ALTERNATIVE APPROACH TO ESTIMATING BIOACCESSIBLE LEAD IN SOIL USING NUTRIENT SOIL TESTS

By

TIPANUN UPANISAKORN

A REPORT PRESENTED TO THE GRADUATE SHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2016

TABLE OF CONTENTS

Page

LIST OF FIGURES

Figure **page**

LIST OF FIGURES

Figure **page**

Abstract

Lead is a toxic element well known to be associated with higher risk of neuro-developmental damage in children under 5 years old. Although the current standard soil lead reference levels are based on the total concentration, the amount of lead that is absorbed by the gastrointestinal tract and that becomes bioavailable is lower than the total soil concentration. Phosphorus additions can effectively reduce the bioavailability of soil Pb even more, but additions of excessive amounts of P pose environmental concerns. *In vitro* methods, which estimate Pb bioaccessibility, may help improve the current remediation methods. Soil tests have been shown to be an effective and accurate means of extracting bioaccessible Pb. *In vitro* methods, including soil tests, still require further validation for application in the context of various soil properties, diverse lead sources and wide range of lead concentrations. These expanded data will help support current remediation research and contribute to a new *in vitro* method that is cost effective and accurate. This paper aims to propose alternative methods to assess the extent to which Mehlich-3, Modified Morgan, and AB-DTPA accurately estimate bioaccessible Pb utilizing soils contaminated with different amounts and sources of Pb.

Introduction

Lead (Pb) is a natural element found in soils, surface water, and groundwater (Ryan et al., 2004). In rocks, Pb is concentrated in ore minerals such as galena (PbS), anglesite ($PbSO_4$) and cerussite ($PbCO_3$), but Pb is widely dispersed in other minerals, including silicates such as K-feldspar, plagioclase and mica, and other accessory minerals such as zircon and magnetite (Plant et al., 2012). Lead is released into the environment first through volcanic activity that occurs at the Earth's mantle and then by the rocks weathering which occurs at the Earth's crust (United Nations Environment Program (UNEP), 2010). Lead is naturally found in soils at concentrations between 50 and 400 mg/kg [United States Environmental Protection Agency (USEPA), 2014]. However, anthropogenic sources of lead have polluted many soils beyond these levels. Sources of anthropogenic Pb pollution include historical mining and smelting practices , past industrial or disposal activities, agrochemicals such as fertilizers and pesticides, sewage sludge applications, and coal fly ash disposal (Plant et al., 2012; UNEP, 2010). Urbanization of land from historical mining/smelting areas can increase human exposure to preexisting high concentration of Pb (1,000 mg/kg) in soil (Brown et al., 2016). Lead can cause damage to neurological function in children through the soil ingestion route. Children have higher rates of lead ingestion because they are more likely to play in the yard with soil and dirt, and put dirty hands or other objects in their mouths (The Centers for Disease Control and Prevention (CDC), 1991). Children in urban areas tend to have higher blood Pb levels than children in rural areas. Over the last 10 years, although the number of children aged 1-5 years with elevated blood Pb levels (BLLs) decreased only 9%, from 600,000 to 535,000 children (Brown et al., 2016; Wheeler and Brown, 2013).

Therefore, the high number of children with elevated BLLs is concerning. In many countries, soil Pb reference values are based on total concentration. However, the absorption of ingested soil Pb into the gastrointestinal tract can occur at the levels lower than the total Pb value. The precise evaluation of available Pb fraction in soil is required. To protect human health especially children living in areas with Pb-contaminated soils, this paper aims to suggest the accurate assessment bioaccessible Pb using inexpensive methods.

Lead contamination sources

The three most commonly identified sources of elevated soil lead concentrations are lead-based paint on exterior surfaces of old buildings, point source emitters such as smelters and Pb battery recycling sites, and lead mine tailings (Department of Toxic Substances Control (DTSC), 2004).

Lead can enter soil from chips and peelings of exterior lead-based paint from older buildings. Soil Pb concentrations can exceed 3,000 mg/kg (University of California Agricultural and Natural Resource (UCANR), 2010). Another example is the community garden in Rainier Valley District of Seattle, WA. The garden is located on an abandoned lot previously occupied by an old water tower that was coated with Pb-based paint. The tower was sand-blasted frequently, so paint chips and Pb residues resulted in the contamination of surface soils to concentrations of between 695-1,854 mg/kg (Defoe et al., 2014).

The Zn and Pb smelter operations in Joplin, MO (located in the Tri-State mining district) also have elevated Pb concentrations (2,892 mg Pb/kg) (Brown et al., 2004). Li et al. (2015) found Pb contamination in soil around a 60 year-old Pb/Zn smelter in a town in Yunnan Province of China at a maximally detected concentration of 2,485 mg/kg.

Mining of the Red Dog lead-zinc-silver deposit in Northwest Alaska, U.S. began in 1989. The process separates metal-bearing minerals (galena and sphalerite) from other minerals in the metal-bearing host rock (Alaska Department of Environmental Conservation (ADEC), 2001). The concentrations of Pb in surface soils sampled along roads, mines, and the port facility around the Red Dog deposits ranged from 4,600 to 5,240 mg/kg (Kelley and Hudson, 2007). A wetland soil along the northwest shores of Bull Run Lake near the Coeur d'Alene (CdA) mining district in northern Idaho was contaminated with 4,500 mg/kg of Pb resulting from mining operations and mine tailings (Strawn et al., 2007). Mine tailings in a sedimentation basin in Tar Creek, Oklahoma, a remedial site in the US Environmental Protection Agency (EPA) Superfund Program, had a Pb concentration of approximately 4,000 mg/kg. A major source of this Pb was coarse material contaminated with Pb that was subsequently used for fill in home gardens and driveways (Brown et al., 2007).

Lead-zinc ores, galena (PbS) and sphalerite (ZnS) are the primary ores found in western Thailand. Secondary minerals also found in the region include cerussite ($PbCO_3$) and smithsonite ($ZnCO_3$) [Economic and Social Commission for Asia and the Pacific (United Nation ESCAP), 2001]. Indiscriminate disposal of mine tailings has resulted in widespread lead poisoning in western Thailand since the early 1980s. One large contaminated area is near a national park where the Lead Concentrate Company built a Pb mining plant that lacked an efficient treatment system and resulted in the pollution of lead in areas adjacent Klity Creek (Assavarak, 2011). Total Pb concentrations in water over the period of 1988-2009 varied from 0.55 mg/l to 0.90 mg/l, but increased to 3.65 mg/l in 2012 as a result of flooding, which suspended sediment lead into water. Total Pb in sediment decreased from ~143,000 mg/kg in 2004 to ~ 83,000 mg/kg in 2012. Total Pb in fish from the creek in 2012 (0.08 mg/kg) decreased from that measured in 1988 (81 mg/kg). Total Pb in shrimp also decreased from 130 mg/kg to 6 mg/kg and in crabs from 450 mg/kg to 30 mg/kg [Pollution Control Department Thailand (PCD), 2013]. The dramatic decreases of Pb in sediment, fish, shrimp and crabs resulted from remediation via excavation of sediment from the creek. However, financial constraints caused the termination of these remediation efforts.

The Thai Ministry of Public Health set the maximally allowable Pb concentrations in food at ≤ 1 mg/kg to coincide with values set by the United States Department of Agriculture (USDA, 2010). The standard for surface water is ≤ 0.05 mg/L, and the standard for soil is ≤ 400 mg/kg (PCD, 2015a; PCD, 2015b). Thus, high concentrations of Pb in water, shrimp, crabs and soil around Klity Creek continued to concern villagers and Ministry of Public Health officials. Sukkayanagit and Panicha-pat (2013) studied the phytoremediation of Pb contaminated soil (1,400 mg/kg) in Klity using sunflower and sorghum for 105 days. The authors measured bioconcentration factors (BCF), defined as the ratio of Pb concentration in plant tissue to soil, of less than 1, suggesting ineffective phytoredemediation. The BCF values of sunflower and sorghum were 0.1 and 0.06, respectively, which similarly suggested unsuccessful phytoremediation using these plants in highly Pb-contaminated soils. Some phytoremediation applications are limited to depths that are within the reach of plant roots (UNEP, 2002). Such a high Pb concentrations in soil still concerns Klity villagers, and the status of bioavailable Pb in this area remains unknown.

Lead health effects

Lead can have numerous negative effects on human health, the most well-known being neurotoxic and neurodevelopmental effects (DTSC, 2004). Neurodevelopmental effects in children, even at low levels of exposure, represent the most critical effect. Other adverse effects include neurological, cardiovascular, renal, gastrointestinal, hematological and reproductive complications (UNEP, 2010). Children can absorb up to 50% of ingested Pb through the gastrointestinal tract, whereas adults can only absorb ~10% via similar mechanisms [World Health Organization (WHO), 2010]. The ingestion of soil and household dust also accounts for high BLLs in children (Minca et al., 2013b). Lead dust covers surfaces and objects that children touch and clings to their hands and toys. Children ingest Pb dust when they put their hands or toys in their mouths; normal behavior for all young children (UNEP, 2010). Children who live in Pb contaminated areas, where soils exceed 500–1,000 mg Pb/kg, often have increased BLLs (Chaney et al., 1989). Lead also causes long-term harm in adults. Around 94% of the total body burden of Pb is accumulated in the bones of human adults, compared to only 75 % in children (Plant et al., 2012). Lead stored in a mother's bones can be released into circulation under the metabolic stress of pregnancy and thus impact the unborn fetus. Throughout pregnancy, Pb readily crosses from the maternal to the infant circulation system, and the blood Pb concentration of the infant becomes virtually identical to that of the mother (Lamadrid-Figueroa et al., 2006). The source of Pb in an infant's blood seems to be a mixture of about two thirds dietary and one third skeletal lead (WHO, 2010).

Lead reduces the binding of proteins in DNA, which alters gene expression. Lead may also damage DNA by producing reactive oxygen species also known as free radicals (Silbergeld et al., 2000). Lead is a suspected human carcinogen. The International Agency for Research on Cancer, IARC (2006), classifies both Pb and inorganic Pb compounds as Group B2 carcinogens: 'probable human carcinogen'. CDC has developed a strategy for monitoring blood Pb level on human health. In 1991, CDC defined a blood Pb concentration of 10 μg/dL in children as the level that triggers the need for community prevention activities, education, and follow-up (CDC, 1991). To protect human health, USEPA set the maximum total Pb concentration in residential bare soil used for children's play at 400 mg Pb/kg (and at 1200 mg/kg total Pb for bare soil anywhere else in the yard) as a soil screening level (SSL) (United States Agency for Toxicology of Substances and Disease Registry (U.S. ATSDR), 2012). In 2010, the Thai Ministry of Public Health tested the level of lead in the Klity Creek region people and found that 36.3% of children and 7.7% of adult still have BLLs more than 25 μg/dL (Thaipublica, 2014). Recently, the CDC lowered the definition of elevated BLLs in children 1–5 years old to 5 μg/dL (Henry et al., 2015). As a result, regulatory policymakers may lower residential soil screening levels (SSL) reference values. The new reference value will impact research in an effort to reduce Pb exposure in children. Such, more data and research on assessment of bioavailable Pb are urgently needed in the context of lowering BLLs.

Assessment of lead contamination

Various soil tests to assess the potential human health risk of Pb in urban soils have been designed to measure most of the total Pb content of soil. The most commonly used methods involve partial dissolution of soil using strong acids and/or pressureassisted microwave acid digestion such as USEPA Method 3051a (USEPA, 2007b) to yield “total Pb.” However, the amount of ingested Pb due to soil contamination Pb that becomes available and absorbed into the blood (i.e., bioavailable Pb) is less than the total content of Pb in the soil (Minca et al., 2013a). Bioavailable Pb is ingested Pb that is absorbed through the gastrointestinal system and becomes available to internal tissues and organs (USEPA, 2007a). Information on bioavailable Pb in soil has more important health implications than the total soil Pb concentration. Thus, an accurate assessment of Pb hazard requires an accurate assessment of Pb bioavailability.

Recently, many studies have been focused on the estimation of bioavailable Pb using *in vitro* methods to overcome cost and ethical issues associated with *in vivo* methods. For example, trials using animals for measurements of soil Pb bioavailability (*in vivo* studies) are expensive (about $30,000 per test soil with swine) and time consuming (Casteel et al., 2006). An *in vitro* method is an alternative way to estimate the bioavailability of metals by simulating processes occurring in the gastrointestinal tract (Kelley et al., 2002). The estimated values obtained from the *in vitro* approaches are called *in vitro* bioaccesible (IVBA) soil Pb. The IVBA can be used to predict the relative bioavailability (RBA) of Pb using regression analysis of *in vitro* methods and corresponding data obtained from *in vivo* studies. Several *in vitro* methods have been developed to predict the bioaccessibility of Pb in soil (Scheckel et al., 2009; Van de Wiele et al., 2007; Oomen et al., 2003; Ruby et al., 1999).

There are two common approaches for *in vitro* extractions to determine bioaccessible Pb. The physiologically based extraction tests, (PBETs)-based methodologies, employ components such as simulated gastric solution at 37 °C, stomach and intestinal pH, solid-to-solution ratio, and mixing to mimic conditions of the fasting human digestive tract (Hooda, 2010). A recent study by Liang et al. (2016) used PBET method to extract bioaccessible Pb. They spiked three soils with Pb at 150 and 1,500 mg/kg and incubated under laboratory condition for 76 weeks. The result show that bioaccessible Pb decreased with aging. The approach of other bioaccessibility extraction methods is not to mimic the human GI tract, but to solubilize levels of trace elements using "simple" solutions that correlate well with the levels of bioavailable elements in the soil. This approach uses the measured bioaccessibility to estimate bioavailability using mathematical relationships collected from *in vivo* studies (Zia et al., 2011). In theory, if a mathematical relationship exists between *in vitro* and *in vivo* trial results, the composition of fluid extraction of the *in vitro* test does not necessarily need to be a close mimic of the GI tract (Smith et al., 2010).

Drexler and Brattin (2007) introduced a modified *in vitro* procedure for the estimation of soil IVBA-Pb that correlated well with the results of bioavailability tests using swine fed mine wastes and smelter contaminated soils. The Relative Bioaccessibility Leaching Procedure (RBALP) uses a simple acidic solution (0.4 M glycine HCl adjusted to pH 1.5) to solubilize contaminants from the soil. The RBALP measurements correlated strongly with *in vivo* relative bioavailability (RBA) values ($r^2 = 0.924$, $p < 0.0001$), with an average absolute error of 10% and an average predictive error of 20%. Thus, the RBALP was deemed effective in providing reliable estimates of lead RBA as predicted by the immature swine model. However, the soil IVBA-Pb can vary depending on the pH and extraction solution conditions. USEPA (2007c) estimated IVBA-Pb using 24 different test materials by performing the *in vitro* method using 0.4 M glycine at pH 1.5, 2.5 and 3.5.

The greatest Pb extraction occurred at pH 1.5. Similarly, Juhasz et al. (2013) estimated IVBA-Pb in soils from a former domestic incinerator waste disposal site and residential land developed on quarry fill material using 5 *in vitro* methods (RBALP, IVG, PBET and 2 modified Unified BARGE (UBM)). The predicted IVBA-Pb values were overestimated when using the RBALP at a pH 1.5 compared to other methods. Lead compounds tend to be more soluble in low pH conditions compared to neutral conditions (Hettiarachchi and Pierzynski, 2004). Recently, the USEPA (2013) released USEPA Method 1340, a standard method for estimating IVBA-Pb in soil. Method 1340 uses 0.4 M glycine and HCl adjusted to pH 1.5. Obrycki et al. (2016) estimated IVBA-Pb using USEPA Method 1340 and the three modified versions of the test (solutions with pH 1.5 and 2.5 and the solution with and without 0.4 M glycine). A historic garden area and urban vacant city lot soils which contained 790-1300 mg/kg Pb were amended with six different phosphate amendments and incubated for 1 year. The amended treatments in both sample areas showed no reduction in IVBA-Pb using 0.4 M glycine at pH 1.5 extraction (USEPA Method 1340). The P treatment tended to lower IVBA-Pb in the pH 2.5 extraction more than pH 1.5. The pH 2.5 without glycine extraction solution yielded the greatest reduction in IVBA-Pb (from 5 to 26%). Obrycki et al. (2016) suggested that a modified USEPA Method 1340 without glycine at pH 2.5 might be a good *in vitro* method to estimate bioaccessible Pb in soil. However, the P treatments showed different results in IVBA-Pb reduction with reference to the different contaminants found in the soil sources. The largest IVBA-Pb reduction was 26% using mono ammonium phosphate at pH 2.5 with glycine extraction on the city lot soil, but not on the garden soil. More data on different soil types, variously amended, are urgently needed.

The relative bioavailability of Pb in contaminated soils can vary with the different physical and chemical forms of Pb, as well as with particle size (Smith et al., 2011). Most studies of bioaccessible soil elements focus on the fine fraction (<250 mm) because that is the size of particles that adheres most readily to children's hands (Zia et al., 2011). However, Morman et al. (2009) collected 20 uncontaminated 'background' soils along north-south and east-west transects across the USA and Canada to evaluate the bioaccessibility of some trace metals. Two separate size fractions, <2 mm and <250 μm, were evaluated to determine if there were significant differences between the extractions following the Drexler and Brattin (2007) procedure. Results for the <2-mm size fraction were not substantially different from the <250-mm size fraction for measures of bioaccessible As, Cd, Cr, Ni and Pb.

Remediation of lead-contaminated soils

To reduce the Pb exposure, researchers have explored *in situ* soil treatments to remediate Pb using various materials, such as biosolids and compost (Chen et al., 2000; Brown et al., 2003; Brown et al., 2004), amorphous iron and manganese oxides (Chen et al., 2000; Garcia et al., 2004), and phosphate (Ryan et al., 2004; Brown et al., 2004; Brown et al., 2005) to reduce the phyto- and bioavailability of soil Pb. Adding compost to Pb contaminated soil reduced the soil total Pb by dilution (Attanayake et al., 2014). Leaf compost added to urban garden soil containing 60 to 300 mg/kg total soil Pb in 2009 and 2010 was diluted by 29 - 52%. The bioaccessible Pb values extracted using *in vitro* method (PBET) at pH 1.5 and 2.5 were not influenced by compost addition. Addition of high-Fe biosolids compost amendment to Pb arsenical pesticide contaminated orchard soil significantly diluted total Pb from 1700 to 1000 mg/kg, but had no impact on bioaccessible Pb (Brown et al., 2012). However, the organic amendments decompose over time, so their effectiveness in long term settings raises some concerns. Phosphorus addition is the most commonly and widely accepted technique to immobilize Pb in soil (Miretzky and Cirelli, 2009) because it can chemically change the lead-form in soil. Various forms of Pb exist in contaminated soils and the soluble species tend to be more bioavailable than insoluble species (Beak et al., 2008). The sorbed Pb [Pb associated with clay mineral, hydrous oxides (Fe and Al oxides) and organic matter] is a common form of Pb in soil (Minca et al., 2013b; Juhasz et al., 2014; Obrycki et al., 2016). Cerrusite and anglesite are highly soluble Pb minerals that may impact the bioavailability in soil (Brown et al., 2012). Recently, Liang et al. (2016) found carbonate-bound fraction and bioaccessible Pb were significantly correlated ($r^2 = 0.95$ and 0.97, $p < 0.05$) in the gastric phase before and after 76-week of aging soil. Similarly, the exchangeable fraction and bioaccessible Pb were significantly correlated ($r^2 = 0.75$ and 0.91, $p < 0.05$) in the intestinal phase. These findings imply that the major sources of bioaccessible Pb are the carbonate and exchangeable Pb fractions. Cerrussite has been identified in Pb contaminated soils from an abandoned Pb/Zn smelter and an orchard where Pb arsenicals were used (Baker et al., 2014; Brown et al., 2012). Phosphate amendments added to contaminated soils reduce the Pb mobility by precipitation of pyromorphite-type minerals [$Pb_5 (PO_4)_3X$; X = F, Cl, B or OH]. The newly formed minerals have very low solubility and bioaccessibility (Kumpiene et al., 2008). Fluoropyromorphite [$Pb_{10}(PO_4)_6F_2$] was formed in soil after amended with phosphate rock containing F (Cao et al., 2004). However, the dissolution of Pb and P in soil impact the rate and effectiveness of Pb-immobilization. Soil pH plays important role on the formation of pyromorphite. Lead-carbonate is more stable at higher soil pH (pH=7), leading to the greater presence of $PbCO_3$. Acidic conditions (pH<5) favor chloropyromorphite formation (Cao et al., 2008).

Different P sources with different solubilities may impact the effectiveness of Pb immobilization. Hettiarachchi et al. (2000) used P to stabilize Pb in five metal-contaminated soils and mine spoils. About 5,000 mg P/kg as triple superphosphate (TSP) or phosphate rock (PR) were applied to the soils. Reductions in bioavailable Pb in stomach phase extractions upon addition of P ranged from 15 - 41% compared to non-amended soil. The phosphorus amendments (including 1% P-TSP, 3.2% P-TSP, 1% Phosphate rock and 1% P-H_3PO_4) were also added to Pb contaminated soil from Zn and Pb smelter in Joplin, MO. All treatments tested in the laboratory significantly reduced bioaccessibility of Pb in soil (Brown et al., 2004). Recently, very highly Pb-contaminated soils (24,300 mg/kg) from abandoned Pb/Zn smelter regions near Dearing, Kansas were treated with liquid and solid P sources and incubated for either 4 or 52 weeks (Baker et el., 2014). Both liquid and soil forms of P can induce Pb phosphate formation and reduce Pb bioavailability in soil. Phosphoric acid (liquid) was the most effective material in inducing formation of Pb phosphate with respect to time and distance of P application. However, soluble sources could increase P leaching and runoff (Baker et al., 2014). Li et al. (2014) enhanced the effectiveness of P amendment of soil by adding different amounts of calcium. The soils were spiked with 1000 mg/kg Pb, incubated for 50 days in the laboratory and available Pb in soil was extracted using DTPA extraction solution. The DTPA-Pb concentrations of P-amended soils decreased as the Ca concentration increased. The addition of soluble Ca to P-amended treatments apparently enhances pyromorphite formation, which can increase the amount of Pb released from the exchange site to compete with Ca-phosphate mineral formation. Thus, available Pb could react with soluble P and form pyromorphite more efficiently. (Li et al., 2014).

The amount of amendment used is a key factor controlling remediation efficiency. The amount of added material is usually expressed as a percent of amendment added to soil (weight ratio), as a concentration of P per kg soil or ton per ha, or as a molar ratio of P/Pb (Kumpiene et al., 2008). The P amendment rate of 1% (w/w) is commonly used for remediating soils in many studies - regardless of total Pb concentration - and typically results in significant reduction of extractable Pb (Brown et al. (2004); Strawn et al. (2007); Geebelen et al. (2003); Ryan et al. (2004)). The application of hydroxyapatite-P to aqueous Pb or Pb-contaminated soil at a P/Pb molar ratio of 3:5 has been suggested for transforming soil Pb into pyromorphite based on theoretical yields (Ma et al., 1993). However, soluble P may not only react with insoluble Pb, but also with other cations to form other minerals, e.g. apatite and manganese hydrogen phosphate ($MnHPO_4$) in the field. Thus, Melamed et al. (2003) suggested increasing the amount of added P to yield a molar ratio of 4.0 P/Pb. Juhasz et al. (2013) used phosphoric acid and rock phosphate to amend soil Pb at a P/Pb molar ratio of 5:1 in soils from 3 different sources; smelter (1700 mg/kg Pb), nonferrous slag (600 mg/kg Pb), and shooting range (8700 mg/kg Pb). The bioavailable Pb, evaluated in an *in vivo* study using adult male mice, decreased in all treated soils. Soils from wheat field were spiked with Pb (1000 mg/kg) and then amended with calcium and/or potassium phosphate at a P/Pb molar ratio of 5:1. Available Pb was estimated using the DTPA solution, and decreased in all P addition treatments (Li et al., 2014). Recently, Obrycki et al. (2016) used six different phosphate amendments at four different P/Pb ratios (5:1, 8:1, 17:1 and 30:1). The reduction of IVBA-Pb in garden soil differed from that of the city lot samples and also varied depending on the *in vitro* method used. Adding large amounts of P, at P:Pb ratios of 17:1 and 30:1, tended to reduce IVBA-Pb more than at lower ratios of 5:1 and 8:1 in both soils at a pH of 2.5 using the modified USEPA Method 1340. Obrycki et al. (2016) hypothesized that soil amended with higher P:Pb molar ratios result in more Pb-phosphate formation. However, consistently higher P:Pb molar ratios treatments did not show consistent Pb-phosphate formation (P=0.136) due to several factors, such as incubation pH, extraction solution pH, and soil mineralogy. The Pb-phosphate formation could not be explained solely by the P:Pb molar ratio. Large additions of P, however, can lead to soil P enrichment and concerns about P leaching to adjacent water bodies. Phosphorus leaching can increase the fertility status of natural waters (eutrophication), and accelerate the growth of algae and other aquatic plants. Therefore, P amendment needs to be carefully used at an appropriate rate.

Soil tests

Many studies have reported correlations between soil test extractable Pb concentrations and total soil concentrations of Pb. The diethylenetriaminepentaacetic acid (DTPA) soil test was first developed and published by Lindsay and Norvell (1978) for characterizing the micronutrient status of neutral and calcareous soils. The ammonium bicarbonate-DTPA (AB-DTPA) was subsequently developed by Soltanpour and Schwab (1977) to simultaneously extract macro-, and micro-nutrients in alkaline soils. Barbarick and Workman (1987) used AB-DTPA to extract trace elements in metal-contaminated soils that were significantly correlated with concentrations of Pb in Swiss chard (*Beta vulgaris L.*) grain (r = 0.83) grown on soils amended with sewage sludge. Similarly, Elrashidi et al. (2003) found that the AB-DTPA extractable Pb and total Pb were significantly correlated in 30 acidic (r=0.995) and 20 alkaline (r=0.745) U.S. soils obtained from 21 states. The authors suggested that AB-DTPA extraction can be used for estimation of phytoavailable Pb in both acid and calcareous soils. Recently, DTPA was used as extraction solution to determine available Pb in Pb-spiked soil (1000 mg/kg) from wheat fields by Zhengzhou (Li et al., 2014), and was shown to have potential as a good estimate of bioavailable Pb.

The Mehlich-3 extract, developed by Adolf Mehlich in North Carolina (Mehlich, 1984), is a well-known and widely used as a soil test by laboratories across the United States to assess the nutrient status of soils. It is effective across a wide range of pH values (Summer, 1999). Moreover, the Mehlich-3 soil test can be useful as a screening tool to estimate total Pb. Figure 1 shows the correlation of Mehlich 3-extractable Pb to total Pb, for 80 soils; the overall r^2 value is 0.869. The correlation for 35 agricultural soils (r^2 = 0.894) was slightly better than that for 45 urban soils (r^2 = 0.844) (Wharton et al., 2012). The Mehlich-3 soil test was also useful for assessing bioaccessible Pb. Figure 2A shows that Mehlich-3–extractable Pb was strongly correlated (r^2 =0.975) with bioaccessible Pb as measured with the standard RBALP method at pH 1.5. Ryan et al. (2004) found the greatest reduction in bioaccessible Pb for soils treated with phosphoric acid at pH 2.5. Therefore, Minca et al. (2013b) used the modified RBALP at pH 2.5 in their study because Pb-phosphate minerals have been identified in residential soils from various natural phosphate sources and such soil samples might have previously received soil P amendments. Mehlich-3 was strongly correlated with bioaccessible Pb (r^2 = 0.938) at pH 2.5 (Figure 2B).

Acidic soils predominate in the Northeast United States (Bruulsema, 2006). The Modified Morgan (MM) soil test was developed as a routine extractant for soils in New England. The extractant is currently used by soil testing labs at the University of Maine, the University of Connecticut ,and the University of Vermont to assess overall acid soil nutrient status and to estimate Pb in soil (Hamel et al. 2003). McBride et al. (2011) compared three simple extractions (including 0.1 M sodium citrate (pH 6), the Modified Morgan solution (1 M ammonium acetate at pH 4.8), and 1 M nitric acid (HNO_3) in 32 soils from urban garden, sewage sludge amended soils and industrial-site soils contaminated with Pb. The MM soil test yielded a strong correlation ($r^2 = 0.84$) between soil test Pb and total Pb in soils ranging in Pb concentration between 10 to 1000 mg/kg (Figure 3). Recently, Minca (2012) found that MM results were strongly correlated with total and bioaccessible Pb, as measured with the standard RBALP at pH 1.5 (r^2= 0.848, Figure 4) and pH 2.5 (r^2=0.904, Figure 5), in 65 urban residential vacant lots (total Pb in soil ~14 to 1200 mg/kg in the < 2 mm soil fraction) in Cleveland, OH. The success of the MM soil test in more highly Pb contaminated soils is unknown.

Future Research

The information discussed above shows that new and improved validation for *in vitro* bioaccessibility testing methods is urgently needed. Most studies have used phosphorus as an amendment in urban areas that contain Pb in concentrations between 600-1200 mg/kg (Minca et al. (2013b); Minca (2012); Obrycki et al. (2016)). More Pb contaminated sources, such Pb smelter and Pb-contaminated mine tailings/mine spoil (>3000 mg/kg Pb), should be examined. The use of P amendments has been studied at different P/Pb ratios (mostly, P/Pb molar ratio of 3:5, 4:1 and 5:1). The reduction of IVBA-

Pb was found for all P treated soils. However, Pb phosphate formation can be influenced by various factors such as the natural form of Pb, the different soil Pb sources, the P availability from the amending agent, soil pH and organic matter (Li et al., 2014). Further, the environmental concerns associated with using large amounts of P for P amendment remain. The appropriate amount of P to use must be determined in tandem with site specific soil parameters such as Pb concentration, pH, organic matter, and moisture content (Scheckel et al., 2013) This will allow for the clarification of P concentrations that ought to be used in order to better protect the environment.

Minca et al. (2013b) found the Mehlich-3 soil test provided a reliable estimate of Pb in soils from 68 vacant residential lots in Cleveland, OH (total Pb ranging from 300 to 2000 mg/kg). Mehlich-3 was well correlated with both total Pb (r^2 = 0.970) and bioaccessible Pb using RBALP (pH 1.5; r^2 = 0.975, pH 2.5; r^2=0.938). The effectiveness of the Mehlich-3 soil test to extract more highly contaminated Pb soils, and soils contaminated with different Pb sources, remains unknown.

The work of Minca et al. (2013b) should be expanded to include soils with different amounts and sources of Pb contamination. Such data will respond to a recent critical review by Henry et al. (2015) calling for studies of *in situ* Pb bioavailability remediation in urban areas. The authors encouraged more research on the issue of soil composition variability because it can influence Pb bioavailability testing and soil amendment effectiveness. Therefore, the Mehlich-3, Modified Morgan, and AB-DTPA soil tests should be evaluated as indicators of bioaccessible Pb in different soils, contaminated with different amounts and sources of Pb, and amended with various ratios of P used in remediation. Using a nutrient soil test to estimate Pb bioaccessibility and as a Pb extraction analysis would be a cost-effective way to approach the accurate assessment of Pb bioavailability in soil. Because there is variability of bioaccessibility testing and *in situ* remediation techniques used in different contaminated sites, the *in vitro* methods need to be validated using a wider variety of soil samples, including sites used in previous studies. For example, data obtained from analyses of Zn and Pb smelter operations in Joplin, MO and from Pb-based paint contaminated soils in Seattle represent good ranges of variability of soil characteristics. The extent of validation of bioaccessiblity testing will improve assessment of bioaccessible and bioavailable Pb and application to specific contaminated sites. The efficiency of P amendment and the bioavailability and bioaccessibility of Pb in specific sites will also allow for broader generalizations in determining the overall soil threat of Pb contamination in a region. The expanded data from past and future research will be valuable in supporting and improving the reliability of Pb remediation methods and Pb bioaccessibility testing.

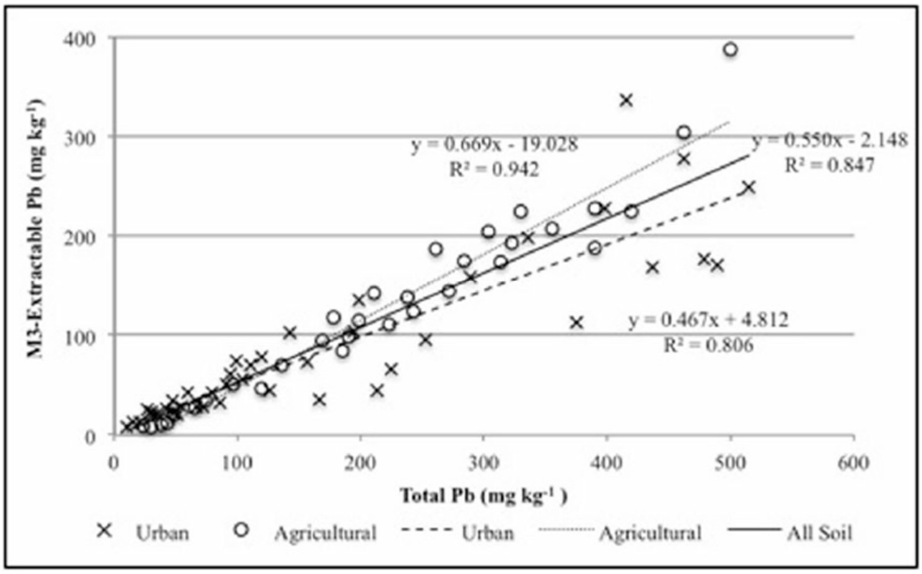

Fig. 1 Correlation of Mehlich-3 (M3) extractable Pb with total Pb in 54 soil samples from urban areas and 35 soil samples from agricultural soils (Wharton et al., 2012).

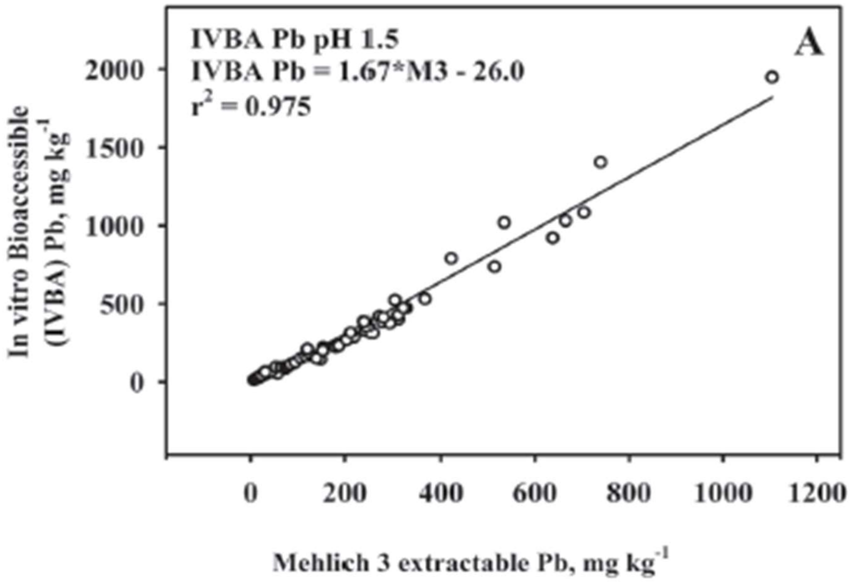

Fig. 2A Correlation of bioaccessible Pb using the Relative Bioaccessibility Leaching Procedure (RBALP) at pH 1.5 with bioaccessible Pb estimated using Mehlich-3 extractable Pb (Minca et al., 2013b).

extractable Pb (Minca et al., 2013b).

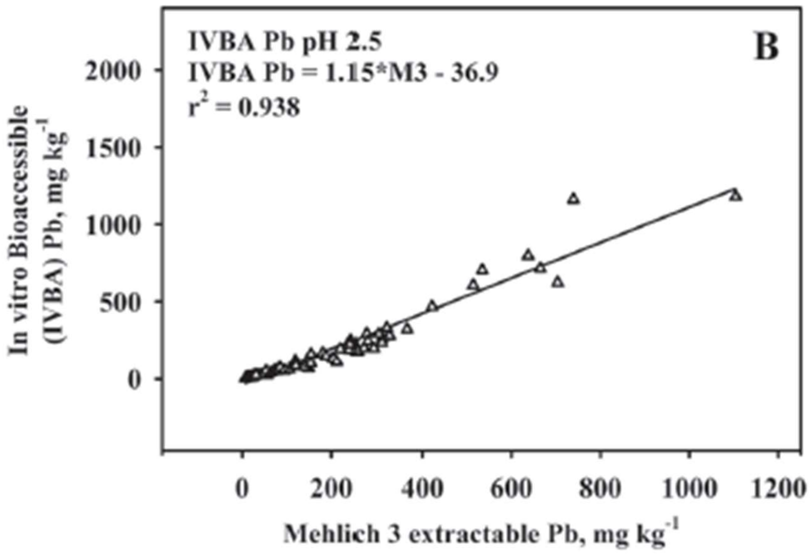

Fig. 2B Correlation of bioaccessible Pb using the Relative Bioaccessibility Leaching Procedure (RBALP) at pH 2.5 with bioaccessible Pb estimated using Mehlich-3 extractable Pb (Minca et al., 2013b).

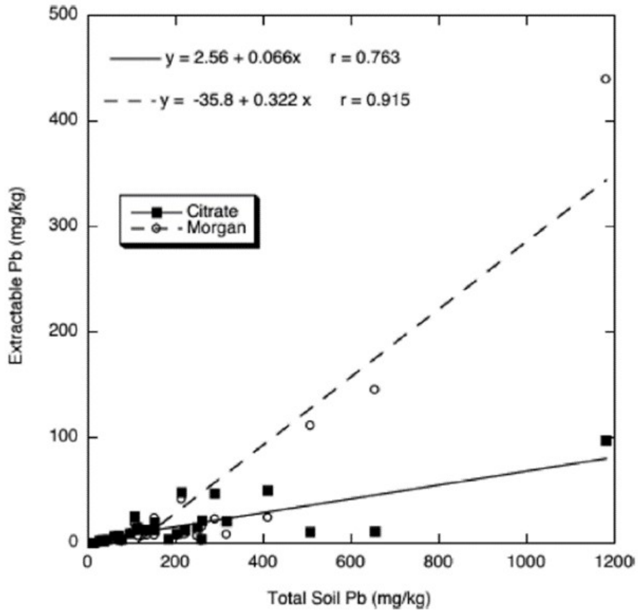

Fig. 3 Relationship of citrate- and modified Morgan–extractable Pb to total Pb in in 32 soils from urban garden soils, sludged soils and Industrial-site soils (McBride et al., 2011).

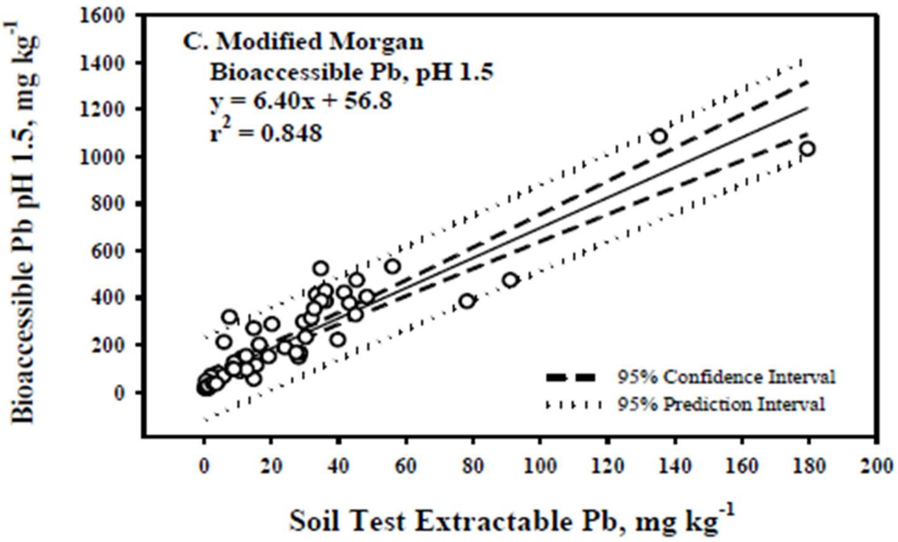

Fig. 4 Relationship between bioaccessible Pb at pH 1.5 and Modified Morgan-extractable Pb in 65 urban residential vacant lots in Cleveland, OH (Minca, 2012). A 95% confidence interval contains the true data of soil test extractable Pb with probability 0.95, whereas prediction intervals provide a 95% interval for the forecast soil test extractable Pb (soil sample not yet collected).

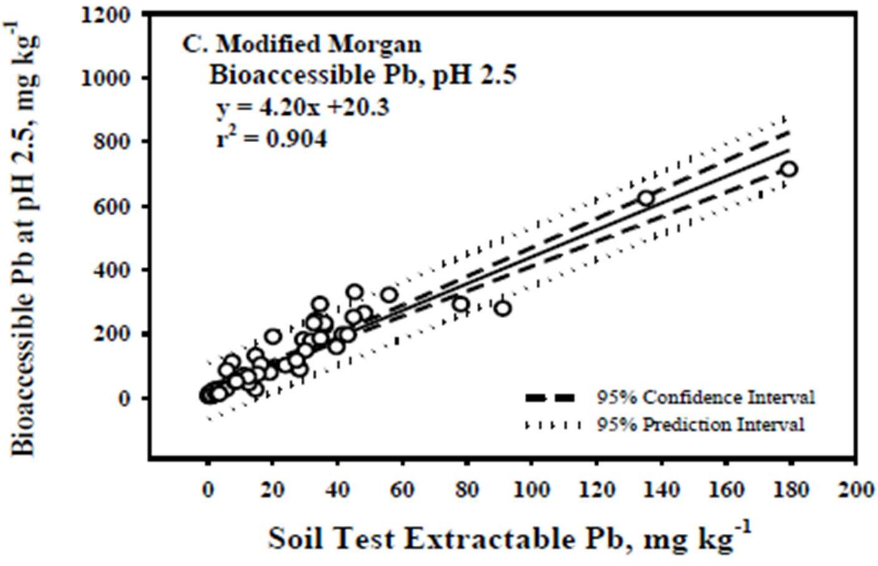

Fig. 5 Relationship between bioaccessible Pb at pH **2.5** and Modified Morgan-extractable Pb in in 65 urban residential vacant lots in Cleveland, OH (Minca, 2012). A 95% confidence interval contains the true data of soil test extractable Pb with probability 0.95, whereas prediction intervals provide a 95% interval for the forecast soil test extractable Pb (soil sample not yet collected).

LIST OF REFERENCES

Alaska Department of Environmental Conservation (ADEC). 2001. Public health evaluation of exposure of Kivalina and Noatak residents to heavy metals from Red Dog mine [online]. Available at https://dec.alaska.gov/spar/csp/docs/reddog/publichealthexpos_102501.htm (verified 4 June. 2015).

Assavarak, P. 2011. Development of measures to mitigate impacts of mining industry: lessons learned from the lower Klity creek, Kanchanaburi province, Thailand [online]. Available at http://arcmthailand.com/documents/documentcenter/20111215-Passanan%20Assavarak_M-08-11-122.pdf (verified 10 Mar. 2015).

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.

Beak D.G., N.T. Basta, K.G. Scheckel, and A.J. Traina. 2008. Linking solid phase speciation of Pb sequestered to birnessite to oral Pb bioaccessibility: implications for soil remediation. Environ. Sci. Technol. 42:779–785.

Baker R. L., G. M. Pierzynski, G.M. Hettiarachchi, K. G. Scheckel, and M. Newville. 2014. Micro-x-ray fluorescence, micro-x-ray absorption spectroscopy, and micro-x-ray diffraction investigation of lead speciation after the addition of different phosphorus amendments to a smelter-contaminated soil. J. Environ. Qual. 43:488-497.

Barbarick, K. A., and S. M. Workman. 1987. Ammonium bicarbonate-DTPA and DTPA extractions of sludge-amended soils. J. Environ. Qual. 16: 125-130.

Brown, S., R.L. Chaney, J.G. Hallfrisch, and Q. Xue. 2003. Effect of biosolids processing on lead bioavailability in an urban Soil. J. Environ. Qual. 32: 100–108.

Brown, S., R.L. Chaney, J.G. Hallfrisch, J.A. Ryan, and W.R. Berti. 2004. *In situ* soil treatments to reduce the phyto- and bioavailability of lead, zinc, and cadmium. J. Environ. Qual. 33: 522–531.

Brown, S., B. Christensen, E. Lombi, M. McLaughlin, S. McGrath, J. Colpaert, and J. Vangronsveld. 2005. An inter-laboratory study to test the ability of amendments to reduce the availability of Cd, Pb, and Zn *in situ*. Environ. Pollut. 138: 34–45.

Brown, S., H. Compton, and N.T. Basta. 2007. Field test of *in situ* soil amendments at the Tar Creek national priorities list superfund site. J. Environ. Qual. 36: 1627–1634.

Brown, S., I. Clausen, M. A. Chappell, K. G. Scheckel, M. Newville, and G. M. Hettiarachchi. 2012. High-iron biosolids compost-induced changes in lead and arsenic speciation and bioaccessibility in co-contaminated soils. J. Environ. Qual. 41: 1612-1622.

Brown, S. R.L. Chaney and G.M. Hettiarachchi. 2016. Lead in urban soils: a real or perceived concern for urban agriculture. J. Environ. Qual. 45:26–36.

Bruulsema T.W. 2006. Soil fertility in the northeast region. Better Crops. 90: 8-10.

Cao, X., L.Q. Ma, D.R. Rhue, and C.S. Appel. 2004. Mechanisms of lead, copper, and zinc retention by phosphate rock. Environ. Pollut. 131 (3): 435–444.

Cao, X., L.Q. Ma, S.P. Singh, and Q. Zhou. 2008. Phosphate-induced lead immobilization from different lead minerals in soils under varying pH conditions. Environ. Pollut. 152:184-192.

Casteel, S.W., C.P. Weis, G.M. Henningsen, and W.J. Brattin. 2006. Estimation of relative bioavailability of lead in soil and soil-like materials using young swine. Environ. Health Perspect. 114: 1162-1171.

Center of Disease Control and Prevention (CDC). 1991. Preventing Lead Poisoning in Young Children: Chapter 1 [online]. Available at http://www.cdc.gov/nceh/lead/publications/books/plpyc/chapter1.htm. (verified 19 Feb, 2016).

Chaney, R.L., H.W. Mielke, and S.B. Sterrett. 1989. Speciation, mobility, and bioavailability of soil lead. Environ. Geochem. Health. 11: 105-129.

Chen H.M., C.R. Zheng, C. Tu, and Z.G. Shen. 2000. Chemical methods and phytoremediation of soil contaminated with heavy metals. Chemosphere. 41: 229-234.

Defoe, P.P., G.M. Hettiarachchi, C. Benedict, and S. Martin. 2014. Safety of gardening on lead- and arsenic-contaminated urban brownfields. J. Environ. Qual. 43: 2064–2078.

Department of Toxic Substances Control (DTSC). 2004. Draft lead report. Hazardous waste management program [online]. Available at https://www.dtsc.ca.gov/HazardousWaste/upload/HWMP_REP_dLead-Rep.pdf. (verified 4 June, 2015).

Drexler, J. W., and W. J. Brattin.2007. An *in vitro* procedure for estimation of lead relative bioavailability: with validation. Hum. Ecol. Risk. Assess. 13: 383-401.

Elrashidi, M. A., M. D. Mays, and C. W. Lee. 2003. Assessment of Mehlich-3 and ammonium bicarbonate-DTPA extraction for simultaneous measurement of fifteen elements in soils. Commun. Soil. Sci. Plant Anal. 34: 2817–2838.

Garcia, M.A., J.M. Chimenos, A.I. Fernandez, L. Miralles, M. Segarra, and F. Espiell. 2004. Low-grade MgO used to stabilize heavy metals in highly contaminated soils. Chemosphere. 56: 481–491.

Geebelen, W., D.C. Adriano, D. van der Lelie, M. Mench, R. Carleer, H. Clijsters, and J. Vangronsveld. 2003. Selected bioavailability assays to test the efficacy of amendment-induced immobilization of lead in soils. Plant Soil. 249: 217–228.

Hamel, S. C., J. R. Heckman, K. L. Shilke-Gartley, and B. Hoskins. 2003. Lead extraction using three soil fertility tests and environmental protection agency method 3050. Commun. Soil Sci. Plant Anal. 34: 2853-2873.

Hardison D. W. Jr., L. Q. Ma, T. Luongo, and W. G. Harris. 2004. Lead contamination in shooting range soils from abrasion of lead bullets and subsequent weathering. Sci. Total Environ. 328: 175-183.

Hettiarachchi G. M., G. M. Pierzynski, and M. D. Ransom. 2000. *In situ* stabilization of soil lead using phosphorus and manganese oxide. Environ. Sci. Technol. 34: 4614-4619.

Hettiarachchi G. M.,and G. M. Pierzynski. 2004. Soil lead bioavailability and *in situ* remediation of lead-contaminated soils: A review. Environ. Prog. 23: 78-93.

Hooda, P. S. 2010. Trace Elements in Soils [online]. Available at http://www.researchgate.net/profile/Peter_Hooda/publication/264555054_Introduction/links/53e733f30cf21cc29fd9bd5b.pdf (verified 4 June, 2015).

International Agency for Research on Cancer (IARC). 2006. Monographs on the Evaluation of Carcinogenic Risks to Humans [online]. Available at https://monographs.iarc.fr/ENG/Monographs/vol87/mono87.pdf. (verified 19 April. 2016).

Juhasz A. L., E. Smith, J. Weber, M. Rees, T. Kuchel, A.Rofe, L. Sansom, and R. Naidu. 2013. Predicting lead relative bioavailability in peri-urban contaminated soils using *in vitro* bioaccessibility assays. J. Environ. Sci. Health., Part A. 48: 604–611.

Juhasz A. L., D. Gancarz, C. Herde, S. McClure, K. G. Scheckel, and E. Smith. 2014. *In situ* formation of pyromorphite is not required for the reduction of *in vivo* Pb relative bioavailability in contaminated soils. Environ. Sci. Technol. 48:7002-7009.

Kelley, K.D., and T. Hudson. 2007. Natural versus anthropogenic dispersion of metals to the environment in the Wulik River area, western Brooks Range, northern Alaska. Geochem. Explor. Environ. Anal. 7: 87–96.

Kelley, M. E., S.E. Brauning, R.A. Schoof, and M.V. Ruby. 2002. Assessing Oral Bioavailability of Metals in Soils. Battelle Press. Columbus, OH.

Kumpiene, J., A. Lagerkvist, and C. Maurice. 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments –a review. Waste Manage. 28: 215–225.

Lamadrid-Figueroa, H., M.M. Tellez-Rojo, , L. Hernandez-Cadena, A. Mercado-Garcia , D. Smith, M. Solano-Gonzalez, M. Hernandez-Avila, and , H. Hu. 2006. Biological markers of fetal lead exposure at each stage of pregnancy. J. Toxicol. Environ. Health Part A 69: 1781–1796.

Li, P., C. Lin, H. Cheng, X. Duan, and K.Lei. 2015. Contamination and health risks of soil heavy metals around a lead/zinc smelter in southwestern China. Ecotoxicol. Environ. Saf. 113: 391–399.

Li, P., K. G. Scheckel, L. Zheng, G. Liu, W. Xing, and G. Xiang. 2014. Immobilization of lead in soil influenced by soluble phosphate and calcium: lead speciation evidence. J. Environ. Qual. 43:468-474.

Liang S., D.X. Guan., J. Li., C.Y. Zhou., J. Luo., and L.Q. Ma. 2016. Effect of aging on bioaccessibility of arsenic and lead in soils. Chemosphere. 151: 94-100.

Lindsay, W.L., and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Amer. J. 42: 421-428.

Ma Q. Y., S. J. Traina, and T. J. Logan. 1993. *In situ* lead immobilization by apatite. Environ. Sci. Technol. 27: 1803-1810.

McBride, M. B., R. R. Mathur, and L. L. Baker. 2011. Chemical extractability of lead in field-contaminated soils: implications for estimating total lead. Commun. Soil Sci. Plant Anal. 42: 1581–1593.

Mehlich, A. 1984. Mehlich-3 soil test extractant: A modification of Mehlich-2 extractant. Commun. Soil Sci. Plant Anal. 15: 1409-1416.

Melamed, R., X. Cao, M. Chen, and L.Q. Ma. 2003. Field assessment of lead immobilization in a contaminated soil after phosphate application. Sci. Total Environ 305: 117–127.

Minca, K. K. 2012. Using soil nutrient tests and 1M HNO_3 to predict total and bioaccessible Pb in urban soils. M.S. thesis. Ohio State Univ. Columbus.

Minca, K. K., and N. T. Basta. 2013a. Comparison of plant nutrient and environmental soil tests to predict Pb in urban soils. Sci. Total. Environ. 445–446: 57–63.

Minca, K. K., N. T. Basta, and K. G. Scheckel. 2013b. Using the Mehlich-3 soil test as an inexpensive screening tool to estimate total and bioaccessible lead in urban soils. J. Environ. Qual. 42: 1518–1526.

Miretzk P., and A. F. Cirelli. 2009. Phosphates for Pb immobilization in soils: a review. Environ Chem Lett. 6:121-133.

Morman, S.A., G. S. Plumlee, and D.B. Smith. 2009. Application of *in vitro* extraction studies to evaluate element bioaccessibility in soils from a transect across the United States and Canada. Appl. Geochem. 24: 1454-1463.

Henry, H., M. F. Naujokas , N. T. Basta, Z. Cheng, G. M. Hettiarachchi, M. Maddaloni, C. Schadt, K. G. Scheckel, C. Attanayake. 2015. Bioavailability-based *in situ* remediation to meet future lead (Pb) standards in urban soils and gardens. Environ. Sci. Technol. 49: 8948−8958.

Obrycki J. F., N. T. Basta, K. Scheckel, B. N. Stevens, and K. K. Minca. 2016. Phosphorus amendment efficacy for *in situ* remediation of soil lead depends on the bioaccessible method. J. Environ. Qual. 45:37–44.

Oomen, A.G., C.J.M. Rompelberg, and M.A Bruil. 2003. Development of an *in vitro* digestion model for estimating the bioaccessibility of soil contaminants. Arch. Environ. Contam. Toxicol. 44(3):281–87.

Plant, J. A., N. Voulvoulis, and K. V. Ragnarsdottir. 2012. Pollutants, Human Health and the Environment: A Risk Based Approach. John Wiley & Sons, Ltd. Chichester, UK.

Pollution Control Department, Thailand (PCD). 2013. Monitoring and remediation of lead contamination in the Klity creek [online]. Available at http://infofile.pcd.go.th/mgt/NewsKity.pdf?CFID=1129246&CFTOKEN=19978266 (verified 9 Mar. 2015)

Pollution Control Department, Thailand (PCD). 2015a. Water quality standards [online]. Available at http://www.pcd.go.th/info_serv/reg_std_water05.html (verified 9 Mar, 2015).

Pollution Control Department, Thailand (PCD). 2015b. Soil Quality Standards [online]. Available at http://www.pcd.go.th/info_serv/reg_std_soil01.html (verified 9 Mar. 2015).

Ruby, M.V., R. Schoof , W.M. Brattin, M. Goldade, G. Post, M. Harnois, D.E. Mosby, S.W. Casteel, W. Berti, M. Carpenter, D. Edwards, D. Cragin, and W. Chappell. 1999. Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. Environ. Sci. Technol. 33: 3697-3705.

Ryan, J. A., K. Scheckel, W. R. Berti, S. L. Brown,S. W. Casteel, , R. L. Chaney, J. Hallfrisch , M. Doolan, P. Grevatt, M. Maddaloni, and D. Mosby. 2004. Reducing children's risk from lead in soil. Environ. Sci. Technol. 38: 19A-24A.

Scheckel, K.G., R.L. Chaney, N.T. Basta, and J.A. Ryan. 2009. Advances in assessing bioavailability of metal(loid)s in contaminated soils. Adv Agron. 104: 1-52.

Scheckel, K. G.; G. Diamond, M. Burgess, J. Klotzbach, M. Maddaloni, B. Miller, C. Partridge, and S. Serda. 2013. Amending soils with phosphate as means to mitigate soil lead hazard: a critical review of the state of the science. J. Toxicol. Environ. Health, Part B. 16 (6):337−380.

Silbergeld, E. K., M. Waalkes, and J. M. Rice. 2000. Lead as a carcinogen: Experimental evidence and mechanisms of action. Am. J. Ind. Med. 38: 316–323.

Smith, B.A., J.L. Kirk, and G.L. Stephenson. 2010. The influence of liquid to soil ratios on arsenic and lead bioaccessibility in reference and field soil. Hum. Ecol. Risk. Assess.16: 149-162.

Smith, E., I. M. Kempson, A. L. Juhasz, J. Weber, A. Rofe, D. Gancarz, and M. Gräfe, 2011. *In vivo-in vitro* and XANES spectroscopy assessments of lead bioavailability in contaminated peri-urban soils. Environ. Sci. Technol.45: 6145–6152.

Soltanpour, P.N., and A.P. Schwab. 1977. A new soil test for simultaneous extraction of macro-, and micro-nutrients in alkaline soils. Commun. Soil Sci. Plant Anal. 8: 195–207.

Strawn, D.G., P. Hickey, A. Knudsen, and L. Baker. 2007. Geochemistry of lead contaminated wetland soils amended with phosphorus. Environ. Geol. 52:109–122.

Sukkayanagit S. and T. Panichapat. 2013. Comparison of growth and lead accumulation of sunflower and sorghum in lead contaminated soil. Khon Kaen University Sci. J. 41: 996-1007.

Summer M. E. 1999. Handbook of Soil Science. CRC Press, Boca Raton, FL.

Thaipublica. 2014. Public Health Reports Levels of lead in the blood of the Klity Creek region people [online]. Available at http://thaipublica.org/2014/05/clity-report-may-2014 (verified 17 Mar. 2015).

United Nations Economic and Social Commission for Asia and the Pacific (United Nations ESCAP). 2001. Atlas of Mineral Resources of the ESCAP Region. Vol. 16, Mineral Resources of Thailand. United Nations Publication. NY.

United Nations Environment Program (UNEP). 2002. Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation [online]. Available at http://www.unep.or.jp/Ietc/Publications/Freshwater/FMS2/3.asp (verified 8 July 2015).

United Nations Environment Program (UNEP). 2010. Final review of scientific information on lead [online]. Available at http://www.environmentalhealthnews.org/ehs/news/2013/pdf-links/UN%202010%20report.pdf (verified 12 Mar. 2015)

United States Agency for Toxicology of Substances and Disease Registry (U.S. ATSDR). 2012. Lead Toxicity What Are the U.S. Standards for Lead Levels? [online]. Available at http://www.atsdr.cdc.gov/csem/csem.asp?csem=7&po=8 (verified 19 Feb, 2015).

University of California Agricultural and Natural Resource (UCANR). 2010. Home gardens and lead [online]. Available at http://anrcatalog.ucdavis.edu/pdf/8424.pdf (verified 12 Mar. 2015).

United States Department of Agriculture (USDA). 2010. Thailand Food and Agricultural Import Regulations and Standards –Narrative [online]. Available at http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Food%20and%20Agricultural%20Import%20Regulations%20and%20Standards%20-%20Narrative_Bangkok_Thailand_1-6-2011.pdf (verified 4 June. 2015).

United States Environmental Protection Agency (USEPAa). 2007. Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment [online]. Available at http://www.epa.gov/superfund/bioavailability/bio_guidance.pdf (verified 11 Apr. 2015).

United States Environmental Protection Agency (USEPAb). 2007. Microwave Assisted Acid Digestion of Sediment, Sludge, soils, and Oils [online]. Available at http://www.epa.gov/solidwaste/hazard/testmethods/sw846/pdfs/3051a.pdf (verified 4 June. 2015).

United States Environmental Protection Agency (USEPAc). 2007. Estimation of relative bioavailability of lead in soil and soil-like materials using *in vivo* and *in vitro* methods [online]. Available at http://www.epa.gov/superfund/soil-bioavailability-superfund-sites-guidance (verified 24 Feb. 2016)

United States Environmental Protection Agency (USEPA). 2013. Method 1340. *In vitro* bioaccessibility assay for lead in soil [online]. Available at https://www.epa.gov/sites/production/files/2015-12/documents/1340.pdf. (verified 19 April. 2016).

United States Environmental Protection Agency (USEPA). 2014. Learn about Lead [online]. Available at http://www2.epa.gov/lead/learn-about-lead (verified 10 Mar. 2015).

Van deWiele, T.R., A.G. Oomen, and J. Wragg. 2007. Comparison of five *in vitro* digestion models to *in vivo* experimental results: Lead bioaccessibility in the human gastrointestinal tract. J. Environ. Sci. Health., Part A Part A 42(9):1203–1211.

Wharton, E.S., A. S. Hannah, M. S. Henry, G. Lydia, L.R. Marquez-Bravo, and Murray B. McBride. 2012. A comparison of screening tests for soil Pb. Soil Science 177(11): 650–654.

Wheeler, W., and M.J. Brown. 2013. Blood lead levels in children aged 1–5 years: United States, 1999-2010. Morb. Mortal. Wkly. Rep. 62:245–248.

World Health Organization (WHO). 2010. Childhood lead poisoning [online]. Available at http://www.who.int/ceh/publications/leadguidance.pdf (verified 4 June. 2015).

Zia, M.H., E.E. Codling, K.G. Scheckel, and R.L. Chaney. 2011. *In vitro* and *in vivo* approaches for the measurement of oral bioavailability of lead (Pb) in contaminated soils: A review. Environ. Pollut. 159: 2320-2327.