potential of exceeding this limit by consumption of collard greens is poor.

Cleaning methods effect on Pb and As concentrations in vegetables

Kitchen cleaned collard greens had greater Pb and As concentrations compared to lab cleaned ones. In lab cleaning, the surfactant used (sodium lauryl sulfate) removes the waxy

cuticle layer from the leaves (Attanayake et al., 2014). With lab cleaning method we have employed, any embedded soil particles in the waxy layer of collard green deposited as dust would be removed. This explains that thorough cleaning of vegetables helps reduce food chain transfer of Pb- and As-like contaminants. No significant difference was found in Pb concentrations of peeled and laboratory cleaned carrots. The majority of Pb in carrots accumulates in the xylem, which is the inner core of the carrot tissues (Codling et al., 2007; Price and Hettiarachchi, 2012). Hence, the slight peeling that we performed may not have influenced the Pb concentration. Attanayake et al. (2014) performed kitchen cleaning, in addition to peeling and lab cleaning, and did not find a significant difference of cleaning methods of carrots. The absence of the cuticle layer in the roots may be the reason for these similar Pb concentrations across cleaning methods.

PAHs concentrations in soils and vegetables

Concentrations of PAHs in soils and vegetables are shown in Table 5.4. In all plots, the concentrations of four to six ring PAHs (molecular weight: 202-278 g mol^{-1}) were higher than the concentrations of two to three-ring PAHs (molecular weight: <178 g mol^{-1}) in soils. This could be because the PAHs with high molecular weight have a higher affinity to soil inorganic and organic constituents and lower volatility than the low molecular weight PAHs (ATSDR, 1995). The effect of compost addition on PAHs concentrations was not clear in the soils collected soon after compost addition when compared to the concentrations of PAHs in control soils. When soil PAHs were determined 376-days after compost addition, the concentrations of PAHs seemed to be reduced in compost-added soils compared to the control soil and soils collected soon after compost addition. This observation was common for both low and high molecular weight PAHs. This reduction of PAHs in compost-added soils suggests that compost

addition had enhanced the degradation of PAHs in soils. It is more likely that the biodegradation of soil PAHs had been enhanced with compost addition, since the soil microbial activity may have increased with providing additional organic C and other nutrients like P; improving soil structure, and oxygen transfer, via compost addition (Haritash and Kaushik, 2009). Further, Wong et al. (2002) described that enhancing dissolved organic C via pig manure application enhanced easily accessible C source for soil microbes and stimulates microbial degradation of soil PAHs. In addition, dissolved organic C could enhance the desorption of PAHs from soil constituents and enhance biodegradation (Bengtsson and Zerhouni, 2003). Slow desorption of organic pollutants has been identified as a major factor that limits their biodegradation (Haritash and Kaushik, 2009). It is also possible that the volatilization of PAHs may also have increased by improving soil physical properties such as aeration and porosity (Wild and Jones, 1993) in the compost-added soils. The reduction of concentration of soil PAHs in compost-added soils collected 376-days after compost compared to the compost-added soils collected soon after compost addition seemed to be higher for low molecular weight PAHs than the high molecular weight PAHs. In composted biosolids-added soil and leaf compost-added soils, the reduction of two to three-ring PAHs were 36 to 49%; it was about 15% for four ring PAHs and about 24% for five to six ring PAHs. In non-composted biosolids-added soils, this reduction was about 72% for two and three ring PAHs, about 53% for four to six ring PAHs. The slow degradation of high molecular weight PAHs, because of their high affinity to soil constituents has been reported previously (Haritash and Kaushik, 2009). Non-composted biosolids seem to be more effective on biodegrading PAHs as compared to composted biosolids and leaf compost (soil sampling for PAHs had not been done in mushroom compost-added soil soon after compost addition, so no such comparisons can be done for mushroom compost). The higher effectiveness of non-

composted biosolids could be attributed to the higher N and P concentrations (Table 5.1) which could help to maintain vigorous microbial activity (Haritash and Kaushik, 2009).

When comparing the concentrations of PAHs in control soil, the concentration of low molecular weight PAHs seemed to be slightly reduced in the 376-days after compost addition soils, while the concentration of high molecular weight PAHs did not change, compared to the soils collected soon after compost addition. This could be probably due to increase of volatilization of the low molecular weight PAHs by soil preparation in the two growing seasons.

The concentrations of PAHs in the vegetables were very low and below the detection limit of the method used. Fismes et al. (2002) reported similar low concentrations of PAHs (range: < 0.1-0.5 mg kg^{-1} in dry weight basis) in lettuce, carrot and potatoes from a pot experiment conducted with soils naturally contaminated with industrial activities and have similar levels of soil PAHs as in this study. In their study, soils with much higher soil PAHs concentrations had shown more accumulation of PAHs in vegetables. The concentrations of PAHs in vegetables grown in naturally contaminated soils reported in the literature seem to be variable depending on the concentrations of soil PAHs and proximity to the source of the soil PAHs (Tao et al., 2004; Rojo Camargo and Toledo, 2003; Voutsa and Samara, 1998). It has been documented that the organic pollutants that are in prolonged contact with soil are well sequestrated and possess low bioavailability (Haritash and Kaushik, 2009). Nam and Kim (2002) showed that molecules of phenanthrene partitioned into/onto humin polymer layers, and later diffuse into micropores in humic-mineral associations, where they get protected with overlying organic polymer layers and become less bioaccessible. The low concentrations of PAHs in vegetables indirectly suggest that the majority of PAHs in this soil would be sequestrated in soil. Haritash and Kaushik (2009) argue that no remediation actions are needed when the

contaminants show low bioavailability. However, in the gardening process, soil preparation disturbs the soil structure, and it could increase the volatilization/mobility of some PAHs in soils. These possibilities must be considered when assessing risk of gardening in contaminated sites. PAH accumulation in plants is mainly happening via atmospheric deposition of particulates that carry PAHs on the waxy cuticle layer of plant aerial parts and/or gaseous PAHs through the stomata (Kipopoulou et al., 1999; Fismes et al., 2002) and refers to as air to plant pathway.

Conclusions

The immediate beneficial effect of compost addition was the dilution of total Pb and As concentrations in soils. Composted biosolids showed the maximum dilution effect; it was high enough to reduce the absolute bioaccessibility of soil Pb and As compared to all other treatments. High concentrations of amorphous Fe and organic C in soils resulted low Pb and As bioaccessibility, regardless of the compost treatments. Composted biosolids which could potentially have the high concentration of active Fe oxides compared to other compost-types effectively reduced bioaccessibility of soil Pb. Increase of soluble inorganic P concentration via fertilizer application seem to induce sparingly soluble Pb-phosphate formation in the bioaccessibility extraction test. In contrast, the P fertilizer application increased As bioaccessibility in composted biosolids-added soils via desorbing the adsorbed As on to active Fe oxides. The biodegradation of soil PAHs was enhanced by compost addition, and this effect was prominent for two to three-ring PAHs than four to six-ring PAHs. Concentrations of PAHs in vegetables seemed to be minimal. The concentrations of Pb and As in the tested vegetables were low, suggesting a low risk of food chain transfer, except for Pb in carrots. Plant Pb accumulation can be quite variable in different cultivars, and hence, cultivar selection could be used to reduce food chain transfer of soil contaminants to humans. Composted biosolids reduced As concentrations in collard greens and none of the other treatments were effective on reducing Pb and As in vegetables. Thorough cleaning of vegetable can reduce food chain transfer of soil Pb and As. In general, gardening in this site carries a low risk of transferring contaminants into humans via soil ingestion and vegetable consumptions.

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Tables and Figures

Table 5.1 Basic properties of site soil and compost-types

Parameters	Soil	Composted biosolids	Non composted biosolids	Mushroom compost	Leaf compost
Total Pb, mg kg^{-1}	475	50.8	25.9	2.5	6.8
Total As, mg kg^{-1}	95	6.2	15.0	<1.4	<1.4
pH (1:10 soil:water)	7.48	6.18	7.26	7.91	7.33
CEC, $cmol_+$ kg^{-1}	13.9	-	-	-	-
Sand, silt and clay, %	72, 27, 1	-	-	-	-
Organic matter, g kg^{-1}	54	212	547	606	641
Available N, mg kg^{-1}	5.2	430	7,310	240	40
Mehlich III-P, mg kg^{-1}	61.2	-	-	-	-
Available K, mg kg^{-1}	50	-	-	-	-
C:N ratio	-	13.8	7.7	13.6	38.6
Total N, mg kg^{-1}	-	8,900	41,350	25,790	9,640
Total P, mg kg^{-1}	-	7,160	22,100	5,540	1,180
Total Fe, g kg^{-1}	-	26.5	26.4	2.1	4.1
Amorphous Fe, g kg^{-1}	28.3	-	-	-	-
$Na_2C_2O_4$ extractable Al, g kg^{-1}	1.3	-	-	-	-
Total Mn, mg kg^{-1}	-	581	411	335	305

Table 5.2 Description of the soil samples collected during the study

Sample set no.	Days after compost addition	Soil sample description
1	7 d	Before planting, 2011 test plot
2	106 d	After harvesting carrots, 2011 test plot
3	315 d	Before planting and fertilizing (samples collected for nutrient analysis at the beginning of the 2012 test plot)
4	351 d	Soils collected before planting and after adding NPK in 2012 test plot
5	484 d	After harvesting carrots in 2012 test plot
6	664 d	Sample set collected from min plots in 2013

Table 5.3 Soil Mehlich III extractable P and organic C concentrations in soils used to determine soil Pb and As bioaccessibility

	Mehlich III extractable P						Organic C		Soil pH (1:10 soil: water extract)			
Days after compost addition	7d	106d	315d	351d	484d	664d	7d	664d	7d	351 d	484 d	664 d
	---------- mg kg^{-1} ----------						---------g kg^{-1}-------					
Composted biosolids	345±10	393±50	379±34	490±10	462±19	443±39	53±3	44±2	6.6±0.1	7.4±0.0	7.8±0.1	7.5±0.0
Non-composted biosolids	316±15	800±66	571±45	839±58	929±172	936±68	47±3	48±2	6.7±0.1	6.6±0.1	6.9±0.2	7.2±0.0
Mushroom compost	273±41	299±60	276±36	430±61	400±81	296±52	58±1	44±3	7.5±0.1	7.0±0.1	7.4±0.0	7.7±0.0
Leaf compost	87±19	102±21	84±20	208±56	127±25	116±17	55±2	45±2	6.8±0.1	7.3±0.2	7.8±0.0	7.8±0.0
Control	71±22	74±15	112±22	159±26	139±17	120±32	44±5	37±1	7.0±0.1	7.3±0.2	7.8±0.1	7.9±0.1

Table 5.4 Concentrations of 16 priority PAHs in soils and vegetables

PAH	Molecular weight	Concentrations of PAHs in soil									Concentrations of PAHs in vegetables †
		Composted biosolids	Non-composted biosolids	Leaf compost	Control	Composted biosolids	Non-composted biosolids	Mushroom compost	Leaf compost	Control	
Days after compost addition		0	0	0	0	376	376	376	376	376	
	g mol^{-1}	---------- mg kg^{-1} ----------									mg kg^{-1}-FW
Two-ring											
Naphthalene	128	0.59 ± 0.06	0.90 ± 0.14	1.00 ± 0.12	0.96 ± 0.15	0.37 ± 0.06	0.26 ± 0.07	0.52 ± 0.09	0.88 ± 0.35	0.58 ± 0.03	<0.01
Three-ring											
Acenaphthylene	152	0.97 ± 0.30	1.00 ± 0.18	1.59 ± 0.38	1.64 ± 0.34	0.91 ± 0.20	0.48 ± 0.16	0.96 ± 0.06	0.69 ± 0.15	1.12 ± 0.37	<0.01
Acenaphthene	153	0.57 ± 0.08	0.42 ± 0.01	0.49 ± 0.03	<0.42	0.07 ± 0.01	<0.07	<0.07	0.22 ± 0.16	<0.07	<0.01
Fluorene	165	0.58 ± 0.09	0.42 ± 0.01	0.49 ± 0.03	<0.42	0.24 ± 0.14	<0.07	0.08 ± 0.01	0.27 ± 0.20	0.11 ± 0.02	<0.01
Phenanthrene	178	2.31 ± 1.13	2.64 ± 0.46	3.02 ± 0.49	2.60 ± 0.15	1.13 ± 0.06	0.75 ± 0.15	1.58 ± 0.09	2.93 ± 1.87	1.78 ± 0.09	<0.01
Anthracene	178	1.22 ± 0.26	1.19 ± 0.15	1.66 ± 0.46	1.52 ± 0.28	0.62 ± 0.12	0.34 ± 0.11	0.64 ± 0.06	0.88 ± 0.33	0.74 ± 0.22	<0.01
Four-ring											
Fluoranthene	202	3.86 ± 1.04	4.14 ± 0.49	5.30 ± 1.21	4.21 ± 0.57	2.50 ± 0.52	1.44 ± 0.48	2.50 ± 0.08	4.00 ± 1.83	3.90 ± 0.79	<0.01
Pyrene	202	3.38 ± 0.84	3.56 ± 0.40	4.76 ± 1.24	3.76 ± 0.58	2.63 ± 0.52	1.73 ± 0.87	2.70 ± 0.15	4.03 ± 1.59	4.48 ± 1.00	<0.01
Banz(a)anthracene	228	2.32 ± 0.57	2.49 ± 0.33	3.19 ± 0.77	2.70 ± 0.39	2.25 ± 0.46	1.32 ± 0.55	2.50 ± 0.14	2.95 ± 0.84	3.40 ± 0.88	<0.01
Chrysene	228	2.90 ± 0.63	3.05 ± 0.42	3.88 ± 0.90	3.44 ± 0.47	2.80 ± 0.56	1.69 ± 0.65	3.03 ± 0.30	3.43 ± 1.05	4.25 ± 0.97	<0.01
Five- or six-ring											
Benzo(b)fluoranthene	252	4.70 ± 1.06	4.51 ± 0.66	5.87 ± 1.31	5.52 ± 0.77	4.13 ± 0.71	2.58 ± 1.05	4.68 ± 0.59	4.45 ± 0.92	5.53 ± 1.61	<0.04
Benzo(k)fluoranthene	252	1.99 ± 0.46	1.92 ± 0.23	2.48 ± 0.56	2.25 ± 0.45	1.63 ± 0.32	1.01 ± 0.41	1.93 ± 0.15	1.87 ± 0.45	2.40 ± 0.35	<0.04
Benzo(a)pyrene	252	3.16 ± 0.75	3.07 ± 0.44	3.99 ± 1.00	3.64 ± 0.65	2.43 ± 0.38	1.66 ± 0.75	2.65 ± 0.26	2.80 ± 0.61	3.65 ± 0.88	<0.1
Indeno(123-cd)pyrene	276	2.14 ± 0.48	2.08 ± 0.30	2.79 ± 0.68	2.58 ± 0.48	1.93 ± 0.35	1.31 ± 0.48	2.13 ± 0.19	2.15 ± 0.49	2.75 ± 0.61	<0.04
Dibenz(a,h)anthracene	278	0.74 ± 0.12	1.70 ± 0.04	1.07 ± 0.28	0.81 ± 0.14	0.67 ± 0.15	0.47 ± 0.16	0.65 ± 0.06	0.78 ± 0.18	0.92 ± 0.21	<0.1
Benzo(g,h,i)perylene	276	2.87 ± 0.26	8.60 ± 0.20	3.35 ± 0.53	2.92 ± 0.34	1.63 ± 0.27	1.12 ± 0.50	1.80 ± 0.23	1.87 ± 0.44	2.28 ± 0.46	0.04

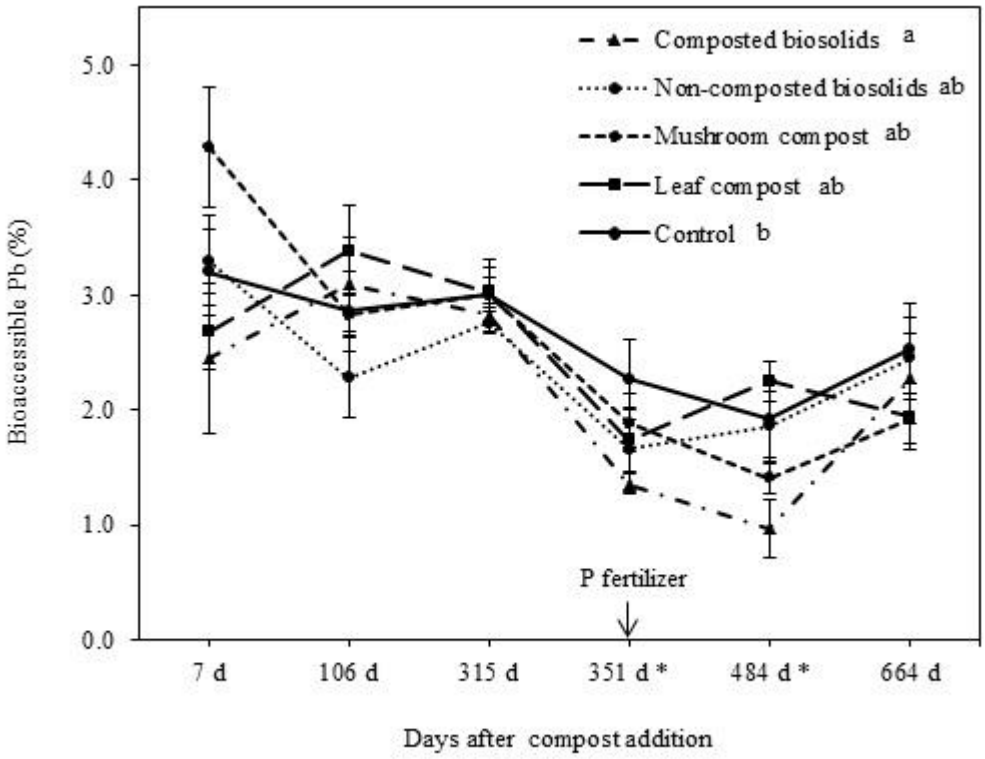

Figure 5.1 Bioaccessible Pb in soil collected various days after compost addition. The compost-type with the same letters were not significantly different and the compost-type with different letters were significantly different at $p<0.1$. The days after compost addition with * were significantly different from the rest at $p<0.05$. The interaction between compost-type and days after compost addition was not significant.

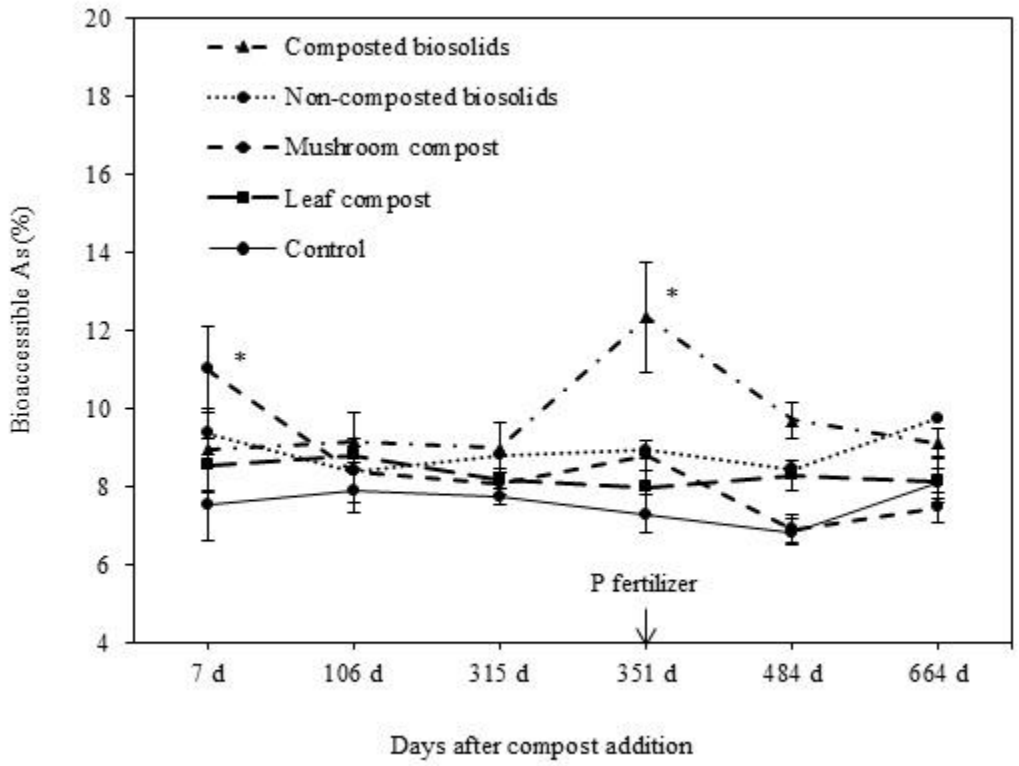

Figure 5.2 Bioaccessible As in soil collected various days after compost addition. * The two values were significantly different from the rest at $p<0.05$.

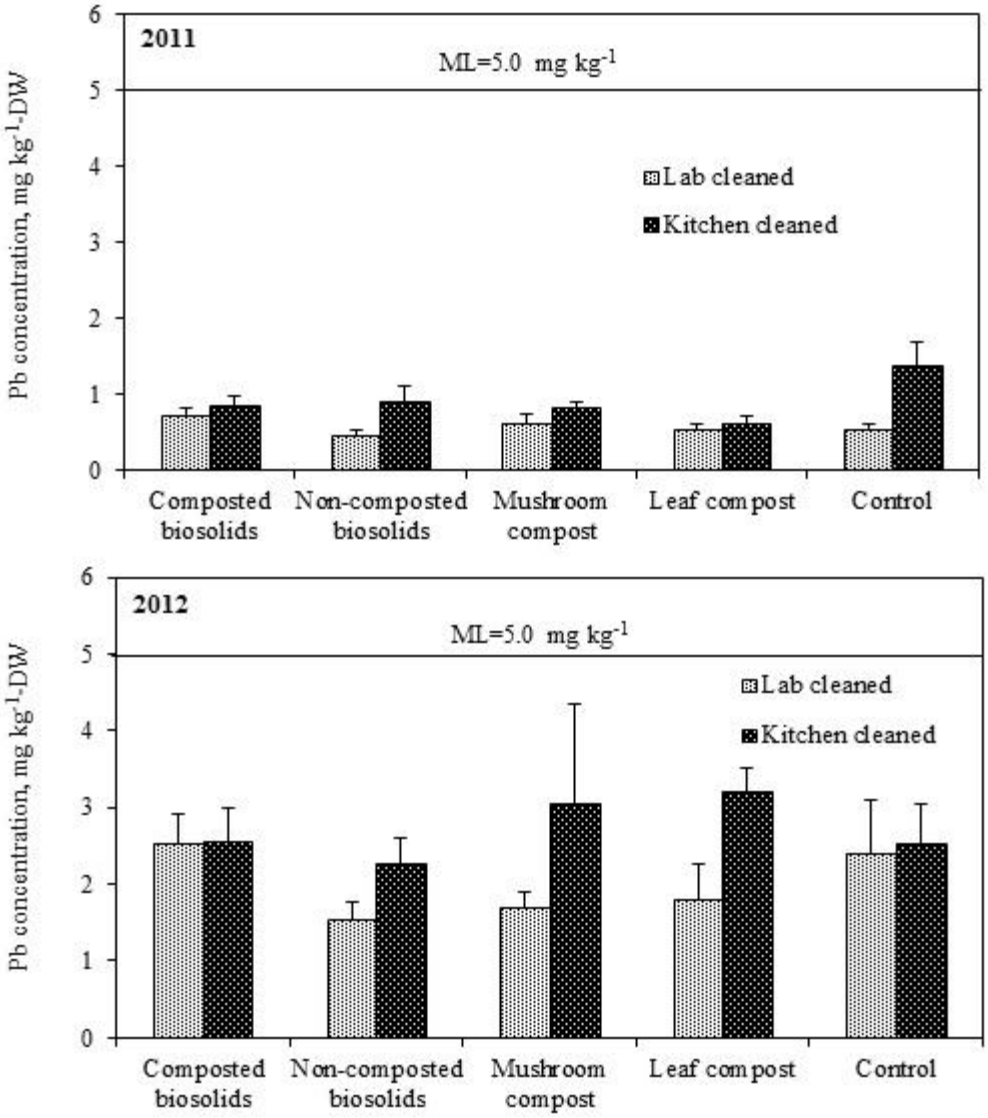

Figure 5.3 Pb concentration in collard greens in 2011 and 2012. Compost-type was not significant; Lab cleaning and kitchen cleaning were significantly different at $p<0.1$. ML= Maximum allowable level of Pb in leafy vegetables, 0.3 mg kg^{-1}-fresh weight (FAO/WHO-CODEX, 1995; 2010 amendment) ML=5.0 mg kg^{-1}-dry weight when moisture content is 94%.

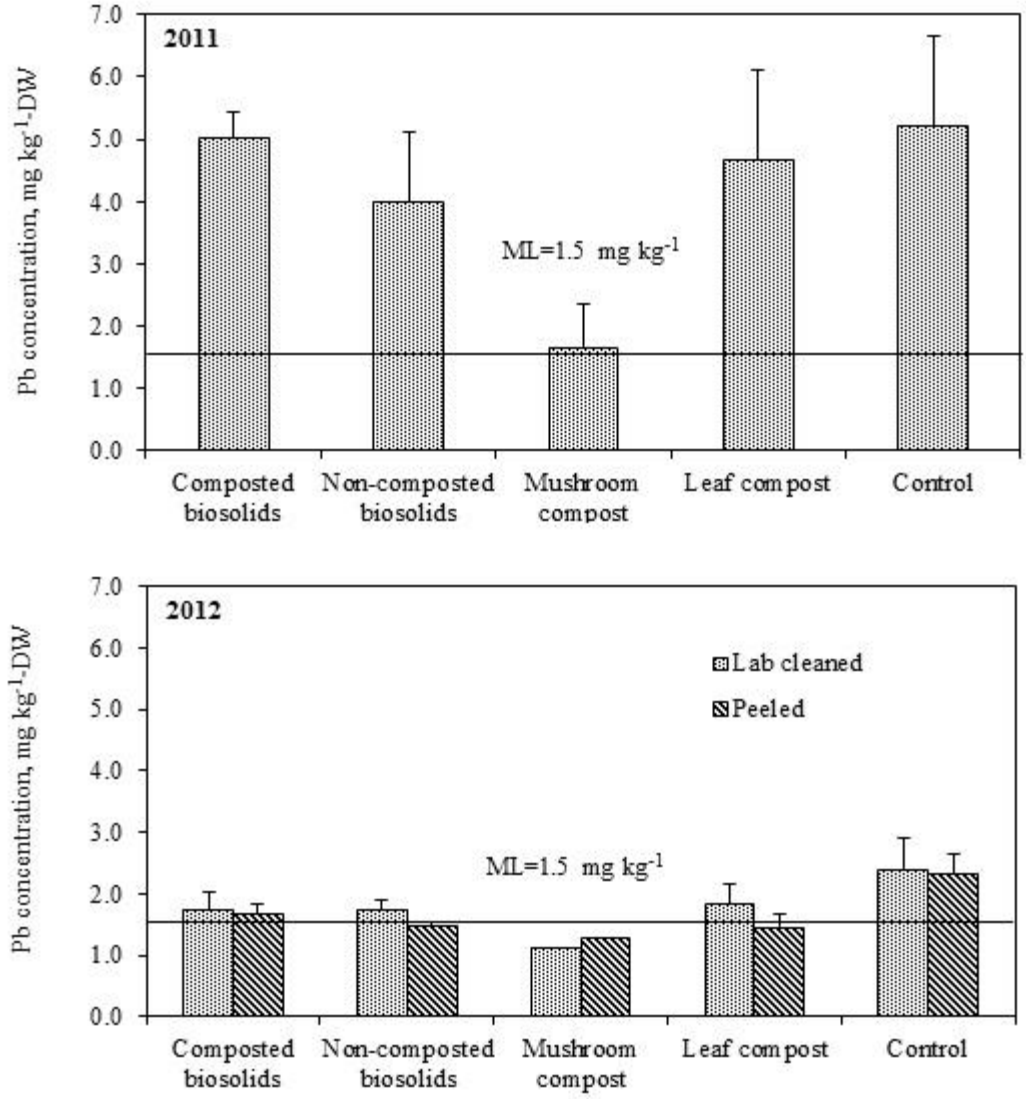

Figure 5.4 Pb concentration in carrots in 2011 and 2012. Only lab cleaning was done in 2011. The compost-type and cleaning methods were not significant. ML= Maximum allowable level of Pb in root and tuber crops, 0.1 mg kg^{-1}-fresh weight (FAO/WHO-CODEX, 1995; 2010 amendment) ML=1.5 mg kg^{-1}-dry weight when moisture content is 93%.

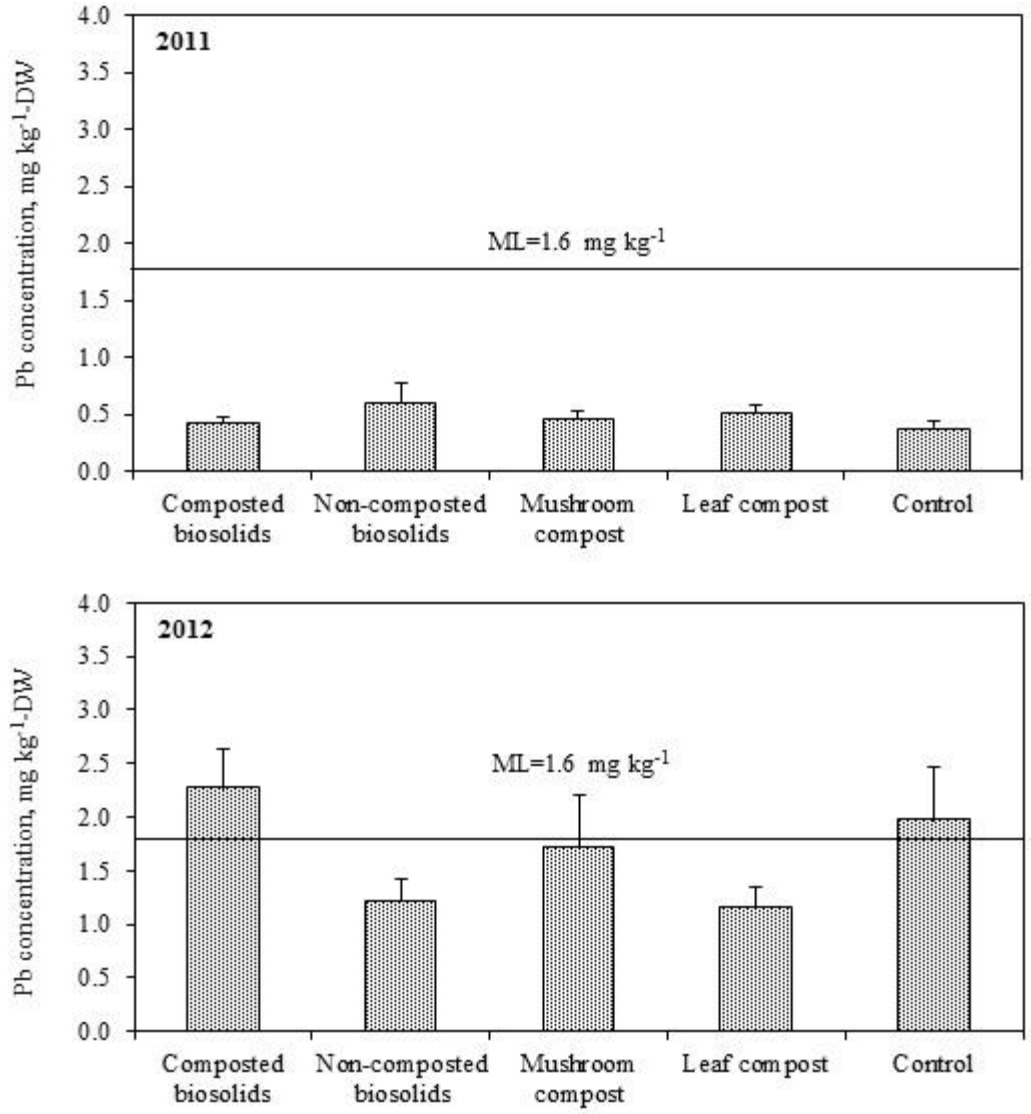

Figure 5.5 Pb concentration in lab cleaned tomatoes in 2011 and 2012. The compost-type was not significant. ML= Maximum allowable level of Pb in fruiting vegetables, 0.1 mg kg^{-1}-fresh weight (FAO/WHO-CODEX, 1995; 2010 amendment) ML=1.6 mg kg^{-1}-dry weight when moisture content is 94%

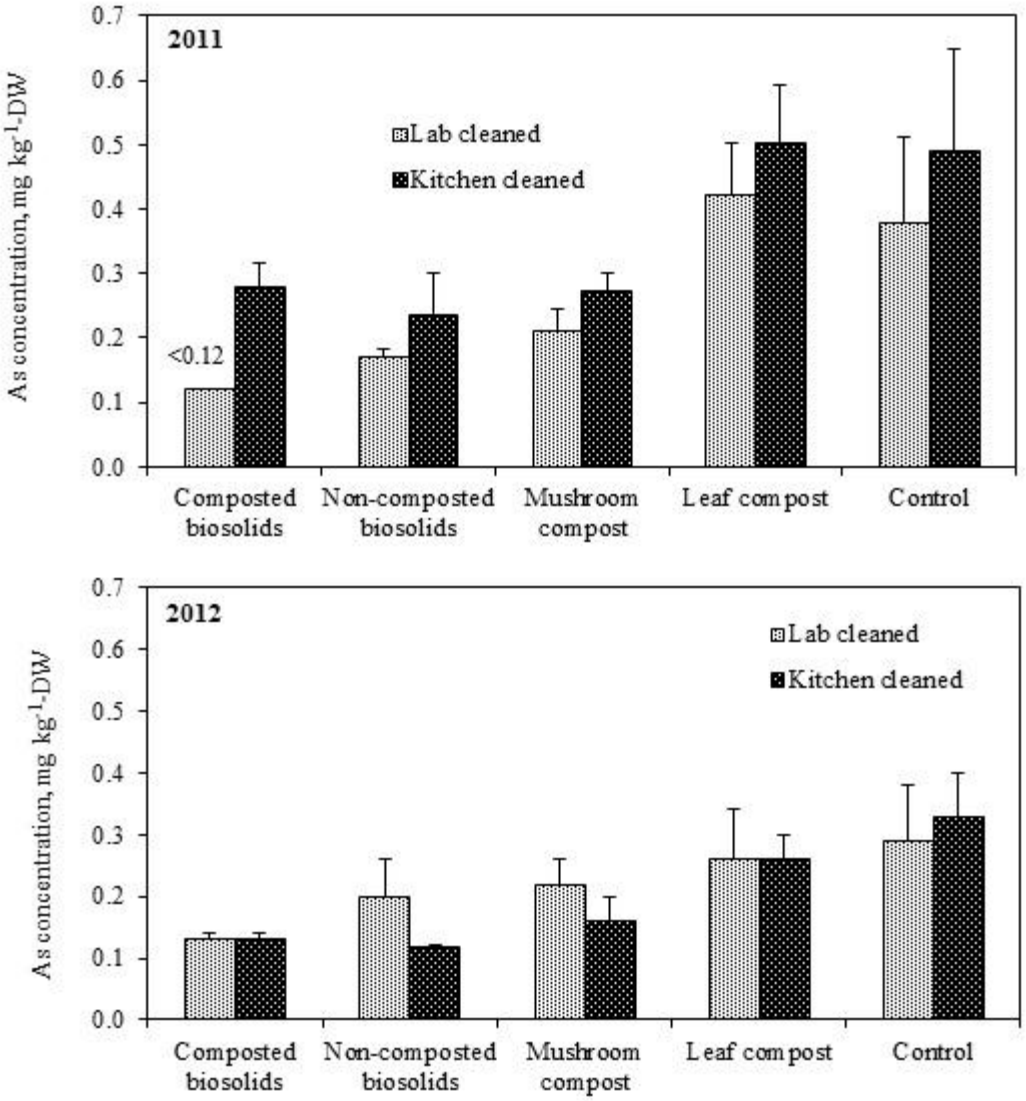

Figure 5.6 As concentration in collard greens in 2011 and 2012. The compost-type was significant at $p<0.05$ in both years. Cleaning method was significant in in 2011 at $p<0.05$. In 2011, all the four replicates of composted biosolids, lab cleaned collard greens had As concentrations of <0.12 mg kg^{-1}

Chapter 6 - Dermal Absorption of Polycyclic Aromatic Hydrocarbons in an Urban Soil: A Qualitative Study of Effect of Soil Matrix and Aging

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic contaminants that are common in urban settings. The carcinogenicity of some of the PAHs and their potential abundance in urban soils raise concerns of risk of transfer of PAHs into humans. Dermal absorption of PAHs has been studied extensively, but majority of those studies were done with pure/simple chemicals and solvents. Dermal transfer of PAHs in complex matrixes like soil largely deviates from the results obtained from simple systems. We assessed dermal transfer of PAHs in a naturally contaminated urban soil giving emphasis on the soil matrix and aging of PAHs. The treatments used were 1) soil contaminated with PAHs at 20 % moisture 2) soil contaminated with PAHs at 40 % moisture 3) contaminated soils mixed with biosolids 4) control soil 5) control soil spiked with PAHs and aged for 0, 1,3 and 12 days (control soil+PAHs: the concentrations of PAHs were matched to the contaminated soil) 6) PAHs dissolved in methanol and propylene glycol (PAHs mix: the concentrations of PAHs were matched to the contaminated soil). An *In vitro* static diffusion cell experiment was conducted to test the transfer of PAHs from above treatments to the receptor fluid (blood) via skin. The exposed skins were observed under a laser scanning confocal fluorescence microscope (Ex: 360nm; Em: 420-480nm) to detect the PAHs accumulation in the skin. No detectable concentrations of PAHs were found in the receptor fluid of contaminated soil, contaminated soil mixed with biosolids, control soil, and control soil+PAHs (12 days). Eleven PAHs out of 16 tested PAHs were transferred into the receptor fluid in PAHs mix treatment. High molecular weight PAHs showed limited capacity to

transfer into the receptor fluid. The control soil+PAHs treatment aged for 0, 1 and 3 day showed only 1-4 PAHs in the receptor fluid showing significant soil matrix and aging effect. The fluorescence microscopy revealed that the PAHs that entered into the skin accumulated in the stratum corneum, in all soil treatments. In general, this study suggests that the dermal transfer of PAHs in naturally contaminated soils is very limited.

Introduction

Polycyclic aromatic hydrocarbons are a group of organic compounds that includes potential carcinogens (ASTDR, 1995). Their chemical structures consist of two or more combined benzene rings. These are by-products of partial burning of fossil fuels, gasoline, and wood etc. Polycyclic aromatic hydrocarbons are primarily released to the atmosphere and later deposited on terrestrial ecosystems like water bodies, sediments, and soils. Urban soils may contain elevated concentrations of PAHs emissions from gasoline combustion, coal burning and other industrial activities. Some wood preservatives, like creosote, contain significant amounts of PAHs. The United States Environmental Protection Agency (USEPA) has identified 16 PAHs as priority pollutants that need to be addressed in site cleanup processes (Khadhar et al., 2010). People can be exposed to PAHs via inhalation, consumption of contaminated food, and dermal absorption (ATSDR, 1995).

Urban gardening has gained much attention over the past decades, and there is a growing need of research on safeness of gardening in potentially contaminated soils. In gardening, consumption of vegetables that absorb (air to plant or soil to plant) and accumulate soil PAHs is a potential transfer pathway for soil PAHs. We analyzed edible portions of collard greens, tomatoes and carrots grown in a contaminated urban gardening site located in Indianapolis, IN for PAHs concentrations and found that the concentrations of PAHs in those vegetables were quite low (Attanayake et al., 2014). Another potential exposure pathway for soil PAHs is dermal absorption. There are numerous *in vivo* and *in vitro* studies that assessed the dermal absorption of PAHs (Storer et al., 1984; Kao et al., 1985; Dankovic et al., 1989; VanRooij et al., 1993). In general, PAHs readily absorb via skin. Stratum corneum is the topmost layer of the skin which

acts as a barrier for solute transfer into deeper layers (Bouwstra et al., 2003). Stratum corneum consists of inactive, keratinized cells surrounded by extracellular lipids (ASTDR, 1995). Lipophilic compounds tend to show higher potential to absorb via skin, because the lipids in the stratum corneum act as the route of absorption for such compounds (Albery and Hadgraft, 1979; Raykar et al., 1988). PAHs have a relatively high octanol-water partitioning coefficient (K_{ow}) which explains the ability of an organic molecule to partition from water to octanol (water to lipid). The PAHs with high molecular weights have a relatively higher K_{ow} than the low molecular weight PAHs, and this would allow high molecular weight PAHs to be absorbing more into skin than low molecular weight PAHs. Turkall et al. (2009) showed higher penetration and accumulation of benzo[a]pyrene (molecular weight: 252 g mol^{-1}) in human skin compared to that of naphthalene (molecular weight: 128 g mol^{-1}) in an *in vitro* study, conducted with and without soil matrix. Further, they reported a higher concentration of naphthalene in the receptor fluid than the concentration of benzo[a]pyrene which could be attributed to the higher capacity of naphthalene to be partitioned into deeper layers with low accumulation in the stratum corneum.

Most of the studies that evaluate dermal absorption of PAHs have been done using pure/single chemicals of PAHs in simple solvents (Kao et al., 1985; Ng et al., 1992; Withey et al., 1993). This approach ignores how PAHs behave when there are several PAHs occur together in a reactive matrix such as soil and the effect of vehicle of transfer/matrix in dermal absorption of PAHs, and hence the generated data may not reflect true absorption of PAHs under situations such as field-contaminated soils. Dankovic et al. (1989) studied dermal absorption of benzo[a]pyrene and benzo[a]pyrene with the presence of eleven other PAHs using a mice model. They found that the half-life of benzo[a]pyrene in skin is lower than when it would absorb alone. This could indirectly affect the amount of benzo[a]pyrene that partition into the skin, since

dermal metabolism of benzo[a]pyrene in the skin was found to be the rate limiting factor of its dermal absorption (Kao et al., 1985). PAHs in soils show relatively low dermal absorption compared to the PAHs in pure solvents (Yang et al. 1989; Wester et al. 1990; Turkel et al., 2009). Sorption of PAHs by soil organic and inorganic constituents is the main factor that limits the dermal absorption of PAHs in soil. Soil PAHs undergo numerous biochemical transformations such as volatilization, biodegradation, photolysis, hydrolysis, abiotic oxidation, and adsorption to organic C depending on their physicochemical properties such as water solubility, volatility, K_{ow}, organic C partitioning coefficient (K_{oc}) (ASTDR, 1995). In general, low molecular weight PAHs are more volatile, and their K_{oc} and K_{ow} are comparatively lower than the high molecular weight PAHs. Therefore, low molecular weight PAHs are more prone to volatilization and abiotic degradation (photolysis and oxidation) (Park et al. 1990; Wild and Jones 1993), whereas high molecular weight PAHs tend to sorb onto soil organic constituents and become less available for biotic or abiotic degradation. In addition to the inherent properties of PAHs, soil properties such as moisture content, organic C concentration, dissolved organic C concentration, clay percentage, aeration, pH, temperature, redox conditions, and concentration of toxic metal also affect the transformation and partitioning of PAHs (Thomas et al. 1989; Wilson and Jones 1993; Bengtsson and Zerhouni, 2003). These soil properties affect biodegradation of PAHs via affecting microbial structure and activities, and providing suitable sorptive sites for binding PAHs. Further, the addition of soil amendments such as biosolids modifies the soil matrix primarily by providing organic C, and subsequently dissolved organic C (DOC), although this increase could only be temporary. In the above context, the dermal absorption of soil PAHs could tremendously influence by soil matrix and its biochemical reactions. Further, aging of PAHs in soils tend to reduce the dermal absorption of soil PAHs (Turkall et al., 2009). The

research published on determining the dermal absorption of soil PAHs in naturally contaminated soils is limited.

The objective of this research was to determine the soil matrix effect on dermal absorption of 16 priority PAHs (suggested by USEPA) using field-contaminated urban soil. Short-term aging effect of PAHs on dermal absorption was also studied with PAHs spiked control soils.

Materials and Method

Soils

Soils contaminated with PAHs were collected from a parcel of land located close to potential urban garden in Indianapolis, IN ($39^{o}46'09.8"$ N and $86^{o}09'31.7"$ W). This site had elevated concentrations of PAHs that mainly originated from creosote on railroad ties that were buried in soils after removing old railroad track. A railroad service station had been located on the site previously. Partial burning of gasoline and other fuels related to industrial activities in the surrounding urban area may also have contributed to the elevated concentration of PAHs in this site. We collected a soil sample from 0-15 cm depth closer to the railroad to use for this experiment as contaminated soil. A control soil sample (0-15 cm depth) was collected from the other end of the site. This control soil also had somewhat elevated levels of PAHs, but the concentration was significantly lower than the concentration of PAHs in the contaminated soil. The concentrations of PAHs in these two soil samples are given in Table 6.1. For more details on soil PAHs extraction and analysis refer to Attanayake et al. 2014. These soils were air dried and sieved using a 2 mm sieve.

Treatments and treatment preparation

The treatment description and their preparation details were as follows. Six treatments were used in this experiment. They were 1) soil contaminated with PAHs at 20% gravimetric water content 2) soil contaminated with PAHs at 40% gravimetric water content 3) contaminated soils mixed with biosolids 4) control soil 5) control soil spiked with PAHs (control soil+PAHs) 6) PAHs dissolved in methanol and propylene glycol (PAHs mix). Except the second treatment above, all the other soil treatments had a gravimetric water content of 20%.

Soils were homogenized by shaking in a rolling shaker (U.S. Stoneware, East Palestine, OH) for overnight. Sub samples weighing 100 g were collected from field contaminated and control soil samples to make the above treatments. All the treatments were made in 250 mL wide mouth amber glass jars. The soil contaminated with PAHs with 20% gravimetric water content and 40% gravimetric water content; and control soil with 20% gravimetric water content was prepared by adding required amounts of de-ionized water. All the other soil treatments had 20% moisture content. A composted biosolids was mixed with the contaminated soil at a rate of 4.5: 1 (w/w) soil: biosolids. Sixteen priority PAH standards were purchased from Sigma Aldrich (St. Louis, MO) to make control soils spiked with PAHs and PAHs mix treatments. The control soil was spiked with PAHs to get the final concentrations of the 16 PAHs in the control soil+PAHs samples, as in the contaminated soil sample. The spiking was done as by mixing required amounts of PAHs in toluene: ethanol (1:4) solvent; and then the mixture was added into dry soil along with required amount of water as suggested by Brian et al. (1998). This treatment was incubated at 25^{o}C for 12 days, and the moisture content of the spiked soil was maintained at 20% by adding required amounts of deionized water every day. Ten g of sub samples were taken from the spiked treatment at 0, 1, 3 and 12 days after spiking. The treatment referred to as ‘PAHs mix’ was prepared by dissolving 16 PAHs in propylene glycol at different proportions to match the

concentrations of 16 PAHs in the contaminated soil sample. Since the original PAH standards were in methanol the final PAHs mix treatment had 3.5: 1 methanol: propylene glycol as a solvent.

In vitro static diffusion cell experiment

We employed the static diffusion cell system using a skin donated by an abdominal plastic surgeon. Human skin from the abdominal area was cut to a thickness of 0.5 mm using a dermatome (Padgett). The skin was stored at -20^{o}C for 5 days until use. It has been found that the human skin permeability would not be compromised when it was stored under -20^{o}C for up to 12 months (Davis et al., 2004). The square sections of (~2 cm×2 cm) skins were mounted on Teflon static diffusion cells. A half a mL of the receptor fluid that was made according to (Riviere et al., 1986) was placed in the receptor chamber of the cell to be in contact with the dermis side of the skin. The receptor fluid, designed to mimic a blood plasma environment, consisted of 13.78 g NaCl, 5.50 g $NaHCO_3$, 0.58 g $MgSO_4.7H_2O$, 0.32 g KH_2PO_4, 0.56 g $CaCl_2$, 0.71 g KCl, 2.40 g dextrose, 90.0 g bovine serum albumin, 0.004 g levofloxacin, and 10 mL heparin made up to 2 L with de-ionized water (Van der Merwe and Riviere, 2005). One g of soil treatments/1 mL of PAHs mix was placed on the epidermis side of the skin. The exposure area of the skin to the treatment was 1 cm^2. The cells were kept at 37^{o}C, which brought up the temperature of the skin to 32^{o}C. The exposure time used was 5 h. Then, the receptor fluid in the cells was collected into a 50 mL Teflon centrifuge tube and the PAHs were extracted, according to Pleil et al. (2010).

Five mL of hexane were added to the 0.5 mL of the receptor fluid contained in the Teflon tube. Next, 0.625 mL of 400 mg L^{-1} deuterated phenanthrene were added as an internal standard. Then, the tubes were capped tightly and vortexed for 20 seconds. Then, tubes were placed on an

orbital shaker to agitate at 300 rpm for 40 minutes, and after that the content was vortexed again. The solution was centrifuged at 3000 rpm for 5 min and frozen at -80^{o}C. Then the solvent was pipetted out leaving the frozen receptor fluid in the centrifuge tube. The pipetted solution was transferred into a glass concentrator vial. The volume of the solution was reduced to < 0.5 mL under N_2 gas flow. Then the solution was carefully transferred into gas chromatography-mass spectrometer (GC-MS) sample vials, and the volume was adjusted to 0.5 mL with hexane. The samples were run in GC-MS (Varian Inc., Foster City, CA) for 16 priority PAHs concentrations. Three replicates for each treatment were employed to check the consistency of the results. However, here we do not intend to provide explanations or conclusions based on in depth quantifications and more qualitative observations are provided in the results and discussions.

Laser scanning confocal fluorescence microscopy

It is well documented that PAHs emit blue fluorescence, when they are excited at ultra-violet light range (Asher, 1984; Wise et al., 1988; Nie et al., 1993; Kumke et al., 1995; Harrison et al., 1996). The maximum excitation/absorption wavelengths of the 16 major PAHs range from 240 to 460 nm and their maximum emission wavelengths range from 310 to 542 nm (Wise et al., 1988; Kumke et al., 1995; Beltran et al., 1998). This fluorescence property of PAHs has been utilized to develop detection and quantification methods for PAHs (Asher, 1984; Wise et al., 1988; Nie et al., 1993). Further, emission of fluorescence by biological tissues upon treating with PAHs has been identified (Peacock 1940; Richter and Saini 1960; Penn et al., 2005). In this experiment, the fluorescence property of PAHs was used to detect the PAHs and their depth of penetration in skins that were exposed to the treatments described in the previous section. The successful localization of PAHs in cultured human respiratory epithelial cells that were contaminated by PAHs in butadiene soot was documented by Penn et al. (2005). The excitation

and emission wavelengths that Penn et al. (2005) used were 360 nm and 420nm, respectively. Since the fluorescence properties of the 16 PAHs reported in the literature are quite variable (Wise et al., 1988; Kumke et al., 1995; Beltran et al., 1998), a pretest was done to test which PAHs can be detected under excitation at 360 nm, and the emission range of 420 to 480 nm, when they are present in human skins. In the pretest, a human skin (0.5 mm) was exposed to 16 PAHs (10 mg L^{-1} in methanol) individually using the static diffusion cell system described above. The exposure time was 5 h. The exposed skins were examined in a fluorescence microscope (Zeiss LSM 5 PASCAL: Carl Zeiss) with 360 nm excitation and 420 to 480 nm emission ranges. The skins treated with the following PAHs emitted strong blue fluorescence: benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene, and dibenz[a,h]anthracene, indicating that those PAHs in the skin could be detected with high sensitivity. A weak fluorescence was observed for the skins treated with fluorene, anthracene, phenanthrene, fluoranthene, and chrysene suggesting that these PAHs in skin could be detected with moderate sensitivity. Naphthalene, acenaphthylene, and acenaphthene did not emit any detectable signal indicating that these PAHs may not be able to detect successfully with this fluorescence set up. In general, the PAHs with high molecular weight were able to detect with satisfactory sensitivity.

A fresh skin from human abdominal area (0.5 mm thick), without freezing or storing, was used for this fluorescence microscopy experiment. Use of fresh skin may help preserving the anatomy of the skin without damaging it from ice crystal formation that may occur during freezing. The skin was exposed to the treatments following the method described in the static diffusion cell experiment, but with longer exposure time (i.e., 12 h). The experiment was duplicated with each treatment to test the consistency of the results. In addition to the above

mentioned treatments, contaminated soil mixed with biosolids treatment was incubated for 30 days at 25^{o}C and used as treatment for this microscopy study to test if the aging of biosolids in soil could reduce the penetration/accumulation of PAHs in skin. The receptor fluid was collected, extracted, and analyzed for PAHs as mentioned above. The results of the receptor fluid were not different from the results of the *in vitro* static cell experiment which was conducted for lower exposure time. We did not attempt to make in depth comparisons here between 5 h- and 12 h-exposure time, because it is not within the objectives of this paper and the replications used in this portion of study may not adequate to make such meaningful comparisons. The 12 h exposed skin was prepared for the microscopic observation as follows. Soil particles on the skin were carefully removed by wiping gently with dry wipes. Then, the skin was immediately frozen at -80^{o}C in a tissue freezing medium using 2-methyl butane chilled with dry ice. The frozen skins were stored at -80^{o}C until the next step was performed. The frozen skin was cross-sectioned using a cryostat (Cryocut 1800, Reichert-jung) at 12 µm thickness. The sectioning was done from the bottom of the dermis to the epidermis to avoid mechanical dragging of any chemicals (including PAHs) from epidermis to dermis while sectioning. Then, the sections were observed under a Leica TCS SP1 confocal laser scanner interfaced to an inverted Leica IMBE inverted microscope (Leica Microsystems Inc. Buffalo Grove, IL) with a 40× Plan apochromat objective at 360 nm excitation and 420 to 480 nm emission range. Our initial attempt was to use a multi-photon laser scanning confocal microscope which would allow us to get quality images with deep optical sectioning eliminating the need of manual sectioning, but with the difficulty of locating such a microscope, the above alternative procedure was used. More details on use and advantages of multi-photon laser scanning confocal microscope can be found in Xu et al. (1996) and Hornung et al. (2004).

Results and Discussion

In vitro Static diffusion cell experiment

This method determines PAHs that transfers from the treatments to the receptor fluid via skin, which represents the PAHs that could potentially circulate in the body with blood. The static diffusion cell system has been widely used to estimate *in vitro* dermal absorption of various chemicals (Franz, 1975; Clowes et al., 1994; Chilcott et al., 2001; Wissing and Muller, 2002; Davies et al., 2004; Larese et al., 2009). This method is capable of simulating the initial dermal absorption process on the skin surface, partition into the stratum corneum, and diffusion process in the stratum corneum, and proven to correlate with *in vivo* study results (Franz, 1975; Bronaugh and Stewart, 1985). Potential disadvantages of this static diffusion cell system are that accumulation of penetrant (study chemical) may occur in the receptor chamber, and the solubility of the penetrant in the receptor chamber fluid may act as a rate limiting factor (Chilcott et al., 2001). In a solution flow-through cell system, continuous flow of receptor fluid is maintained throughout the experiment time, therefore, the above mentioned potential disadvantages can be overcome. However, we employed the static diffusion cell system, since our pretests indicated that the concentrations of PAHs that transfer from soil to receptor fluid is minimal and; hence the effect of PAHs accumulation in the receptor fluid could be insignificant; also flow through cell system would result much more diluted concentrations of PAHs in the receptor fluid which would lead to employ additional steps to improve the detection limits of the PAHs detection in the receptor fluid. Both static and flow through diffusion cells are described by the Organisation for Economic Co-operation and Development (OECD) guidelines for use in skin penetration studies (OECD, 2004).

The PAHs that transferred to the receptor fluid is given in Table 6.2. No detectable levels of PAHs were transferred from contaminated soil with both 20 and 40% moisture contents, contaminated soil mixed with biosolids, control soil+PAHs after 12 days of spiking, and control treatments. So the dermal absorption of PAHs in this field-contaminated soils seems to be insignificant. Previous research has also reported lower dermal absorption of soil PAHs in the soil matrix (Yang et al. 1989; Wester et al. 1990; Turkel et al., 2009). Four to five times lower dermal absorption of benzo[a]pyrene in crude oil was found when crude oil was mixed with soil than the benzo[a]pyrene in crude oil alone, in an *in vivo* study conducted using a rat model (Young et al., 1989). Similarly, significantly lower dermal absorption of benzo[a]pyrene was observed when it was mixed with soils than the benzo[a]pyrene in acetone using an *in vivo* study on monkeys (Wester et al., 1990).

The PAHs mix treatment showed the maximum transfer of PAHs compared to the other treatments. PAHs mix treatment transferred 11 tested PAHs to the receptor fluid, except acenaphthene, fluorene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene. The concentrations of acenaphthene (0.1 mg L^{-1}) and fluorine (0.3 mg L^{-1}) were the lowest in the PAHs mix treatment. The dose of these two PAHs may not have been high enough to show any significant penetration, although their smaller sizes (low molecular weight) would help it to partition into skin. The other three PAHs that did not transfer to the receptor fluid from PAHs mix treatment had relatively high concentrations (Table 6.2), but their molecular weights were the highest among the 16 PAHs. Molecular weight is a main physicochemical property that determines the capacity of a solute to penetrate via skin, as Magnusson et al. (2004) reported from a regression analysis that was conducted with the objective of finding the relationship of maximum flux across the skin and different

physicochemical properties of solutes. The high molecular weights of the above mentioned three PAHs may have restricted their penetration via skin. On the other hand, high molecular weight PAHs have a higher affinity to lipids, as indicated by their higher K_{ow} values. The stratum corneum, the topmost layer of skin has extracellular lipids that could potentially act as a route for dermal absorption, and that could potentially accumulate the high molecular weight PAHs. Accumulation of high molecular weight PAHs in the stratum corneum could restrict the partitioning of PAHs into deeper layers of the epidermis and dermis allowing the transfer of those PAHs to the receptor fluid. In this study, when considering the PAHs that were detected in the receptor fluid of the PAHs mix treatment, high molecular weight PAHs had a higher initial dose compared to the low molecular weight PAHs, but the concentration of high molecular weight PAHs in the receptor fluid was much lower than that of the low molecular weight PAHs. For example, ~ 3.5% of the initial dose of acenaphthylene (molecular weight: 152 g mol^{-1}) was found in the receptor fluid compared to only ~0.34% of the initial dose of benzo[a]pyrene (molecular weight: 252 g mol^{-1}) and ~0.17% of benzo[b]fluoranthene and benzo[k]fluoranthene (molecular weight: 252 g mol^{-1}; these two were quantified together as they were a doublet in the chromatogram). Similarly, from an *in vitro* flow through cell experiment conducted using human skins, Turkall et al. (2009) showed higher penetration and accumulation of benzo[a]pyrene, compared to that of naphthalene. Further, Turkall et al. (2009) reported a higher concentration of naphthalene in the receptor fluid than the concentration of benzo[a]pyrene in the receptor fluid which can be attributed to the higher capacity of naphthalene to be partitioned into deeper layers with low accumulation in the stratum corneum. In the above context, the higher size (molecular weight) and K_{ow} in the high molecular weight PAHs may have collectively contributed to their lower/insignificant concentrations in the receptor fluid.

When PAHs were added to the control soil, the transfer of PAHs via skin was significantly restricted as found with control soil+PAHs treatment. In the PAHs mix treatment, 11 PAHs out of 16 PAHs were transferred via skin, whereas only 4 PAHs (i.e., naphthalene, acenaphthylene, fluoranthene, and pyrene) found to be transferred in the control soil+PAHs treatment soon after spiking (0 day). Moreover, the concentrations of the PAHs that were detected in the receptor fluid were much lower in control soil+PAHs treatment (0 day) than PAHs mix. Figure 1 shows the concentration difference of acenaphthylene in receptor fluids in different treatments using chromatograms. Sorption of PAHs on to organic and inorganic constituents of soils may have largely contributed to lower the dermal absorption of PAHs in soil (Gardner et al., 1979; Karickhoff et al., 1979). Further, the sorption of high molecular weight PAHs on to soil organic C is high compared to low molecular weight PAHs, due to the relatively higher, K_{oc} values. This could be the reason that the PAHs with higher molecular weight (>202 g mol^{-1}) did not transfer to the receptor fluid in control soil+PAHs (0 day) treatment.

As the incubation time increases, the PAHs in the control soil+PAHs treatment became much less bioavailable, most likely because the sorption of PAHs on to soil constituents becomes more significant with time. Figure 6.2 shows the reduction in concentration of acenaphthylene in the receptor fluid of control soil+PAHs treatments as PAHs age in soil. In our experiment, acenaphthylene is the only PAH that appeared in the receptor fluid 3 days after spiking in the control soil+PAHs treatment. Acenaphthylene had the highest concentration (6.7 mg kg^{-1}) among the low molecular weight PAHs. A high dose, low molecular size, and relatively low K_{oc} and K_{ow} of acenaphthylene may collectively have contributed to this observation. At 12 days after spiking, no detectable levels of PAHs were found in the receptor fluid, indicating a significant aging effect of PAHs in soils with regard to dermal absorption. Similarly, Turkall et al. (2009)

reported that the total penetration (in the receptor fluid and accumulated in the skin) of benzo[a]pyrene reduced by about 56% when the PAHs were aged for 3 months in soil compared to the total penetration of benzo[a]pyrene immediately after addition to soil.

Laser scanning confocal fluorescence microscopy

This approach helps us to determine the depth of penetration and localization of PAHs in the skin. The wavelength setup we used resulted in a relatively higher background emission from dermis and lower background emission from the stratum corneum and epidermis (Figure 6.3); therefore, imaging was done separately for epidermis (including the stratum corneum) and dermis, having a different exposure time of the skin section for the excitation wavelength. This approach gave a high sensitivity for PAHs detection in the stratum corneum and a relatively lower detection sensitivity in the dermis. Also, only the high molecular weight PAHs were detected with adequate sensitivity as explained in the materials and methods section.

In all exposed skin samples, except the skin exposed to the control soil, the stratum corneum emitted blue fluorescence, indicating some accumulation of PAHs in the stratum corneum (Figure 6.4-6.7). The stratum corneum has extracellular lipid layers which can accumulate lipid soluble molecules. Since PAHs are lipophilic, especially high molecular weight PAHs that have a high K_{ow}; PAHs seems to accumulate in these lipids layers. Mixing biosolids with contaminated soil and aging of biosolids with soils for 30 days did not change the fluorescence intensity observed in the stratum corneum (Figures 6.4 and 6.5) suggesting that biosolids may not have changed the accumulation of PAHs in stratum corneum, significantly. Although biosolids may provide additional sorptive sites for PAHs, biosolids could increase dissolve organic C concentrations which could enhance the mobility of PAHs in soil, at least initially. Further, an increase of dissolved organic C could enhance the desorption of PAHs from

sorptive sites and induce biodegradation of PAHs in soil (Bengtsson and Zerhouni, 2003). Because of these complex and contrasting effects, we may not have seen any significant difference of dermal absorption in biosolids-added soil treatments compared to those not receiving biosolids. Furthermore, it is possible that some small dissolve organic molecules penetrated into the skin, and contributed to this fluorescence signal, since we cannot rule out the possibility of penetrating/accumulating small molecules of soil dissolved organic C in skin. We tested this hypothesis and a slight blue fluorescence signal was observed when organic matter rich top soil (soil organic C: 22 g kg^{-1}) and humic acid (Sigma Aldrich) was excited at 360nm. Fluorescence of humic and fulvic acid, when excited in the UV range of wavelengths, has been reported by Sierra et al. (2005). However, it is highly unlikely for large molecules derived from soil organic matter to penetrate into the skin, because of their high molecular weights.

In the control soil+PAHs treatment (0 and 12 days after spiking) fluorescence was observed both in stratum corneum and dermis (Figures 6.6 and 6.7) suggesting the presence of PAHs in both places. However, no PAHs were observed in the receptor fluid 12 days after spiking. This could be because the concentration of PAHs in the receptor fluid was below the detection limits of the GC-MS method we employed, while the detection limit of fluorescence microscope was sensitive enough to capture small amounts of PAHs.

Although PAHs accumulated in the skin could metabolize/degrade over time (Dankovic et al., 1989), there is a potential that at least a portion of PAHs accumulated in the stratum corneum systematically transfer to the deeper layers and circulate in the body as they could penetrated to the deeper layers eventually (OECD, 2000; Yourick et al 2004). Further research needs to be done to test the fate of accumulated PAHs in skin under complex matrixes like soil.

Conclusions

The *in vitro* static diffusion cell experiment suggested that the transfer of soil PAHs from the tested naturally-contaminated soil to humans via skin was insignificant. Soil matrix and aging of PAHs in soil clearly limited the dermal absorption of PAHs, suggesting the importance of using real samples when assessing the risk of dermal absorption of soil PAHs in field-contaminated materials. The potential for transfer/partitioning of high molecular weight PAHs in to the receptor fluid was lower than that of the low molecular weight PAHs, because of the large size and relatively high K_{ow} (i.e., high lipophilicity) of high molecular weight PAHs. The high molecular weight PAHs tend to accumulate in the lipids of the stratum corneum, and show low diffusion and partitioning in to the receptor fluid, due to their higher K_{ow}. The fluorescent microscopy study revealed that PAHs in contaminated soil penetrated the skin and accumulated in the stratum corneum, although PAHs concentration in the receptor fluid was insignificant. Further, research needs to be done to investigate the systematic diffusion and partition of the accumulated PAHs in the stratum corneum.

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Tables and Figures

Table 6.1 Concentration of 16 priority PAHs in contaminated and control soil samples

PAH	Molecular weight	Concentration of PAHs	
		Contaminated soil	Control
	g mol^{-1}	----------mg kg^{-1}----------	
Naphthalene	128	0.8	0.0
Acenaphthylene	152	6.7	0.5
Acenaphthene	153	0.1	0.0
Fluorene	165	0.3	0.1
Phenanthrene	178	2.1	0.3
Anthracene	178	3.2	0.4
Fluoranthene	202	11.0	0.9
Pyrene	202	15.0	1.1
Chrysene	228	15.0	0.9
Benz[a]anthracene	228	15.0	0.7
Benzo[b]fluoranthene	252	26.0	1.0
Benzo[k]fluoranthene	252	14.0	0.6
Benzo[a]pyrene	252	18.0	0.9
Indeno[1,2,3-cd]pyrene	276	13.0	0.7
Dibenz[a,h]anthracene	278	4.0	0.2
Benzo[g,h,i]perylene	276	11.0	0.7

Table 6.2 PAHs that transferred to the receptor fluid via skin in the *in vitro* steady fluid experiment

PAH	Molecular weight	Concentration of PAHs	PAHs mix	Control soil+PAHs			
				Day 0	Day 1	Day 3	Day 12
	g mol^{-1}	mg kg^{-1}					
Naphthalene	128	0.8	■	■	■		
Acenaphthylene	152	6.7	■	■	■	■	
Acenaphthene	153	0.1					
Fluorene	165	0.3					
Phenanthrene	178	2.1	■		■		
Anthracene	178	3.2	■				
Fluoranthene	202	11.0	■	■	■		
Pyrene	202	15.0	■	■	■		
Chrysene	228	15.0	■				
Benz[a]anthracene	228	15.0	■				
Benzo[b]fluoranthene	252	26.0	■				
Benzo[k]fluoranthene	252	14.0	■				
Benzo[a]pyrene	252	18.0	■				
Indeno[1,2,3-cd]pyrene	276	13.0					
Dibenz[a,h]anthracene	278	4.0					
Benzo[g,h,i]perylene	276	11.0					

Filled cells represent the transfer of PAHs to receptor fluid.

No detectable levels of PAHs were transferred to receptor fluid in the following treatments: Contaminated soil with 20% and 40% moisture, contaminated soil mixed with biosolids, Control soil.

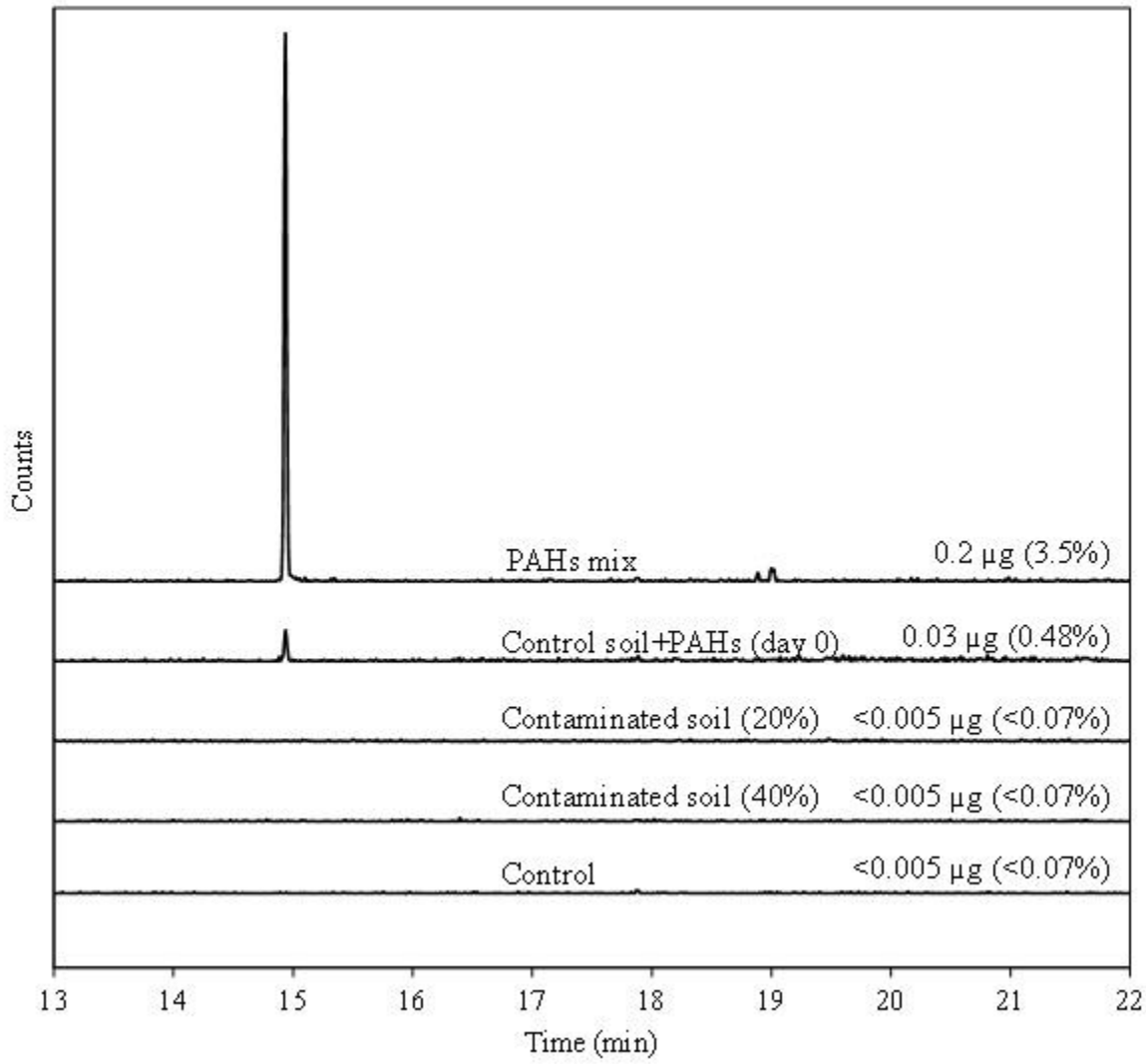

Figure 6.1 Chromatograms of acenaphthylene in receptor fluids. The concentration of acenaphthylene in all the treatments was 6.7 µg g^{-1}, except in control treatment which had 0.5 µg g^{-1} of acenaphthylene. The weight soil/volume of PAHs mix was 1 g/1 mL. The volume of the receptor fluid used in the *in vitro* static diffusion cell was 0.5mL. The microgram number explained the amount of acenaphthylene in 0.5 mL of receptor fluid. The percentage value explained the amount of acenaphthylene in 0.5 mL receptor fluid as a percentage of acenaphthylene in 1 g of soil

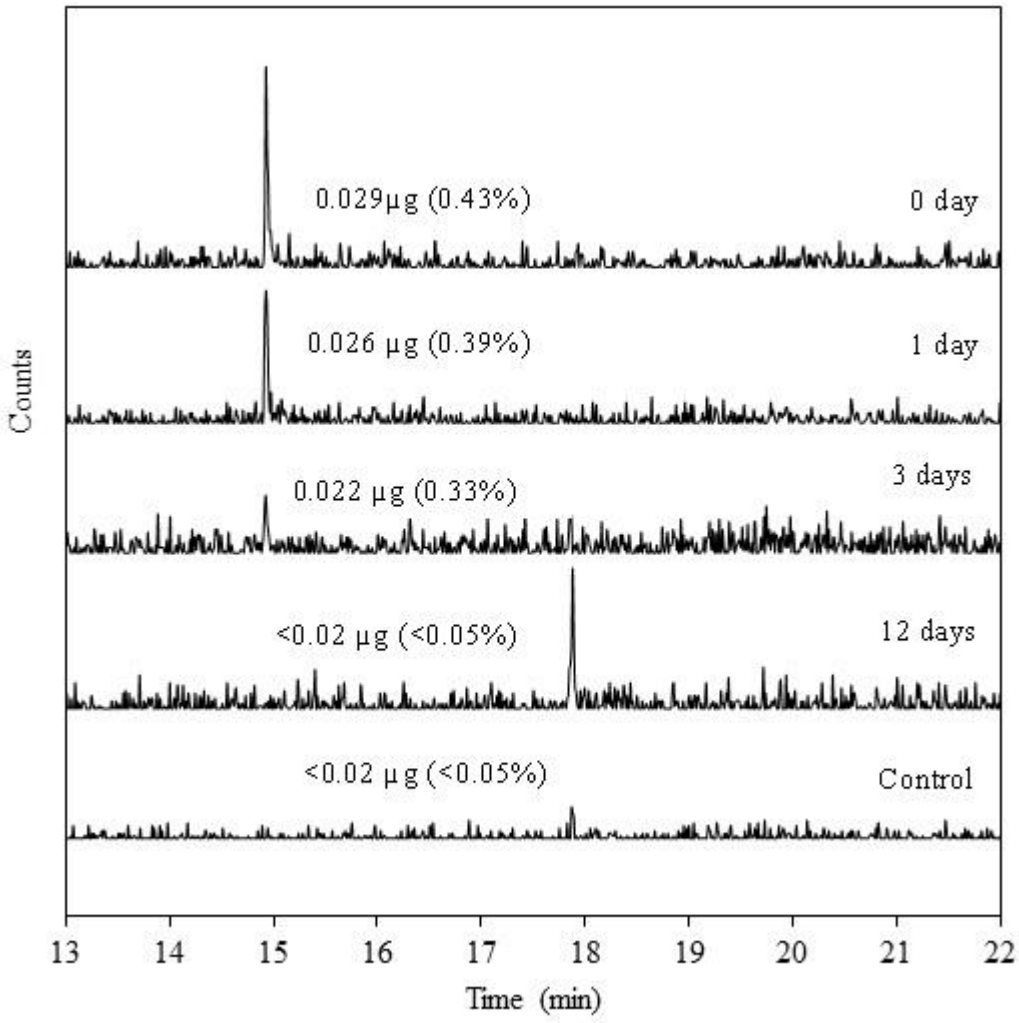

Figure 6.2 Chromatograms of acenaphthylene in receptor fluids of control soil+PAHs treatment 0, 1, 3, and 12 days of spiking. The concentration of acenaphthylene in the control soil+PAHs treatment was 6.7 µg g^{-1}. The weight of control soil+PAHs treatment and the volume of receptor fluid used in the *in vitro* static diffusion cells were 1 g and 0.5mL respectively. The microgram number explained the amount of acenaphthylene in 0.5 mL of receptor fluid. The percentage value explained the amount of acenaphthylene in 0.5 mL receptor fluid as a percentage of acenaphthylene in 1 g of soil

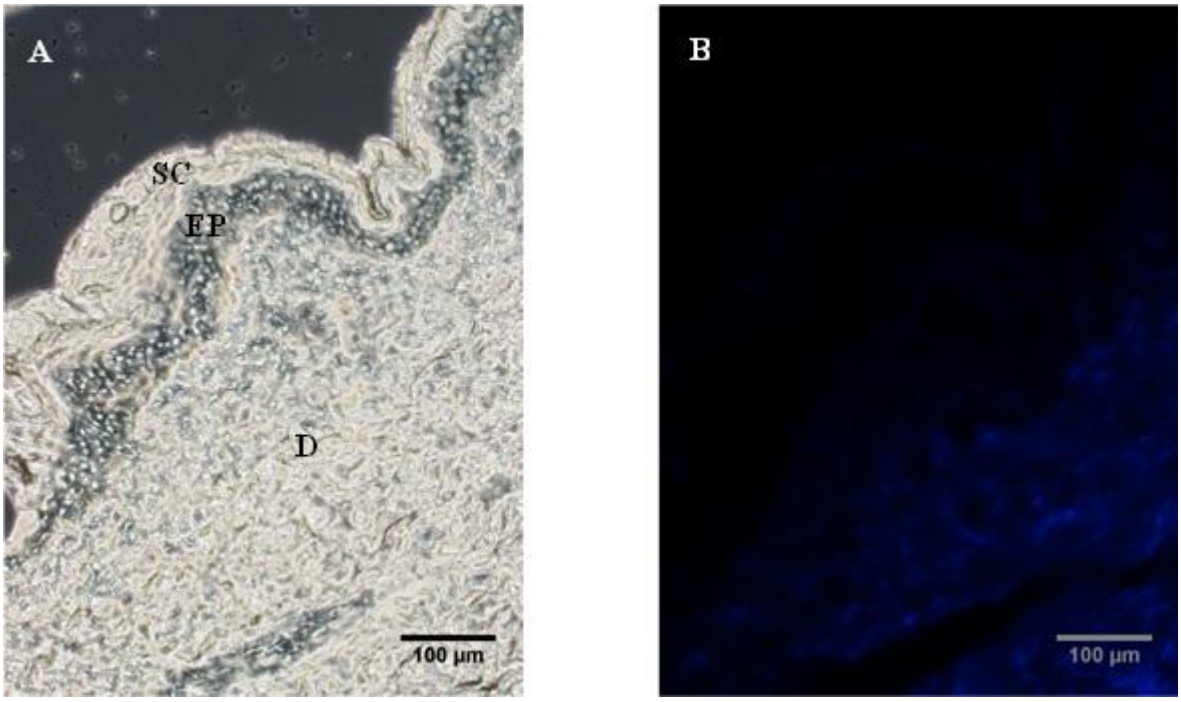

Figure 6.3 Untreated human skin used for the experiment. A: Transmission microscope image. B: Fluorescence microscope image. Excitation wave length was 360 nm. Emission wavelength range was 420 to 480 nm. SC: Stratum corneum, EP: Epidermis, D: Dermis

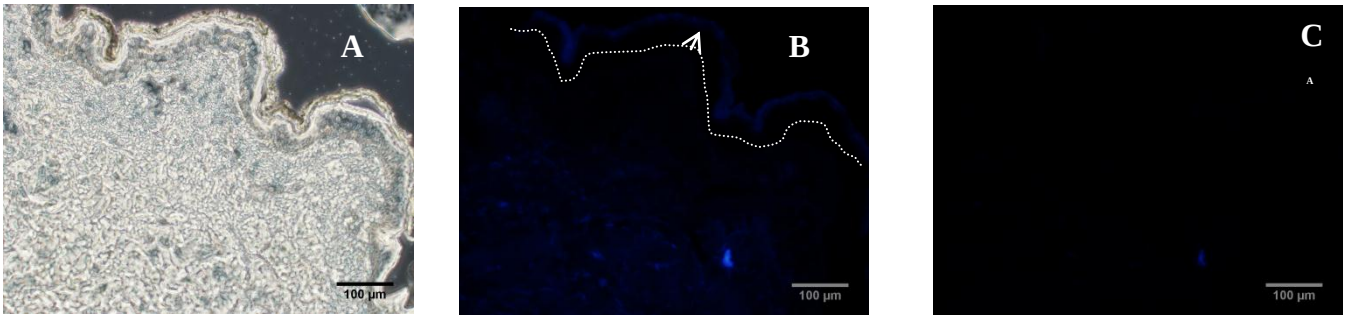

Figure 6.4 Microscope images of skins exposed to contaminated soil for 12 h. A: Transmission microscope image. B: Fluorescence microscope image generated to observe PAHs in the stratum corneum. Excitation wave length was 360 nm. Emission wavelength range was 420 to 480 nm. C: Fluorescence microscope image generated to observe PAHs in the dermis

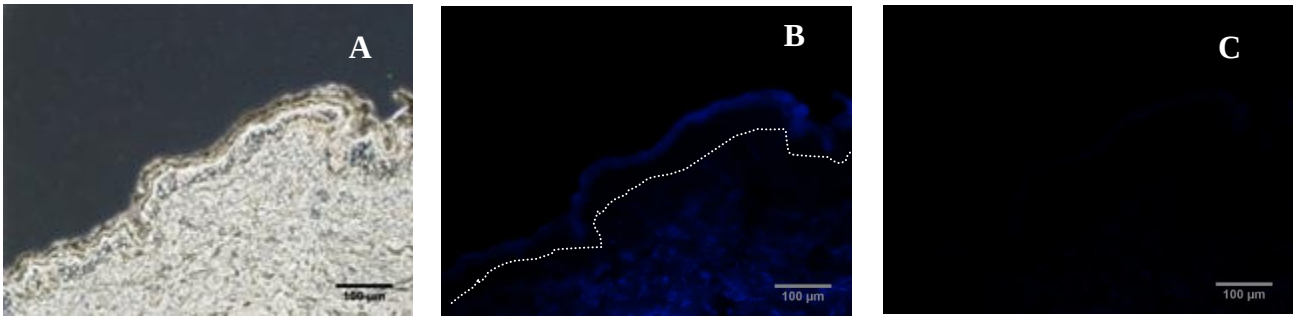

Figure 6.5 Microscope images of skins exposed to contaminated soil mixed with biosolids for 12 h. A: Transmission microscope image. B: Fluorescence microscope image generated to observe PAHs in the stratum corneum. Excitation wave length was 360 nm. Emission wavelength range was 420 to 480 nm. C: Fluorescence microscope image generated to observe PAHs in the dermis

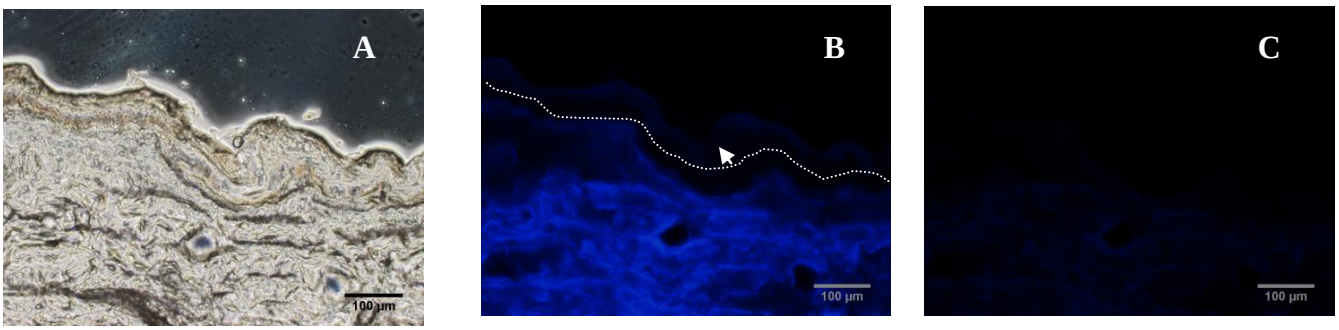

Figure 6.6 Microscope images of skins exposed to control soil+PAHs, 0 days after spiking treatment for 12 h. A: Transmission microscope image. B: Fluorescence microscope image generated to observe PAHs in the stratum corneum. Excitation wave length was 360 nm. Emission wavelength range was 420 to 480 nm. C: Fluorescence microscope image generated to observe PAHs in the dermis

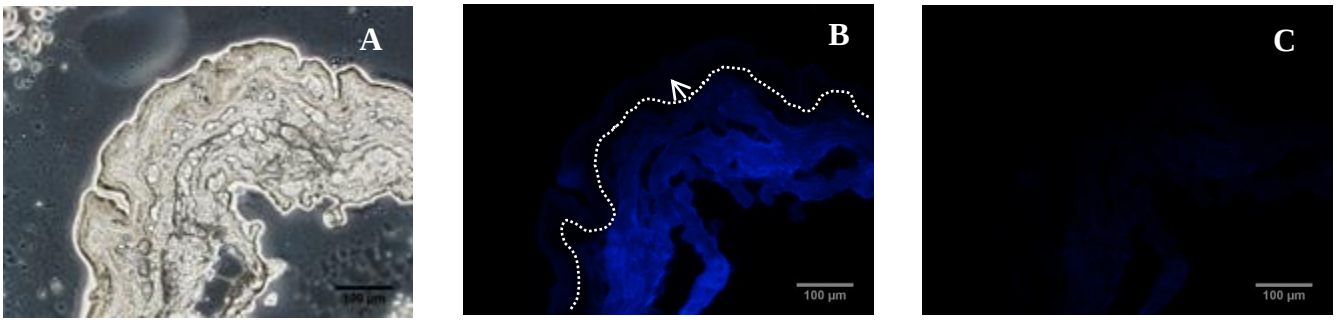

Figure 6.7 Microscope images of skins exposed to control soil+PAHs, 12 days after spiking treatment for 12 h. A: Transmission microscope image. B: Fluorescence microscope image generated to observe PAHs in the stratum corneum. Excitation wave length was 360 nm. Emission wavelength range was 420 to 480 nm. C: Fluorescence microscope image generated to observe PAHs in the dermis

Chapter 7 - Summary and conclusions

Deindustrialization of metropolitan areas has resulted in a large amount of vacant urban brownfields. Urban agriculture is gaining attention as a means of revitalization of brownfields in urban areas. Reuse opportunities for vacant lands include (1) urban agriculture/gardening that improves the availability of healthy, fresh foods will improve nutrition and health of residents and (2) creation of parks, playgrounds and other commons. Urban agriculture can present challenges because of the possibility of soil contamination. Three common contaminants in urban areas may include (1) lead from the past use of Pb paint and gasoline, (2) arsenic from As containing wood preservatives and pesticides, and (3) polycyclic aromatic hydrocarbons-a byproduct of burning materials such as coal, wood, and oil. The experimental components presented in this thesis conducted to gain a better understating of the plant and human bioavailability of three urban soil contaminants.

The field studies suggested that the risk of exposure for soil Pb, As and PAHs in urban soils via food chain transfer is minimal. Only root crops tend to accumulate potentially harmful concentrations of soil Pb. Therefore, growing root crops in Pb-contaminated urban soils is not recommended. Vegetables grown in urban gardens could be contaminated superficially with contaminated dust deposition on them. Food chain transfer of soil contaminants via vegetable consumption can be further reduced by cleaning vegetables thoroughly before consumption/preparation. The effect of compost addition on concentration of Pb and As in vegetables within the study period (2 years) seems to be mainly through improving soil fertility parameters such as plant nutrient concentrations in soil, soil structure and workability, and water holding capacity etc. Although the compost might have increased the binding capacity of soil Pb

and As, and change soil Pb and As speciation, they were not evident through vegetable Pb and As concentrations.

Bioaccessibility of soil Pb and As in the studied urban soils were low compared to the bioaccessibilies recorded in literature for highly contaminated mine impacted soils. Therefore, the risk of exposure to soil Pb and As via direct ingestion of urban soils contaminated with residential and industrial activities could be much lower than the mine impacted soils. Absolute bioaccessibility of soil Pb and As can be further reduced by adding compost, due to significant dilution of the soil matrix by compost addition. In general, compost addition does not seem to affect soil Pb and As bioaccessibility (as a percentage of total concentrations) significantly, mainly because of the inherent low soil Pb and As bioaccessibilities in tested urban soils.

X-ray absorption fine-structure spectroscopy study on soil Pb speciation revealed that the majority of soil Pb in the tested urban soils is in the sorbed forms: Pb sorbed to Fe oxy(hydr)oxides and organic C. Compost addition seems to increase the fraction of soil Pb sorbed to organic C over time. Several changes occurred for Pb speciation during the *in vitro* bioaccessibility extraction test: the fraction of Pb sorbed to organic C survived and/or increased, the fraction Pb sorbed to Fe oxy(hydr)oxides reduced, and the additional available P provided via compost induced the formation of hydroxypyromorphite during the *in vitro* bioaccessibility extraction test. The Pb speciation changes that occurred under extreme pH levels of the *in vitro* extraction/digestive system suggest the importance of validation the *in vitro* bioaccessibility method for its applicability for amended urban soils.

The dermal absorption of PAHs in naturally contaminated urban soils seems to be low, because of the effects of the soil matrix and aging of PAHs in soils. Stratum corneum of the human skin is capable of acting as a barrier for PAHs transfer via skin by accumulating majority

of PAHs in it. The comparison of dermal absorption of PAHs in simple solvent and soil suggested that the effects of the soil matrix and aging of PAHs in soil should be incorporated in accessing the risk of dermal transfer of soil PAHs.

Appendix A - Chapter 3

Figure A.1 Location of community garden in the Washington Wheatley neighborhood in Kansas City, Missouri.

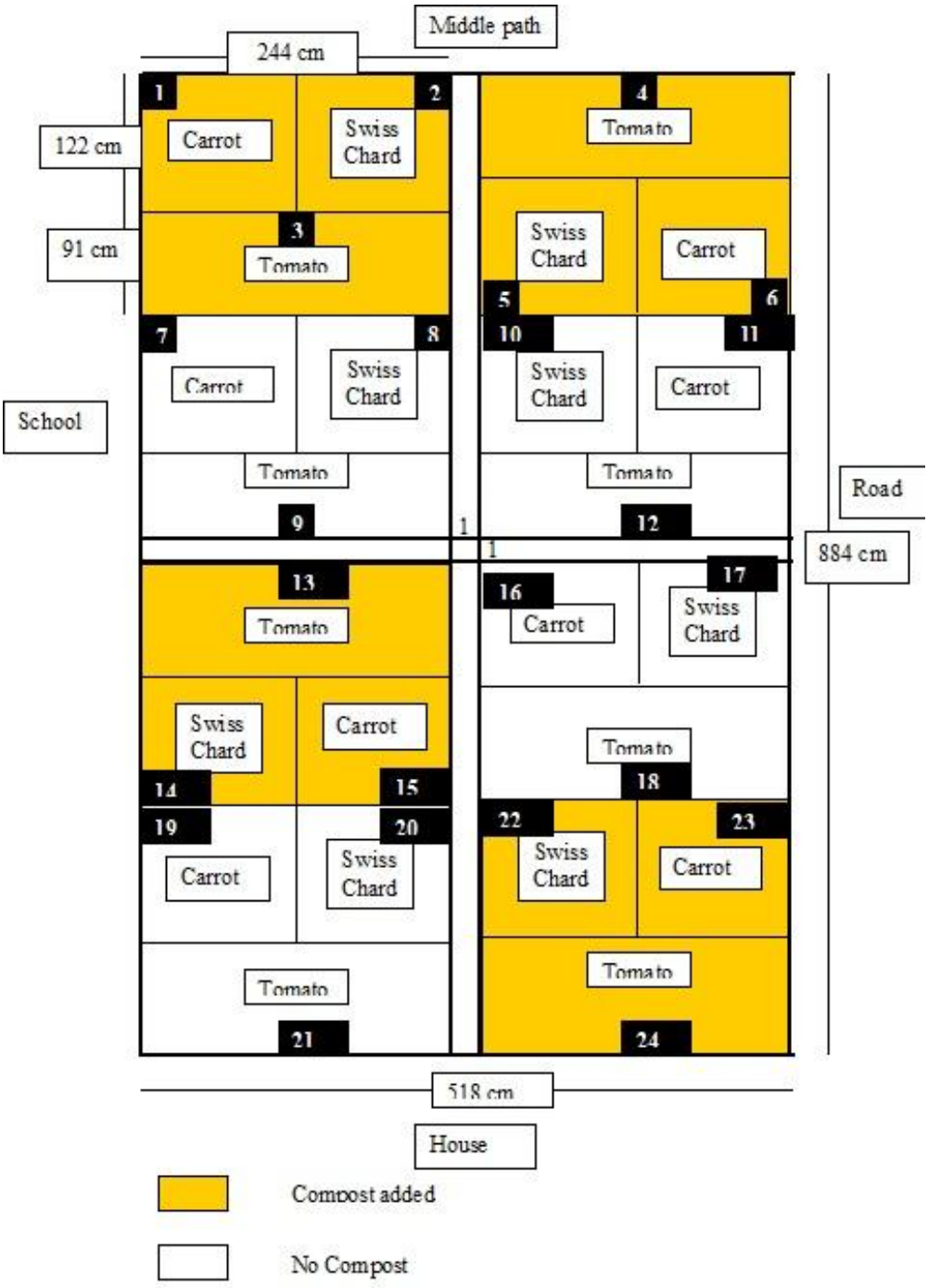

Figure A.2 Test plot arrangement in 2010.

Appendix B - Chapter 4

Figure B.1 Location of urban garden test site in Philadelphia.

Appendix C - Chapter 5

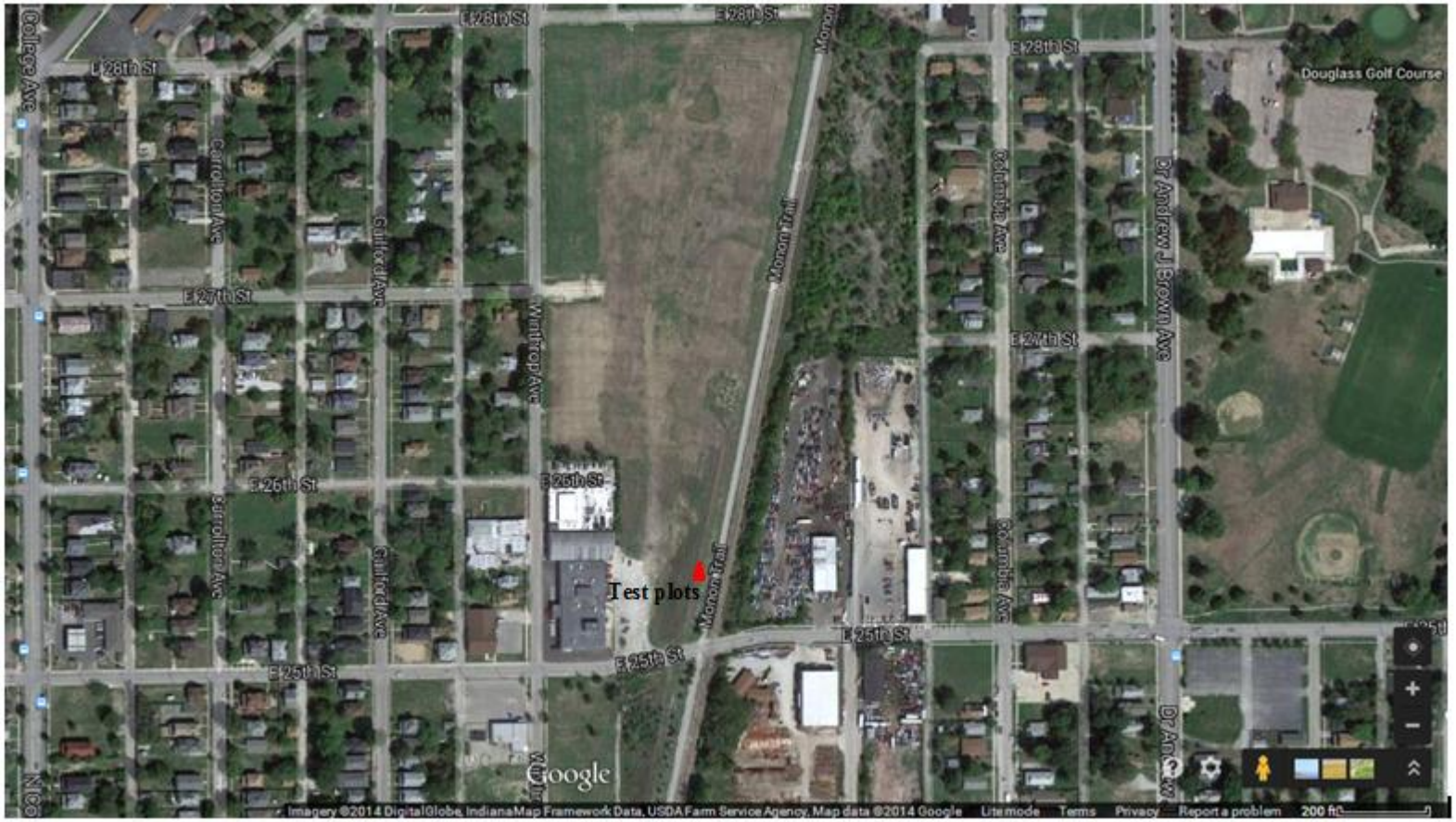

Figure C.1 Location of the Monon test plots in Indianapolis, IN.

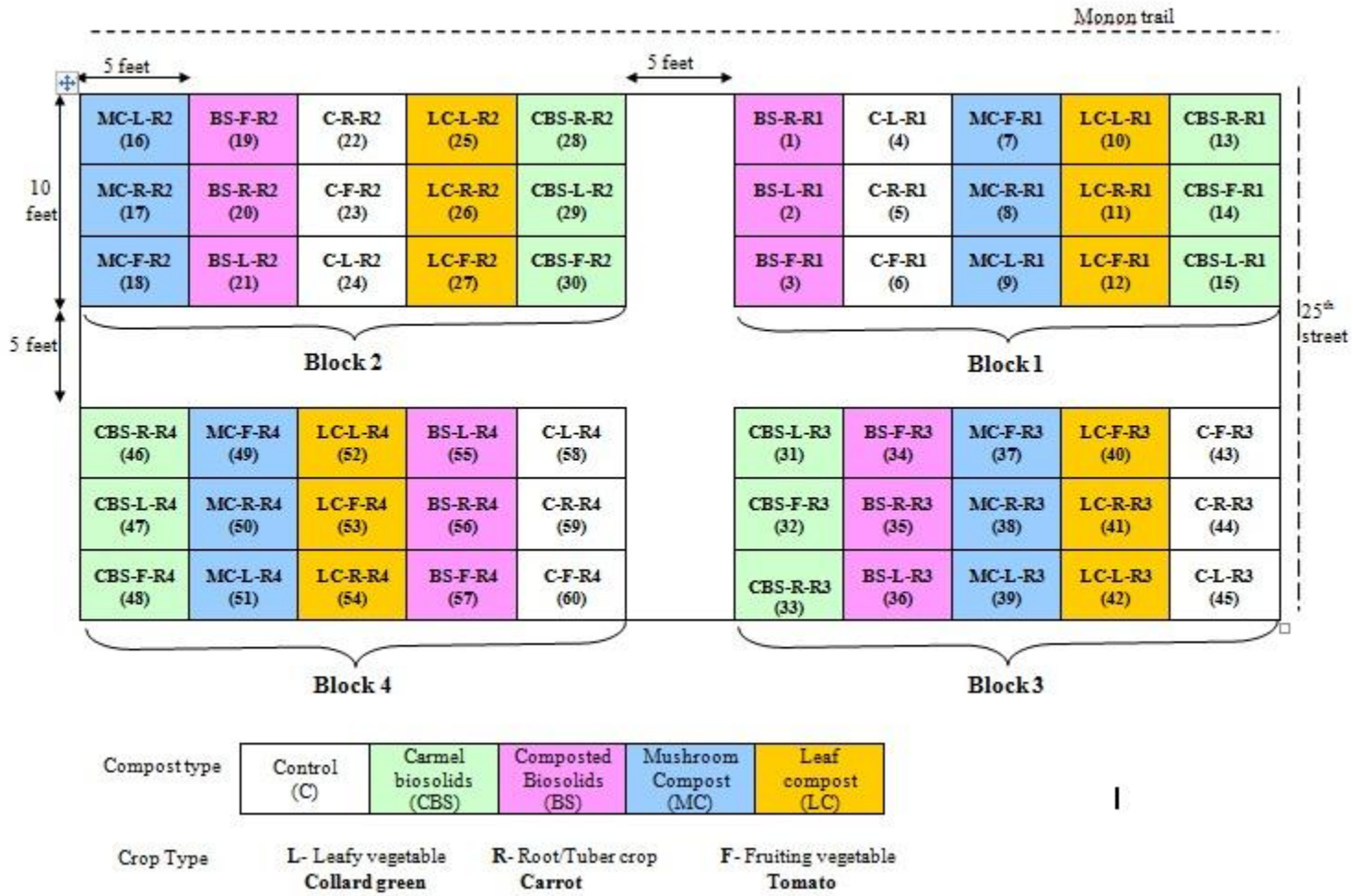

Figure C.2 Plot diagram of the Indianapolis test site.

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