Understanding mechanisms of changes in lead bioaccessibility in amended alkaline soils through integration of multiple techniques and evaluating methods for house dust collection and assessing dust lead bioaccessibility

by

Wekumbure Gedara Chandima Wekumbura

B.S., Sabaragamuwa University of Sri Lanka, 2008
M.S., University of Peradeniya, 2010
M.Phill., University of Peradeniya, 2017

AN ABSTRACT OF A DISSERTATION

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Abstract

Soil contamination by trace elements, particularly lead (Pb), presents a global concern, especially affecting young children who are highly susceptible to Pb toxicity through inadvertent ingestion of Pb-contaminated soils and dust. First two studies aimed to assess the efficacy of organic and inorganic soil amendments in reducing bioaccessible Pb concentrations in four soils collected from arid to semi-arid regions with prevailing soil alkalinity. This research also sought to examine the impact of these amendments on changes in Pb speciation and associated in vitro Pb bioaccessibility across four soils with varying Pb concentrations. One soil sample (EP4) was collected from a near ASARCO smelter with a high total Pb concentration (~3000 mg kg^{-1}) and three other soil samples (EP1, EP2, EP3) were collected from residential areas of El Paso, Texas, exhibiting Pb concentrations mild to moderate (750 to 150 mg kg^{-1}). These soils were treated with two organic (Class A, Fe-rich biosolids and compost) at two rates (5 and 10%) and two inorganic P fertilizers (triple superphosphate, TSP and monoammonium phosphate, MAP) at two rates (Pb:P molar ratio 1:4 and 1:6) and incubated for up to 24 weeks. Bioaccessible Pb concentrations were periodically measured using a modified physiologically based extraction test. Fractionation and speciation of Pb were determined through sequential chemical extraction and synchrotron techniques, including X-ray absorption spectroscopy (XAS) and spatially-resolved micro X-ray diffraction (µ-XRD) and µ-XAS, aided by micro X-ray fluorescence (µ-XRF), after 24 weeks.

Results demonstrated a significant reduction in bioaccessible Pb levels and pH over time in EP4 soil treated with biosolids, compared to untreated soil, with biosolids showing the most pronounced reduction in bioaccessible Pb. Biosolids treatment consistently reduced the

bioaccessible Pb fraction in soils from residential areas (EP1, EP2 and EP3). Sequential extraction and synchrotron analysis confirmed the formation of Pb-associated P and Fe minerals, including pyromorphite and Fe (hydro)oxides sorbed Pb. Class A Fe-rich biosolids were most effective in reducing bioaccessible Pb and transforming Pb into residual fractions across all soils (EP1, EP2, EP3, and EP4), regardless of the type of soil and total Pb concentration. The reduced bioaccessible Pb was primarily associated with pyromorphite, Fe (hydro)oxides sorbed Pb, and Pb bound to organic complexes. Overall, applications of biosolids, TSP, and MAP were effective in diminishing exchangeable Pb fractions and forming stable fractions in alkaline soil.

Third study addressed limitations associated with standardized dust wipes in measuring important lead parameters such as bioaccessible Pb in household dust samples. To address these limitations, two types of simulated household dust samples (Pb-soil contaminated and Pb-paint contaminated) were created and compared using a standard dust wipe method and a modified micro vacuum method. Results indicated no significant difference between methods in higher Pb concentration dust mixtures (1200 and 1000 mg kg^{-1}); however, in lower Pb concentration dust mixtures (800 to 400 mg kg^{-1}), the dust wipe method yielded significantly higher lead loading. This difference may be attributed to the dust-transferring procedure in the micro vacuum method. Bland and Altman plots indicated good agreement between dust wipe and micro vacuum methods, suggesting potential for developing a standardized micro vacuum method that is low-cost, efficient, and suitable for detailed dust analysis. Furthermore, the fourth study compared two in vitro methods for estimating the proportion of bioaccessible Pb in dust collected by dust wipes: the US-EPA's in-vitro bioaccessible assay (IVBA) method at two pH values (1.5 and 2.5) and the physiologically based extraction test (PBET) at pH 2.5. Results showed significant differences in estimated bioaccessible Pb levels between extraction methods, with PBET

demonstrating greater discrimination, particularly in lead-paint contaminated dust. In conclusion, this thesis work provides valuable insights into the efficacy of organic and inorganic soil amendments in reducing bioaccessible Pb concentrations and altering Pb speciation in alkaline soils. Additionally, it proposes improvements in dust collection methods for more accurate Pb analysis in household dust samples, highlighting the importance of selecting appropriate extraction methods for assessing bioaccessible Pb in environmental samples.

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Approved by:

Major Professor
Dr. Ganga M. Hettiarachchi

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Dedication

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Chapter 1 - Introduction

Environmental pollution is globally recognized as the second most pressing issue (UN, 2024), with water, air, and soil being critical components susceptible to degradation from various toxins, including plastics, trace elements, and organic and inorganic chemicals. These pollutants originate from diverse sources such as mining and smelting, industries, fossil fuel combustion, atmospheric deposition, and domestic and industrial wastes. Soil contamination with potentially toxic trace elements, particularly in urban and industrial areas, is a significant aspect of environmental pollution (Purves, 1972; SSSA, 2024). While trace elements naturally occur in soil at low levels, certain land use practices such as mining and smelting, agriculture and waste deposition can elevate their concentrations to levels posing health risks (Chilinski et al., 2022). Contamination with elements like copper (Cu), lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg) and zinc (Zn) is often considered irreversible (Purves, 1972), with anthropogenic activities heavily contributing to the sources and accumulation of such contaminants in soil (Hettiarachchi et al., 2024). Lead and Zn mines and smelters, both active and inactive, are widespread globally, with many ceasing operations years ago. However, the environmental legacy of these activities remains a significant concern. These trace elements pose health risks to individuals exposed to them through inhalation and ingestion within areas affected by metallic trace element contamination (Chopin et al., 2003; Camizuli et al., 2018; Briffa et al., 2020; Piekut et al., 2020).

Soils near industrial sites, urban areas, and residential communities across the United States and globally have been contaminated by Pb from various sources such as leaded gasoline, lead-based paint deterioration, emissions from smelters, waste recycling, and other industries (Burt et al., 2011; Laidlaw et al., 2017; CDC, 2022). Exposure to Pb in soil and associated dust is widespread, with a significant proportion of urban children in specific neighborhoods exhibiting blood lead levels (BLLs) exceeding the reference level, established by the U.S. Centers for Disease Control (CDC) (Frank et al., 2019; Hauptman et al., 2021; Olowoyo et al., 2022). The CDC's updated blood Pb reference value for children is 3.5 µg/dL (CDC, 2022), while no blood Pb concentration is considered safe for children. Additionally, new screening values for soils in residential properties are also recommended, reducing from the earlier level of 400 parts per million (ppm) to 200 ppm. For residential properties with multiple sources of Pb exposure, the US Environmental Protection Agency (US EPA) has set the screening value at 100 ppm (US EPA, 2024).

Lead serves no essential function in the human body. Lead poisoning, contributing to approximately 0.6% of the global disease burden, poses significant challenges for child health worldwide, with even low levels of Pb exposure correlating with adverse effects on intelligence, behavior, and learning due to brain and nervous system damage (Lidsky and Schneider, 2003; WHO, 2010; CDC, 2022). Further, young children (< 5 years old) have the potential for exposure to toxic substances through non-dietary ingestion pathways other than soil ingestion. Repeatedly putting objects or their fingers in their mouths, whether out of curiosity or habit, can lead to exposure to potentially harmful substances in their surroundings. This behavior may result in a higher intake of toxicants compared to other means of ingestion or skin contact (US EPA 2002). Indoor dust is comprised of small solid particles, together with organic and inorganic inert materials, which are usually characterized by fine size fractions (< 100 µm) that settled under the influence of gravity (Turner and Ip, 2007; WHO, 1999). Research investigating human exposures to Pb has suggested that settled house dust may be a significant source of indoor exposure (Maertens et al., 2004). Trace metal contaminants in indoor dust pose a significant potential exposure risk to people because of the time spent indoors and the readily ingested and inhaled fine-grained composition of indoor dust (Doyi et al., 2020). Thus, ingestion and inhalation of contaminated soil and house dust from various sources are the primary exposure pathways of Pb in children (Hettiarachchi and Pierzynski, 2004; Özkaynak et al., 2022; Zartarian et al., 2023).

The ionic form of lead (Pb^{2+}) and metallic lead (Pb^0) are the common forms of Pb released into the environment (Wuana and Okieimen, 2011). In a moderately oxidizing environment, Pb forms minerals like lead oxides, hydroxides, carbonates, sulfides, phosphates, and lead metal oxyanion complexes. In soil, Pb^{2+} is the most common and reactive form of Pb. (Nriagu, 1978). Reactive Pb^{2+} and other soluble complexes interact with soil constituents through processes such as sorption, precipitation, nucleation, solid solution formation, and redox reactions (Li et al., 2022). Except for the total contaminant concentration, the most important fraction of contaminant is the bioaccessible fraction. A portion of a substance or element in soil that is available for absorption into living organisms is known as a bioaccessible fraction (Hettiarachchi & Pierzynski, 2004; Scheckel et al., 2009). Bioavailability of metal(loid)s is defined as the extent to which bioaccessible metals adsorb onto or absorb into and across the biological membrane of an organism (McGeer et al., 2004). The toxicity and mobility of Pb compounds are associated with their solubility, and in this regard largely influenced by soil properties and the distribution of Pb species (Kumar et al., 2022). Soil biogeochemical processes such as immobilization, adsorption, and precipitation significantly influence the bioaccessible fraction of Pb, thereby affecting its solubility and potential absorption by living organisms. (Hettiarachchi & Pierzynski, 2004; Miretzky & Fernandez-Cirelli, 2008).

Various ex-situ and in-situ remediation methods are available for treating Pb-contaminated soil, involving physical or chemical techniques. Soil replacement involves removing and replacing the contaminated soil entirely. Soil isolation utilizes physical barriers to isolate contaminated soil or adding clean soil to cover contaminated soil, while soil leaching involves washing away Pb-contaminated soil with specific reagents to remove heavy metal complexes and soluble ions. Phytostabilization, utilizes plants to stabilize contaminated soil and prevent erosion, with perennial plant species having robust root systems preferred for this method (Egendorf et al., 2020; Lan et al., 2020). However, majority of these methods are expensive, inefficient, and environmentally unsustainable, especially for widespread mild to moderate levels of Pb contamination. Chemical stabilization, or in situ stabilization of Pb, involves adding immobilizing agents to alter soil Pb into less bioavailable forms, thus reducing environmental risk. Soil amendments influencing soil physiochemical properties are ideal strategies for managing Pb-contaminated soil (Hettiarachchi & Pierzynski, 2004; Lwin et al., 2018; Palansooriya et al., 2020).

Applying soil amendments to Pb-contaminated soil typically increases and stabilizes the fraction of total Pb in the soil solid phase compared to the soil solution phase. Organic soil amendments containing humic acids, high amounts of P, Fe, etc., such as biosolids, composts, manures, and biochar, are commonly used to immobilize soil Pb by transforming it from highly bioavailable forms to less bioavailable fractions associated with organic matter, metal oxides, or carbonates (Lwin et al., 2018; Palansooriya et al., 2020).

Phosphate-containing materials are among the most common and popular inorganic soil amendments used for managing Pb-contaminated soil (Mayer et al., 2022). Various natural and synthetic materials as P sources with different solubilities have been successfully employed to remediate Pb-contaminated soils to reduce Pb bioavailability and bioaccessibility. Phosphate amendments decrease Pb mobility primarily through ligand exchange, ionic exchange, and precipitation of pyromorphite minerals (Hettiarachchi & Pierzynski, 2004; Park et al., 2011). The redistribution of Pb from more chemically labile forms to non-labile phases results in decreased bioavailability and totoxicity, indicating the effectiveness of P amendments in Pb-contaminated soil.

Applying of inorganic and organic sources of P for in-situ stabilization of soil Pb is feasible if the P amendment would present long-term decreases in Pb bioaccessibility. However, there are some concerns, such as the success of these treatments on different soil conditions. Past studies commonly utilized in-situ soil amendments for remediation of Pb-contaminated soils characterized by very high Pb concentrations, contamination from identifiable Pb sources (Yang & Mosby 2006; Brown et al., 2007; Moseley et al., 2008; Sanderson et al., 2016; Alasmary, 2020), or soil spiked with Pb-nitrate-like soluble Pb chemicals (Martínez-Villegas et al., 2004; Alamgir et al., 2011; Lwin et al., 2018). In addition, researchers have consistently shown a trend of utilizing acidic soil and amendments aimed at reducing soil pH (Yang et al., 2001; Sipos *et al.,* 2005; Tang et al., 2009; 2013; Obrycki et al., 2016; Zhou et al., 2020; Mayer et al., 2022). Conversely, studies on calcareous and high-pH soils have indicated limited effectiveness of P treatments. This ineffectiveness is attributed to decreased solubility and increased precipitation of Ca-P products (Penn & Camberato, 2019) as well as the constrained availability of free P and Pb for reaction (Yang et al., 2001).

We chose two types of inorganic and organic P amendments for our laboratory incubation studies in this dissertation research. We collected soil contaminated with Pb from an area characterized by arid climatic conditions and dry, alkaline soil. This area also hosts an old smelter yielding lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn), and antimony (Sb) during its > 110-year history (Darby, 2012; Shekhter, 2013), which is highly likely to have generated an accumulation of Pb along with various other trace elements. In addition to evaluating changes in Pb bioaccessibility and extractability over time, bulk X-ray absorption spectroscopy (XAS) and spatially-resolved micro X-ray diffraction (μ-XRD) and micro XAS aided by micro X-ray fluorescence (μ-XRF) were used for further verification of speciation changes of soil Pb. Sequential chemical extraction was also used to compare the soil Pb fractionation and association with Pb its bioaccessibility.

The accumulation of Pb-contaminated house dust, primarily from legacy sources like leaded paints, leaded gasoline, and industrial sites, poses significant health risks. Effective dust collection methods are crucial for tracing sources and implementing mitigation measures. Wipe methods and vacuum devices are commonly used for dust sampling, each with advantages and limitations. Standardized methods for utilizing dust wipes to collect and measure total Pb in household dust are well-established. However, the application of dust wipes for measuring bioaccessible Pb is less defined. With the recognition that no level of Pb is safe for children and growing fetuses, there is a need for in vitro methods to estimate the fraction of bioaccessible Pb in dust collected by dust wipes. These methods are crucial for generating more informative Pb toxicity data, essential for health, environmental, and mitigation purposes.

Wipe methods are efficient for surface sampling but lack data on dust loading and bioavailable Pb fraction. Vacuum methods offer more comprehensive data on Pb concentration, loading, and dust loading across various surfaces. Studies have shown higher collection efficiency and precision with high-volume vacuum samplers compared to low-flow vacuum methods, while vacuum methods, such as micro vacuum, provide valuable insights for Pb risk assessment, mitigation efforts, and for other critical measurements, such as bioaccessible Pb in residential environments. Thus, evaluating the micro vacuum sampling method would be an alternative method that can be used efficiently and precisely while getting valuable information that the wipe method could not offer.

Overall in this dissertation, two studies were undertaken to investigate the influence of soil amendments on changes in Pb speciation and associated in vitro Pb bioaccessibility in soils with differing Pb concentrations. Additionally, two studies were conducted to determine the optimal method for assessing in vitro Pb bioaccessibility of dust wipe samples and to develop dust collection methods for Pb-contaminated household dust.

Objectives

1- The objectives of the first study (Chapter 3) were to characterize and evaluate the effects of reducing bioaccessibility of Pb in Pb/As/Cd/Zn/Cu co-contaminated alkaline smelter-impacted soil using soil amendments. A laboratory-based study was conducted with two main objectives: i) Assessing the impact of various P-containing organic and inorganic soil amendments on the Pb bioaccessibility over time. ii) Evaluating the relationship between changes in soil Pb speciation and Pb bioaccessibility.

2- The objectives of the second study (Chapter 4) were to evaluate the effects of reducing Pb bioaccessibility in mild to moderately Pb-contaminated residential soils using soil amendments.

The laboratory-based investigation aimed to assess two key aspects: i) The influence of various P-containing organic and inorganic soil amendments on the Pb bioaccessibility over time, and ii) The correlation between the formation of Pb species and the reducing Pb bioaccessibility.

3- The objective of the third study (Chapter 5) was to compare the two main approaches currently suggested in the literature for determining in vitro bioaccessible Pb in household dust wipe samples, including the USEPA's in vitro bioaccessible assay (IVBA) Method 1340 at two pH values (IVBA 1.5 and IVBA 2.5 pH); and the physiologically based extraction test (PBET 2.5 pH) to develop a method that can use in determining in vitro bioaccessible Pb in household dust wipe samples.

4- The objective of the fourth study (Chapter 6) was to develop a modified method for household dust collection that is simpler, more precise, and more reliable than the currently recommended dust wipe sample method that will allow collecting not only dust Pb concentration and dust loading but provide adequate quantities of dust for other critical measurements, such as bioaccessible Pb levels, in the collected dust.

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Chapter 2 - Literature Review

Characteristics of lead

Geogenic origin of Pb

Lead (Pb) is a naturally occurring element, holding the 36th position in terms of abundance among elements in the earth's crust, characterized by an atomic number greater than 60 (Ghazi and Millette, 1964). Lead is found in more than 200 known minerals, but naturally, these minerals are rare and their occurrence can vary in different geological formations (Lovering, 1976). Elemental lead in its pure metallic form is relatively rare on Earth, constituting only a small percentage (about 0.002%) of the Earth's crust by weight. Lead is more commonly found in ores where it is combined with other metals (Davidson, et al., 2014).

Lead naturally forms through the radioactive decay of uranium (U) and thorium (Th) through by radon (^{222}Rn) (Acharya, 2013; Ghazi and Millette, 1964). The variability in lead isotopic composition across global Pb ore deposits is influenced by factors such as the concentrations of thorium and uranium, the rate of isotopic Pb decomposition into a more stable form, initial isotope ratios, the composition of reservoir Pb, and the duration of reservoir evolution before Pb fractionation from the system through geochemical processes (Ghazi and Millette, 1964; Blum and Erel, 2003; Hettiarachchi et al., 2024).

The average crustal (un-mineralized rock) abundance of Pb is around 15-16 mg kg^{-1} (Lovering, 1976). The lead content in common rock types found in the Earth's crust varies, ranging from approximately 30 mg kg^{-1} in granitic rocks, rhyolite, and black shale to about 1 mg kg^{-1} in evaporite sediments, basalt, and ultramafic igneous rocks such as dunite. Natural coal and petroleum are recorded at around 10 mg kg^{-1} and 1 mg kg^{-1} of Pb, respectively (Fleischer, 1976). Lead ores, including well-known minerals such as galena, anglesite, and cerussite, typically have a significantly higher lead content, about 8% (King, 2021). In contrast, unmineralized rock types lack concentrated lead minerals, have a much lower lead content, approximately 15 ppm. Additionally, lead is present in trace amounts in various common rock-forming minerals. Potash feldspar, whether in the form of orthoclase or microcline, contains the highest lead content among the commonly occurring silicate minerals (Lovering, 1976). In natural soils, Pb concentration varies within the range of 15- 200 mg kg^{-1} (Zimdahl and Skogerboe, 1977) with a global average concentration of approximately 29 mg kg^{-1} lead in soils worldwide (Ure and Berrow, 1982). Natural processes, including sea waves, windblown dust, and wildfires, can introduce lead particles into the environment, leading to occasional increases in concentration exceeding background average values (Pacyna and Pacyna, 2011). There are some exceptions including soils derived from Carboniferous black shales, which can contain up to 200 mg kg^{-1} of soil (Alloway, 2013).

Lead is found in concentrated and easily accessible Pb ore deposits. Galena (lead sulfide, PbS) is the primary constituent found in sulfide ore deposits. The lead dissolved from primary minerals tends to combine with carbonate or sulfate ions, forming lead carbonate or lead sulfate depending on the environmental conditions and availability of those ligands. The formation of anglesite (lead sulfate, $PbSO_4$), cerussite (lead carbonate, $PbCO_3$), and minium (lead oxide, Pb_3O_4) results from the oxidation of galena near the surface (Ghazi and Millette, 1964). Lead is often in association with other minerals and alongside Zn, Cd, Fe, and other metals in ore deposits. Alternatively, it may be adsorbed by ferric hydroxide (Wuana and Okieimen, 2011; ITP, 2013).

Chemical and physical properties of Pb

Lead is one of the earliest metals discovered by the human race for its utility (Ghazi and Millette, 1964; Flora et al., 2012). Lead is a dense element that typically presents itself in pure form as a bluish-white metal with a bright luster, or alternatively, as a silvery-white or soft gray metal with a specific gravity of 11.34 g cm^{-3}. Lead in the periodic table is grouped in IVa period six, and has an atomic number 82 (Britannica, 2024). The valence shell electron configuration of Pb is $6s^2$ $6p^2$, which permits it to have three possible oxidation states: Pb (II), Pb (III) and Pb (IV). Lead can exist in the Pb^0 oxidation state in metallic Pb such as Pb pipe. The most stable oxidation state found over a wide range of environmental conditions and in soils is Pb^{2+}. Naturally occurring Pb is a mixture of isotopes with mass numbers 204, 206, 207, and 208, with 207 being the most abundant. The atomic weight of lead is 207.21 g mol^{-1}. The melting and boiling point of lead are 327°C and 1755°C respectively. Thus, Pb is very soft, highly malleable, ductile, a poor conductor of electricity, and is very resistant to corrosion. The chemistry of inorganic Pb compounds is generally similar to that of the Group 2(II) or alkaline earth metals (UNEP, 2010; ATSDR, 2021a).

Common uses of Pb

Direct and two-stage processes are used in extracting Pb metal. Pre-processing includes grinding ore materials followed by dense-medium separation, froth-flotation, and drying. The direct process followed in melted lead sulfide is oxidized in the furnace to form lead monoxide. The lead monoxide is then reduced to lead metal by the adding of carbon in the form of coke or coal gas. In the first stage, of the two-stage process, the ore is roasted to oxidize the lead sulfide. During the second stage, the lead oxide is reduced to obtain the metal using a coke-fired blast furnace (CEW, 2024). In addition, lead can be recycled. After the lead has served its original purpose, it can be collected, melted down, and reformed into new products through a process called secondary smelting. During secondary smelting, the collected lead-containing materials (lead-acid batteries, scrap metal, and other leaded products) undergo a smelting process to separate the lead from other components and impurities (Cohen, 2024). Recycled lead helps to conserve resources, reduce the environmental impact of lead mining, and mitigate the potential hazards associated with lead disposal.

Lead has been used for various purposes throughout history, owing to its unique properties. Lead-acid batteries, construction materials including lead pipes, and lead flashing, ammunition (bullets and shot for firearms), radiation shielding, cables and sheathing, solders, stained glass, and some nuclear, medical, and industrial applications are the current uses of lead (IARC, 2006; Bača and Vanýsek, 2023). The diverse applications of a Pb metal derive from its distinctive characteristics, which include resistance to corrosion and malleability, high density, electrical conductivity, low melting point, and its capacity to form robust and durable connections (ILZSG, 2023; Britanica, 2024).

Former applications of Pb, such as its inclusion in gasoline and jet fuels as antiknock agents (tetraethyllead and tetramethyllead), lead-based paint containing lead-based pigments (lead(II) chromate, lead(II,IV) oxide, and lead(II) carbonate), and the use of inorganic insecticides (lead hydrogen arsenate or lead arsenate), currently serve as legacy sources of environmental lead, posing ongoing human health hazards (ASTDR, 2021a; US EPA, 2021). In addition, Pb is still found in some cosmetics products, folk medicine, pottery glazing, cookware, utensils, paints, and solder in cans (CDC, 2022).

Sources of lead contamination

The ubiquitous nature of Pb causes its widespread presence in every corner of our environment, encompassing the air, soil, water, and even the interiors of households. Lead enters the environment through both natural and anthropogenic sources. Natural sources encompass sedimentary rocks, volcanic eruptions, soil formation, rock weathering, seawater, and the decomposition of plants. On the other hand, anthropogenic sources consist of activities related to industry, agriculture, mining, as well as the use of domestic and consumer products. These two categories collectively contribute to the introduction of Pb into the environment. While natural sources of Pb generally remain within acceptable limits in the environment, the primary contributor to environmental contamination results from human activities. There are two major sources of Pb contamination, point and non-point sources. Point sources are readily identifiable as they release pollutants into the environment from a distinct and confined location as the name implies. On the other hand, nonpoint sources are more challenging to identify and address because they emanate from multiple locations simultaneously (US EPA, 2021).

Point sources

Mining and smelting

Products from mining and the particles generated during ore smelting activities can give rise to atmospheric particles of Pb (Adnan et al., 2022; Schindler et al., 2022; Guillevic et al., 2023).

These particles have the potential to be transported through various means, including wind, gravity, runoff water, or even anthropogenic methods such as vehicle transport, covering several kilometers. Mining and smelting operations serve as major point sources, generating significant volumes of mine and metallurgical wastes rich in toxic trace elements such as Pb, As, Cd, Cu, and Zn. These wastes become contributors to Pb contamination, spreading into the environment through different mechanisms. Mining and smelting activities exert a significant impact on soil, especially when mining and metallurgical wastes are abandoned on soils without adequate protection. The soil serves as the immediate repository for these discarded materials, intensifying the environmental consequences of such practices (Rieuwerts and Farago, 1995; Wuana and Okieimen, 2011; Fry et al., 2020; Ettler, 2016; Guillevic et al., 2023).

Leaded paints

Lead carbonate and Pb chromate known as "white lead,", and "chrome yellow," were commonly used as pigments in paint to maintain a fresh appearance and resist moisture-induced corrosion (Gliozzo and Ionescu, 2022). Due to their anti-corrosion properties, these paints were applied to various construction structures in urban and household settings (O'Connor et al., 2018). Lead-based paint remains one of the most prevalent and hazardous sources of Pb exposure for young children in the United States (CDC, 2022). Despite the ban on Pb-based paints in the U.S.A. for household use in 1978, homes painted or constructed before 1978 continue to pose a risk of exposure for both adults and children, highlighting the persistent challenges in mitigating Pb exposure risks. The peeling, cracking, or wear resulting from rubbing or friction of lead-based paint can lead to the deposition of Pb paint chips and dust. This can occur on windows, doors, floors, porches, stairways, and cabinets, creating potential environmental exposure pathways.

Lead flashing, drain pipes, or water pipes, commonly found in older construction, serve as additional sources of lead contamination, extending beyond residential housing to various types of structures. Renovation, repair, or painting activities have the potential to generate lead dust that can spread both inside and outside of buildings, as well as into the surrounding soil. These activities may disturb existing lead-based paint, contributing to the dissemination of Pb particles in the surrounding environment (Dignam et al., 2019; US EPA, 2022; US EPA, 2024).

Leaded gasoline

Lead-based antiknock gasoline additives became widely utilized globally from the mid-1920s to the mid-1980s, with leaded gasoline emerging as a significant source of Pb exposure especially for the urban population (Falta et al., 2005). The addition of tetraethyl lead (TEL) or tetramethyl lead (TML) was employed to boost the octane number and enhance the "anti-knock" property of gasoline (Stolark, 2016). Gasoline sold during this period typically contained an average Pb concentration of around 0.6 g L^{-1}. The phase-out of leaded gasoline commenced in 1973 in the USA and was completed in 2021 (Falta et al., 2005). Although leaded gasoline was phased out during the 1980s and 1990s, some uses persist, such as piston-engine airplanes, and certain off-road vehicles such as racing cars, farm equipment, and marine engines (US EIA, 2022). Lead constitutes over 20% of the total mass of fine particles emitted during the combustion of leaded gasoline, with approximately 75% of this Pb being directly released into the atmosphere (Pacyna and Pacyna, 2011). The rise in Pb levels is predominantly linked to vehicle emissions and roadside sources at that time. This emphasizes the significant contribution of vehicular activities to releasing of Pb particles, impacting air quality and subsequent atmospheric deposition on soil and water bodies. Contamination levels exhibit a proportional relationship with fuel consumption per unit area, with the dispersion by wind contributing to the gradient observed in contamination distribution.

Agrochemicals

Among the agricultural sources of Pb, pesticides, inorganic fertilizers, manures and sewage sludge are the most common. Lead-arsenate ($PbHAsO_4$) was widely used as a pesticide on fruit tree orchards to combat gypsy and codling moth caterpillar infestations throughout the United States between the 1890s and 1950s (Peryea and Creger, 1994; Schooley et al., 2008; Higgins et al., 2022). Estimates suggest that the cumulative application of arsenical pesticides in an orchard over the entire period of their use may have reached up to 22.4 g m^{-2} of As and 100 g m^{-2} of Pb (Robbinson et al., 2007).

Phosphorus fertilizers are derived from phosphate rocks that inherently contain trace elements, such as Cd, Pb, Zn and As. Prolonged and excessive fertilizer use leads to the accumulation of Pb-like trace elements in significant amounts. The global concentration of Pb in phosphorus fertilizers varies from 1 to 300 mg kg^{-1}, while in manures, it ranges from 2 to 60 mg kg^{-1} (Alengebawy et al., 2022). In addition, certain micronutrient fertilizers and liming materials have been reported to have elevated levels of Pb and other trace elements (Franklin et al., 2005).

Sewage sludge (now regulated and referred to as biosolids) is considered a by-product and waste of municipal and industrial wastewater treatment (Fijalkowski et al., 2017). In intensive farming, biosolids and sewage sludge serve as valuable sources of nutrients and substantial organic matter supplementation. The concentrations of trace elements in biosolids are influenced by the nature and intensity of industrial activity, along with the specific processes utilized during sewage sludge treatment. The land application of biosolids in farming may be restricted due to their toxic trace element content. It is important to note that applying biosolids derived from industrial waste and inadequately treated sources can resulting the accumulation of trace metals in soils, even with short-term application (Silveira et al., 2003; Jordán-Vidal et al., 2020; Popoola et al., 2023). Current regulations and guidance are in place to ensure that biosolids undergo proper processing, handling, and land application procedures, mitigating potential risks to human health. Class B biosolids, may contain minimal pathogens that quickly diminish after soil application, meeting safety standards. Class A biosolids are pathogen-free before application, with metal content requirements identical to Class B. Federal regulation 40 CFR Part 503 outlines these regulations in the USA. Exceptional Quality (EQ) biosolids have stricter metal concentration limits than Class A or B, with pathogen levels akin to Class A biosolids (US EPA, 2023).

Miscellaneous sources

Lead dust and particles have been reported in proximity to areas where pottery glazes are used, where Pb shot is employed at shooting ranges, and where improper storage and processing of Pb materials occur in recycling operations (Hettiarachchi et al., 2024). Lead has been a favored choice in munitions manufacturing for centuries, appealing to its high density, low melting point, and anti-corrosive properties. These characteristics contribute Pb to bullets having enhanced strength and power at extended ranges compared to less dense materials. Additionally, easy molding of bullets and shots, minimal corrosion within gun pipes, high flexibility, and relatively low cost, make Pb an ideal material for bullet production. An average shot released from small-caliber military bullets contains approximately 97% metallic Pb (Alasmary, 2020). The abrasion of Pb bullets and corrosion of remaining shells, bullets, and bombs has the potential to release significant quantities of Pb into the environment (Hardison et al., 2004; Li et al., 2015). The issue of Pb contamination in shooting range soils is extensively documented, ranking as the second-largest contaminator following the battery industry (Bai and Zhao, 2020). Lead ammunition, extensively used in hunting and shooting, constitutes a major, largely unregulated source of Pb discharged into the environment in the USA. Initially considered a threat to gaming birds, it now poses risks to the health of various terrestrial species, and humans who directly or indirectly consume them (Arnemo et al., 2016).

Non-point sources

Lead-contaminated dust and particles of Pb, originating from various point sources, can be transported through wind and runoff water, settling in new locations where their origin becomes challenging to identify and named as non-point sources. Emissions from industrial sites, vehicles, and dust from contaminated soil can be efficiently transported over long distances through windstorms and storm runoff (US EPA, 2022).

Soil and household dust are major sources of children's Pb exposure

Lead is a trace element that has been widely used in various industries, and its extensive use has led to significant environmental contamination. Mining, smelting, manufacturing and recycling activities, a wide range of products and legacy sources are currently important sources of environmental Pb contamination. Human exposure to Pb remains a significant public health concern worldwide, and exposure can occur through various pathways. Drinking water coming through Pb pipes and ingestion and inhalation of contaminated soil, dust, food and some traditional medicines are some key sources of human Pb exposure (US EPA, 2022). Lead, being an unwanted trace element, seems to serve no essential purpose and is deemed potentially harmful to the body. Therefore, there is no safe level of exposure to Pb. Lead toxicity disrupts several body functions, primarily impacting the central nervous, hematopoietic, hepatic, and renal systems, leading to the development of severe disorders (Kalia & Flora, 2005). Acute toxicity related to occupational exposure is now uncommon but chronic toxicity is significantly more prevalent (Flora et al., 2012).

Lead exposure, particularly in young children under the age of 6, can cause serious neurological and developmental issues, and chronic exposure can lead to various health problems. Children at this age are particularly vulnerable to the toxic effects of Pb due to their rapid growth and development. Even low levels of exposure can lead to developmental and cognitive impairments in young children than adults. Pregnant women exposed to Pb may experience adverse effects on fetal development (WHO, 2024).

Due to strict rules and regulations on food and the adaptation of special water treatment practices very less incidents of Pb poisoning are reported concerning food and water (LeBrón et al., 2019; CDC, 2023; US EPA, 2024). However, increasingly low levels of children's Pb exposure are reported worldwide, including in the United States (Abelsohn and Sanborn, 2010; McFarland et al., 2022). One of the major sources identified for low levels of Pb exposure among the children includes contaminated soil and dust (Mielke et al., 2019).

Children’s Pb exposure pathway

The complete exposure pathway concept was introduced by WHO, which enables to identification of 5 components of Pb exposure, 1. source of contamination; 2. environmental medium and transport mechanism; 3. point of exposure; 4. route of exposure; and 5. exposed population (WHO, 2010; CDC, 2024). Exposure to Pb and Pb chemicals in humans can occur through inhalation, ingestion, dermal absorption, absorption from retained or embedded leaded foreign body, and trans-placental (endogenous) routes (CDC, 2024). Ingestion and inhalation are the major sources of ongoing Pb exposure among children (ATSDR, 2023).

Household dust, either indoor or outdoor, contaminated by old Pb-based paint, Pb-contaminated soil, and Pb dust on clothes from working sites is a common risk (US EPA, 2022). The threshold for Pb-contaminated dust that is considered dangerous for children remains unclear. (Lanphear et al., 1996). Many researchers have reported the significance of dust ingestion and/or inhalation as a health concern, emphasizing the importance of this pathway in terms of subsequent Pb exposure for children, over the last four to five decades (Wani et al., 2015; Hauptman et al., 2017). Young children are prone to ingesting substantial amounts of dust as they often stay indoors (Doyi et al., 2020; 2020; Isley et al., 2022; Zhao et al., 2020), play on the ground, and frequently put their hands and objects into their mouths (CDC, 2023). The US EPA estimates that children under the age of 6 consume twice as much dust (20 mg per day compared to 10 mg for adults) and are up to ten times more vulnerable to contaminated dust exposure (US EPA, 2017). Therefore, house dust stands out as one of the primary sources of Pb exposure in children under the age of 5 years. (CDC, 2022). Calabrese et al., (1991) reported that 11% of toddlers may display pica behavior, where they eat nonfood items, and could potentially consume up to 10-13 g of soil per day (Calabrese et al., 1991). As a result, ingestion and inhalation emerge as significant contributors to children's substantial Pb exposure, often originating from unregulated sources and occurring at levels that are less common today.

Household dust contamination with Pb

Lead-contaminated house dust is identified as the major source of exposure for children in the United States (CDC, 2023). Dust contaminated with Pb is primarily generated from leaded paint remains in older buildings and during activities like paint stripping. Additionally, it arises from Pb processing activities such as smelting, recycling, burning of leaded fuel, and soil contaminated with Pb. House dust can become contaminated with old lead-based paint that is present on surfaces frequently in motion or subject to bumping, rubbing, or deterioration. Home repair activities such as scraping, sanding, or heating can contribute to this contamination. Lead-contaminated soil can be tracked from outdoor areas into the indoor environment. Additionally, Pb dust on clothing worn at job sites and from hobbies involving the use of Pb materials can contribute to indoor contamination (US EPA, 2024).

A study conducted in the USA, alongside with the American Healthy Homes Survey (AHHS) to estimate Pb exposure from house dust and soil, revealed that the predominant Pb species in house dusts was highly bioavailable Pb (100%) in the form of (hydroxy)carbonate, indicative of Pb-based paint (Sowers et al., 2021) that was based on in vivo Pb relative bioavailability assessment (RBA). Research conducted by Doyi et al., in 2019 and 2020, found that around 23% of vacuum dust samples from Sydney, Australia, contained Pb concentrations exceeding 300 mg kg^{-1}, which is the Australian guideline for standard residential soil. Additionally, the mean Pb bioaccessibility surpassed 40% based on gastric phase physiologically based extraction technique (PBET) (Doyi et al., 2019 and Doyi et al., 2020) and Pb is mainly derived from garden soil and leaded paint (Dong et al., 2014). Similarly, a study conducted in Canada revealed that 10% of homes had elevated Pb concentrations, with values of 447 mg kg^{-1}, and anomalous concentrations as high as 1730 mg kg^{-1}. These homes also exhibited higher Pb bioaccessibility ranging from 75% to 81%, in contrast to those with background values (63% bioavailable and 58 mg kg^{-1} background soil level). This heightened bioaccessibility may be associated with a higher proportion of Pb carbonate and Pb hydroxyl carbonate (Rasmussen et al., 2011). The mean concentration of Pb in house dust was found to be 195 mg kg^{-1}, which is 7-10 times higher than the background soil Pb concentration in the area and dust Pb bioaccessibility ranged from 53% to 91%, in Shanghai, China. The Pb isotope results indicate that the Pb in household dust is primarily derived from contemporary coal combustion and solid waste incineration, rather than from common legacy Pb sources such as Pb from petrol and paint (Dong et al., 2022).

Soil Pb contamination

Soil is a crucial endpoint for many trace elements, serving as a significant repository for their accumulation. Soils in diverse settings, including rural, urban, forest, and protected areas, are subjected to contamination with trace elements attributed to atmospheric deposition and the dispersion of contaminants through wind (US EPA, 2023). Direct soil Pb contamination arises from human activities such as the improper disposal of industrial wastes, the use of fertilizers and pesticides, sewage water irrigation, vehicle exhaust emissions, and the mishandling and disposal of lead-containing products like batteries and leaded paints. The increasing contamination leads to a significant accumulation of Pb in soils, particularly in the topsoil, due to the persistent nature of Pb. However, the most common type of contaminant in urban soil is Pb from the historic use of leaded gasoline and paint. Furthermore, sources like solid-waste incineration, anthropogenic fill material or debris, the use of pesticides such as lead-arsenate, and construction materials contribute significantly to Pb contamination in major cities with a long industrial history (Landes et al., 2023). Soils close to mining and smelting activities, waste incineration sites, as well as areas used for military and sport shooting, are often highly contaminated with Pb. Background soil Pb concentrations are less than 30 mg kg^{-1}, but soil lead levels in many urban areas exceed 200 ppm. The EPA's standard for lead in bare soil in play areas is 400 mg kg^{-1} by weight and 1200 mg kg^{-1} for non-play areas (US EPA 2020). As of 2024, the US EPA has revised its recommended screening levels for Pb in soil at residential properties. The updated guidance has lowered the reference screening level from 400 mg kg^{-1} to 200 mg kg^{-1}. This adjustment aims to enhance the standards for investigating and cleaning up Pb-contaminated soil in residential areas, with a specific focus on protecting the health of children. At residential properties with multiple sources of Pb exposure, US EPA will generally use 100 mg kg^{-1} as the screening level (US EPA, 2024b).

Lead in soil can be ingested through hand-to-mouth activity, which is common among young children. Additionally, consuming vegetables that may have come into contact with splashed soils and dust and taking up Pb from the garden soil poses a greater risk as Pb uptake and transfer to the aboveground portion from the garden soil is less significant (Attanayake et al., 2014). Furthermore, Pb in soil can be inhaled if it becomes airborne or is tracked into houses, leading to the spread of contamination.

Plant-available, bioavailable and bioaccessible Pb

A large number of studies have been conducted to investigate the toxicity of Pb to soil organisms (plants, invertebrates, and microorganisms) and human health. Researchers have observed a strikingly large variability in the levels of Pb causing toxicity. Some studies indicate incipient toxicity at Pb levels approaching natural background concentrations, while other studies have not identified Pb-related effects even at concentrations exceeding 1000 mg Pb kg^{-1}. Considering bioavailability in relation to Pb toxicity standards is crucial for treatment applications, although total lead-based quality standards currently play a key role for policymakers. Risk assessment solely based on total Pb concentration is unreliable and tends to overestimate the potential human health risks associated with soil contamination. This is because it doesn't account for factors such as bioavailability and specific forms of Pb present in the soil. Striking a balance is essential as these standards can be either under or over-conservative based on specific factors and considerations (Alengebawy et al., 2021; Oorts et al., 2021; Hettiarachchi et al., 2024).

From ingested soil, a portion of total soil Pb in soil solid is potentially available for absorption by the human body (bioaccessible Pb) (Farmer *et al.*, 2011). Human gastric pH is around range from 1.3 to 4 depending on the fasting to fed state, but average is ~2 (Tutuian et al, 2002). Therefore, there is a higher tendency to dissolve Pb in soil solid fractions when they are loosely bound into soil particles and sensitive to pH-dependent dissolution. Lead concentration in soil solution as well as potentially desorbable Pb in the solid fraction is equally important when considering the bioavailability of Pb.

Total and plant-available Pb

Total Pb typically represents all forms of Pb found in any materials such as soil, sediments, plants, or organisms. The total lead content in soil, sediments, plants, or animals is largely estimated by completely dissolving these materials using strong extractants, such as concentrated nitric and perchloric acids, aided by high heat and pressure. In addition, several direct methods/ or instruments have been developed to measure Pb concentrations in these materials (Rieuwerts et al., 1998). Aqua regia and USEPA Method 3051a are the commonly employed wet chemical methods that are used in measuring total Pb in soil and sediment samples. One of the direct analytical methods, specifically the US EPA Method 6200, employs field-portable X-ray fluorescence spectrometry (pXRF) for the in-situ analysis of Pb along with various other elements (Method 6200, 2007). However, measuring the total Pb concentration alone does not provide sufficient information for a comprehensive understanding of fate and transport in the environment. This aspect has been subject to detailed studies over several decades.

Depending on the physical and chemical properties of the soil, Pb can interact with soil constituents and engage in various soil biogeochemical processes. These include sorption between solid surfaces such as minerals, Fe and Mn oxides, and organic matter. Additionally, Pb may be involved in precipitation, nucleation, formation of solid solutions, and redox reactions within the soil (Li et al., 2022a). Therefore, soil solution availability of Pb ions for plant uptake is limited.

The plant-available Pb or phytoavailability can be defined as a portion of soil Pb available for plant uptake. Generally, Pb can exist in the soil as a free ion (Pb^{2+}) in soil solution; complexed with carbonates, oxides and hydroxides; occupying ion exchangeable sites and specifically adsorbed onto inorganic soil constituents (clay minerals); as structure of silicate minerals; and bound to organic matters (McLean and Bledsoe, 1992). The availability of Pb^{2+} ions in the soil solution and their ultimate fate may be determined by one or more soil processes and the dissolution of the lead particles in the soil solution phase. Dissolved Pb may very rarely leach through the soil profile, and this occurs only if it remains in a soluble form (ATSDR, 2021b). It may also be taken up by plants. Although, Pb is neither a vital element nor plays any significant role in cell metabolism it is naturally absorbed and accumulated in several parts of a plant when

Pb exists in the soil as a free ion (Pb^{2+}) in soil solution or Pb-chelate complex (Wu et al., 1999; Egendorf et al., 2020). The majority of Pb absorbed from the soil through their roots is sequestered in root tissues and rarely transported to other parts of the plant.

Bioavailable and bioaccessible Pb

The extent to which a substance or element in the soil is available for absorption into living organisms, such as humans, animals, or plants is known as bioavailability (Hettiarachichi and Pierzynski, 2004). The bioavailable fraction of soil Pb is crucial for human health, as it represents one of the primary exposure pathways through incidental ingestion of soil. This is particularly concerning for children under the age of 6, given their frequent hand-to-mouth activities and enhanced pharmacokinetics. The bioavailability of Pb in a specific medium can be expressed either in absolute terms (absolute bioavailability, ABA) or in relative terms (relative bioavailability, RBA). The fraction or percentage of a chemical that is ingested, inhaled or applied on the skin surface that actually is absorbed and reaches the systemic circulation is known as ABA (Kelly et al., 2002; ATSDR, 2021c; Hettiarachchi et al., 2024). Absolute bioavailability represents the exact value of the ingested dose of Pb that crosses the gastrointestinal epithelium and becomes available for internal distribution in the body specific to the ingested medium (equation 1). If the absorbed value is expressed in a frame of reference for describing the relative amount of Pb ingested through soil that gets absorbed into the systemic circulation of the body, the term used is RBA. A measure of the difference in extent of absorption among two or more forms the chemical or medium is RBA (Kelly et al 2002). Therefore, RBA is the ratio of ABA for soil Pb to that of a water-soluble reference form of Pb, typically lead acetate (OSWER 9285, 2007; US EPA, 2021) (equation 2). The absorbed amount of Pb can vary from 0% to 100%, due to various factors. US EPA assumed that RBA of soil Pb compared to soluble Pb forms is 0.6 (60%) based on 30 % soil Pb bioavailability (ABA) divided by 50 % soluble Pb bioavailability (ABA) (ITRC, 2017; US EPA, 2021).

$$ABA\ (\%) = \frac{absorbed\ dose}{adminstered\ dose} \times 100 \qquad \text{(Equation 1)}$$

$$RBA\ (\%) = \frac{absorbed\ fractin\ from\ soil}{absorbed\ fraction\ from\ dosing\ medium\ used\ in\ the\ study} \times 100 \qquad \text{(Equation 2)}$$

Bioaccessible Pb is defined as the fraction of ingested Pb dissolved in the gastrointestinal tract and that is in a chemical form that can be absorbed by the small intestine resulting in Pb bioavailability. The bioaccessibility of Pb is influenced by factors such as solubility, speciation, and the presence of modifying soil properties, which can lead to the formation of insoluble solid phases compounds (Zhang et al., 2019; Lake et al., 2021). Although, RBA Pb can be directly measured using acceptable animal in vivo models (e.g., juvenile swine, adult mouse, primate), laboratory in vitro soil extraction-based methods have also been developed to predict RBA Pb. In these approaches in vitro bioaccessible Pb is measured, and a validated in vivo-in vitro regression equation is then used to predict RBA Pb. Therefore, in vivo methods measuring RBA Pb provide valuable information for risk assessors. In contrast, in vitro methods simulate the gastrointestinal condition to measure bioaccessibility. Bioaccessibility is typically expressed as a fraction of the total Pb content, providing a percentage that represents the amount accessible. This percentage can be further converted to relative bioavailability (Hettiarachichi and Pierzynski, 2004; Scheckel et al., 2009). Valid in vitro methods have been established and those were validated by correlating to animal feeding models.

Measuring bioavailable/ bioaccessible Pb

For human health risk assessment purposes and as an indicator of treatment success, measuring bioaccessible Pb is crucial. Relative Pb bioavailability measurement helps to understand the chemical and physical behavior and forms of Pb in exposure matrices (e.g., food vs. water vs. soil) (Casteel et al., 2006). Several studies have correlated in vitro and in vivo bioavailability estimates (RBA) for lead in their studies. Some studies have applied various in vivo methods and in vitro bioavailability estimation methods for Pb and some other trace elements. The ABA and RBA are measured in in vivo animal models other than predicted from in vitro bioaccessibility assays. In vitro bioaccessibility assays estimate bioaccessibility from measurements of in vitro solubility of soil Pb (ITRC, 2017).

In vivo methods

Most of the in vivo research conducted so far has primarily focused on oral bioavailability study methods that utilize soil to investigate metal absorption in laboratory animals (Kelly et al., 2002). Bioavailability data from actual human soil feeding tests is scarce due to regulatory, ethical and risk-associated factors (Wragg et al., 2011). While in vivo animal studies have been conducted, they are generally expensive, time-consuming, face ethical constraints, and often have limited soil availability. These studies typically measure Pb concentrations in various body parts or excreta after administering doses over a specified period of time and intervals (Zia et al., 2011; Denys et al., 2012).

Measuring oral bioavailability in soil Pb can be carried out using multiple methods simultaneously. This includes measuring blood Pb concentration, assessing Pb levels in feces and urine as a fraction of the dose, and determining Pb concentrations in various tissues such as bone, kidney, liver, etc. Nevertheless, numerous studies have employed measurements of blood, bone, liver, and kidney Pb concentrations following the ingestion of contaminated soil (Casteel et al., 2006; Deshommes et al., 2012; Bradham et al., 2018). These concentrations are then compared with those obtained from soluble lead acetate to calculate RBA. These studies predominantly utilized weaning rats and swine as animal models because, to the extent possible they mimic the physiology of children. Weanling swine would be a preferable choice in Pb animal feeding studies for surrogate bioavailability in children. This is because they closely resemble children in terms of stage of development, weight, omnivorous behavior, and physiological maturity. Additionally, studying blood Pb concentration as a time series without adversely affecting the health status of swine makes them a more suitable animal model. Weanling rat also used in Pb absorption studies due to rapid growth and active bone formation resembles the period in children in which they are most vulnerable to Pb toxicity (Kelly et al., 2002; Denys et al., 2012; Deshommes et al., 2012). Except for blood all other biological tissues were collected and tested after the study was terminated. The feeding, diet, and health status of animals need to be well-monitored in vivo studies. Reference standards include animals dosed with soluble Pb is Pb(II) acetate[$(CH_3CO_2)_2$ Pb.$3H_2O$] (Kelly et al., 2002).

In vitro methods

The history of in vitro extraction tests is closely tied to assessing the dissolution of metals in the gastrointestinal tract environment. This approach has been involved in studying the bioavailability of iron in the context of nutritional studies, particularly focusing on its release from food in the digestive system (Kelly et al., 2002). The principle of in vitro models is that the extraction procedure should simulate the gastrointestinal tract and its conditions. In vitro methods are primarily employed to measure the bioaccessible fraction, which can then be used to estimate bioavailability. Several in vitro bioaccessibility tests are specifically designed to simulate the human digestive process, encompassing three fundamental phases: mouth, stomach, and small intestine (Oomen et al., 2003; Wragg and Cave, 2003, Dean and Ma, 2007).

The test materials intended for evaluation, such as soil, undergo extraction in a low pH solution for a specific duration, simulating the residence time and incorporating mechanical shaking to mimic the agitating conditions of the stomach. Following the initial low pH extraction, the pH is elevated to near neutral levels, and the incubation process persists for a specified period designed to replicate the conditions of the small intestine. The extraction solution, prepared to mimic distinct phases of the gastrointestinal tract, includes a combination of enzymes and organic acids. To regulate pH, HCl and NaOH is employed in the extraction solution at each phase. Most current in vitro tests aim to replicate pediatric gastrointestinal conditions, specifically focusing on fasting states (Kelly et al., 2002).

The critical design factors thoroughly investigated in the in vitro method development include solution chemistry, extraction temperature and duration, mixing rate, and the particle size of the test material (Drexler and Brattin, 2007; Ruby et al., 1996). Thus, there are two major differences in in vitro methods: variability in the composition of the extraction solution and the pH used in the stomach phase. Common batch extraction methods that closely mimic the chemical environment of the human gastrointestinal system include the physiologically based extraction test (PBET) initially developed by Ruby et al. (1996), the in vitro gastrointestinal method (IVG) (Rodriguez et al., 1999), the Dutch National Institute for Public Health and the Environment method (RIVM) (Versantvoort et al., 2004), mainly used in Europe, and the unified BARGE method (UBM) developed by the bioaccessibility research group of Europe (BARGE). However, these methods have differences in the composition of each extraction solution. To evaluate Pb bioaccessibility and bioavailability through an in vitro method, comparing and correlating the results with in vivo data is essential. These methods have been validated for Pb, As, and occasionally Cd with in vivo models.

The methods, relative bioaccessibility leaching procedure (RBALP) which was developed specifically for Pb in soils (Drexler and Brattin, 2007) and the test Method 1340: in vitro bioaccessibility assay for Pb (IVBA) or solubility/bioavailability research-consortium (SBRC) (Method 1340, 2017; Ruby et al., 1999) in soil are physiologically relevant pH of the stomach but uses a glycine buffer as the extraction medium. The time, temperature, solid-to-solution ratio, and extraction/ rotation speed are all most similar in all these methods.

Over the past two decades, numerous studies have employed in vitro methods to measure bioaccessibility. However, there are differences in the composition of extracting solutions, pH, and soil fractions used across these studies. Additionally, the scientific community has not reached a consensus on a standard method for assessing Pb bioaccessibility. Correlation and comparisons of studies across different methods are challenging due to these variations. Even within the same method, differences arise, with two studies employing different pH values or focusing on distinct soil fractions (Obrycki et al., 2016). Based on current recommendations and wider acceptability, the in vivo correlation between PBET and IVBA has gained prominence, representing two contrasting methods employed in recent studies.

Physiologically Based Extraction Test (PBET)

The Physiologically Based Extraction Test (PBET) serves as an in vitro test designed to predict the bioavailability of metals from a solid matrix, initially developed by Ruby et al., 1996. It integrates gastrointestinal tract parameters representative of a human, encompassing stomach and small intestinal pH and chemical composition, soil-to-solution ratio, stomach mixing, and stomach emptying rates (Ruby et al., 1996). The initial study showed that for Pb, the PBET results exhibit a linear correlation with outcomes from an in vivo (Sprague-Dawley rat) model, with an r-squared value of 0.93 between in vitro and in vivo results (n=7). Modifications were then added by later studies by Medlin, 1997 into this study (Medlin, 1997; Attanayake et al., 2017).

The gastric solution for the PBET is a combination of different ratios of pepsin, citrate, malate, lactic acid, and acetic acid and HCl. During the gastric phase of PBET (PBET-G) extraction, a critical step is to maintain the pH within the range of 2.5±0.5. This is achieved by periodically adding HCl as necessary. Following a 1-hour PBET-G extraction, the reaction solution's pH is adjusted to 7 by adding $NaHCO_3$. Subsequently, porcine bile salts and pancreatin are introduced, initiating the intestinal phase, which is allowed to proceed for 1 and 3 hours. Both extractions are recommended to be performed at a temperature of 37°C, which is identical to the human body temperature. This ensures that the experimental conditions closely mimic physiological processes. Additionally, a soil-to-solution ratio of 1:100 was employed, and thorough mixing was adopted as part of the experimental procedure (Ruby et al., 1996; Basta et al., 2016; Attanayake et al., 2017).

Researchers have observed a notable decrease in Pb bioaccessibility during the intestinal phase, attributed to pH neutralization leading to Pb precipitation (Zia et al., 2011; Boros et al., 2017). As a result, the intestinal phase generates greater variability. Therefore, many later studies chose to focus exclusively on the gastric phase for their investigations (Hettiarachchi et al., 2003; Li and Zhang, 2013; Boros et al., 2017; Cao et al., 2019; Du et al., 2020).

In Vitro Bioaccessibility Assy (IVBA)

The In Vitro Bioaccessibility Assy (IVBA), also known as SBRC (Solubility Bioavailability Research Consortium), is a method developed through collaboration among regulators, academics, and industry members. Its purpose is to estimate the relative bioavailability of Pb. This collaborative effort involved various stakeholders to enhance the understanding of Pb bioaccessibility and contribute to effective assessment methods. The EPA has validated an IVBA for predicting soil Pb RBA in the context of human health risk assessment. The recommendation is to utilize this assay for characterizing site-specific soil Pb RBA (Method, 1340). The recommended assay involves a gastric-phase extraction of soil in a simple extraction medium composed of a glycine buffer with pH adjusted to 1.5 using HCl, although pH adjusted to 2.5 also gave comparable r^2 values when correlated with in vivo verification studies. The specified extraction procedure involves mixing the soil with 0.4 M glycine in an end-over-end shaker at 37°C for a duration of 1 hour.

However, several studies have stated that the IVBA method with a pH of 1.5 is ineffective when applied to soils treated with P for risk assessment, giving unexpectedly higher bioaccessibility (Obrycki et al., 2016). These findings suggest limitations in the applicability of the method to certain soil conditions, particularly those treated with phosphorus.

Advantages and disadvantages of in-vitro methods

In current risk assessment studies, there is a growing preference for utilizing in vitro models that simulate the gastrointestinal (GI) tract as an alternative to traditional in vivo animal or human models. In vitro methods offer significant advantages, including time-saving and cost-effectiveness. These methods can be conducted within a day, incurring only a fraction of the expenses associated with in vivo studies. However, a notable drawback of in vitro methods is that, as screening tools, they are primarily designed to measure the bioaccessible contaminant fraction. This measurement may not provide a direct assessment of bioavailability. The reliability of in vitro bioaccessibility tests in risk assessment studies can be questionable due to the multitude of available tests and variations in gastric conditions used in these tests. The reliability of in vitro methods depends on their correlation with in vivo models. Some methods exhibit correlation only for single elements, making it challenging to study multielement contamination and its associated effects with in vitro models (Kelly et al., 2002; Zia et al., 2011; Ollson et al., 2018).

Soil processes controlling Pb bioavailability

Soil can be characterized as unconsolidated material covering the Earth's surface. It serves as a primary source providing water and vital plant nutrients crucial for plant growth and the sustenance of terrestrial living organisms. Additionally, soil plays a crucial role in receiving, filtering, and processing waste products. Its capacity to regulate the movement of toxic substances helps prevent their entry into groundwater or living organisms (Essington, 2015). The natural abundance of Pb is confined to very low concentrations, although it is present in various types of rocks, soils, and sediments (NASEM, 2017; Lovering, 1976). Elevated levels of soil Pb are attributed to human activities, specifically mining, smelting, industrial waste disposal, and the persistence of historical sources such as leaded gasoline and paint (Alloway, 2013; Haque et al., 2021). Metallic lead (Pb(0)), Pb(II), lead oxides and hydroxides, and lead metal oxyanion complexes are the most common forms of Pb that are released into the environment (Wuana and Okieimen, 2011). Based on their physical and chemical properties, Pb compounds can engage in various interactions with soil constituents, participating in several soil biogeochemical processes.

Apart from the total contaminant concentration, the bioaccessible fraction is considered the most crucial fraction of a contaminant that is available for absorption into living organisms (Hettiarachchi and Pierzynski, 2004; Scheckel et al., 2009). The gastric pH enhances the dissolution of Pb in soil solid fractions, particularly when it is loosely bound to soil particles and susceptible to pH-dependent dissolution. Both the Pb content in the soil solution and the Pb present in the solid fraction are crucial factors in determining the bioavailability of Pb. The toxicity and hazard posed by Pb in soils are primarily influenced by soil properties and the relative distribution of Pb species (Kumar et al., 2020). The main soil processes include adsorption onto organic and inorganic constituents and precipitation of slightly soluble complexes. Additionally, Pb compounds may be involved in precipitation, nucleation, the formation of solid solutions, and redox reactions within the soil environment. These interactions contribute to the complex dynamics of Pb in soil biogeochemistry. (Hettiarachchi & Pierzynski, 2004; Miretzky & Fernandez-Cirelli, 2008; Borgese et al., 2013; Li et al., 2022a), and dilution with the help of external material addition. In general, soil Pb can be held in cation exchange sites in clay fraction, precipitated with oxides of iron and manganese, present as complexed with phosphate and carbonates, or bound with insoluble organic species (McLean & Bledsoe, 1992; González-Costa et al., 2017). Lead from both natural and human-made sources initially dissolves in the soil solution and subsequently transfers to the solid phase through the above-mentioned processes. Conversely, Pb in the solid phase can move back into the solution phase through desorption and dissolution of the soil solid fraction. The boundaries between these processes are not clearly defined, dynamic and evolving conditions of the soil environment continually influence them.

Adsorption

Adsorption via cation exchange

Lead is commonly found in the highly stable form of Pb^{2+}, representing a divalent cation. This divalent state is a prevalent and stable form of Pb in various environmental contexts. Ion exchange is primarily influenced by the extent of negative charges present on the surfaces of soil colloids and the availability of various cation species, along with their respective charges in the solution and on the soil surface. The interplay of these factors plays a significant role in the ion exchange processes occurring in the soil (Lee et al., 1998). The negatively charged sites on soil colloids attract the Pb^{2+} cations, forming electrostatic bonds. The availability of more negatively charged sites, indicated by a larger Cation Exchange Capacity (CEC), enhances the tendency for Pb retention in the soil (Sumathi et al., 2010).

Negative charges on soil colloidal surfaces can be either pH-dependent or permanent. To maintain a balance between negative and positive charges, cation exchange is preferred. The negative charge on the surface is counterbalanced by an equivalent quantity of cations from the soil solution, helping to preserve the overall charge balance (Bradl, 2004). Cations form weak, electrostatic bonds with the soil surface and can be easily exchanged with other cations. The strength of cation retention on colloidal surfaces is influenced by factors such as valency (charge) and hydrated radii (larger in cations with smaller ionic radii) when they have equal charge. Lead demonstrates one of the highest affinities for soils among heavy metals, attributed to both its strong retention on the soil surface through weak, electrostatic bonds and its favorable combination of valency (charge) and hydrated radius. As a result, competition, even in the presence of competing cations, has a minimal effect on Pb due to its strong retention on the soil surface (Sipos et al., 2005).

Specific Adsorption

The formation of covalent bonds between metal cations and charged mineral surfaces is a process known as specific adsorption. Both organic and inorganic colloids from soil solid fractions play a role in specific adsorption, and this phenomenon is highly dependent on pH. Highly selective adsorption sites specifically adsorb metal cations which have affinities for particular sites despite competition and total concentration. Lead has superior adsorption compared to Cd, Zn and Cu. However, increasing metal concentrations and increasing saturation of surface sites, can limit the adsorbing surfaces for particular metals, thus, soil solution concentration might increase (Appel and Ma, 2002; Bradl, 2004; Sipos et al., 2008). Over time, the labile Pb fractions in soil, including water-soluble and ion-exchangeable fractions, are transformed into organic-bonded, and oxide-bonded forms of Pb. This conversion reflects a shift from more easily accessible and potentially harmful Pb fractions to more stable and less environmentally harmful states (Ming et al., 2012). While adsorbed Pb can be relatively inert, lead oxides, sulfates, and carbonates exhibit high solubility in acidic to circumneutral environments. Soil Pb in these solid forms can pose a significant environmental risk due to the potential for dissolution and release into the surrounding environment. The solubility characteristics of these Pb compounds make them more mobile and accessible, contributing to their negative environmental impact (Yan et al., 2019). Bargar et al. (1997) and Roe et al. (1991) revealed that Pb could adsorb to goethite by creating inner-sphere surface complexes with the surface hydroxyl (-FeOH) groups, based on X-ray Absorption Fine Structure Spectroscopy (XAFS) and X-ray Absorption Spectroscopy investigations. This interaction involves the formation of bonds between Pb and the hydroxyl groups on the goethite surface (Roe et al., 1991; Bargar et al., 1997).

Precipitation

Precipitation is another soil process that forms a solid metal-containing phase together with a metal cation and an anion such as phosphate, carbonate, or sulfate. Metals may also be precipitated as metal hydroxides, removing OH^- ions from the solution (Neina, 2019). Lead is preferred to precipitate with the presence of excess phosphate and sulfate ions in the soil solution. Pyromorphite or Pb-phosphate minerals could precipitate in a wide variety of soils and sediments. These precipitated Pb products are highly stable in a wide variety of environmental conditions, including a wide range of pH. Lead is also adsorbed and coprecipitated with Fe and Mn oxides and hydrous oxides (Traina & Laperche 1999; Basta et al., 2005; Hettiarachchi & Pierzynski, 2004).

The orthophosphate ion can form sparingly soluble solid precipitates with Pb and various other toxic metals. The application of phosphates has been explored extensively to assess the effectiveness of using phosphates to mitigate the mobility and bioavailability of Pb in different soil environments. Typically, Pb-phosphates exhibit significantly lower solubility compared to common Pb-solids such as galena, anglesite, cerussite, litharge, and crocoite found in soils contaminated by mining and smelting activities and paint. According to log Ksp, solubility of the pyromorphites decreases fluoropyromorphite > hydroxypyromorphite > bromopyromorphite >chloropyromorphite, (−72, −77, −78, and −84, respectively). The precipitation of pyromorphite is a process where Pb^{2+} in a solution reacts with phosphate ions to form the mineral pyromorphite ($Pb_5(PO_4)_3X$) (equation 3). The X would be halogen (F, Cl, Br) or hydroxyl (Traina & Laperche 1999). The reaction indicates that the P-induced precipitation of pyromorphite was greatly influenced by the pH (H^+) (Hettiarachchi and Pierzynski, 2004).

$$5Pb^{2+} + 3H_2(PO_4)^- + H_2O \rightarrow Pb_5(PO_4)_3OH + 7H^+ \quad \text{(Equation 3)}$$

Complexation and Chelation

Complexation involves the formation of polyatomic structures and can divided into outer-layer complexation and inner-layer complexation. This process involves the formation of specific metal-ligand interactions via complexation between metal ions and functional groups (Nie et al., 2021). Complexation may be in the form of chelation, where the complex-forming ligands form two or more coordination bonds with the metal ion. Dissolved Organic Matter (DOM) in soil consists of organic matter with various molecular weights, including organic acids and humic substances. These DOMs contain surface functional groups such as carboxyl and hydroxyl groups, facilitating the transformation of Pb into insoluble forms together with soluble forms (Yamada & Katoh 2020). Organic ligands include citric, oxalic, or gallic acids or fulvic and humic acid fractions in soil organic matter. The most common inorganic ligands include hydroxide and chloride ions. Metal complexing ligands can bind to the soil surface or may remain in the solution due to low affinity for the soil’s solid surface. Lead forms strong complexes with organic matter, so it is often found in complete retention within the surface layer of organic matter-rich soil when having higher atmospheric Pb deposition. In acidic soils, the complex or chelate formation may play an important role (Rieuwerts et al., 1998).

Soil properties controlling soil Pb bioavailability

The combinations of mineral substances, organic matter, water, air, and microorganisms in soil collectively determine its three major properties: chemical, physical, and biological. These properties interact to influence the overall characteristics and behavior of the soil environment. The total soil Pb concentration is influenced by various factors, including the natural inherent mineral soil Pb content and additional sources resulting from human activities. However, the solubility, bioavailability, and mobility of Pb are governed by several geochemical and biogeochemical processes. The efficiency, type, and rate of these processes are influenced by inherent soil properties and environmental conditions. Soil Pb bioavailability is primarily determined by the partitioning of metals between the solid and solution phases (Miretzky and Fernandez-Cirelli, 2008).

Soil pH

Soil pH can be considered the "master variable" in soil chemistry because of its significant influence on various reactions that govern Pb solubility. It plays a crucial role in determining the bioavailability and mobility of Pb in the soil. The pH of the soil directly affects the chemical speciation of Pb, influencing its interactions with other soil components and ultimately determining its environmental behavior (Apple and Ma, 2002; Uchimiya et al., 2020). Soil pH is a measure of soil acidity or alkalinity, represented by the inverse log of the Hydrogen ion concentration on a scale from 0 to 14. The mechanism underlying soil pH involves the movement of elements between solid and liquid soil phases through precipitation-dissolution reactions. This dynamic process influences the availability and mobility of various elements, including Pb, within the soil environment. The soil solid phase comprises primary and secondary mineral fractions (inorganic) with sizes ranging from < 2µm clay size fraction to >2mm large gravels and rocks and, organic fractions including plant and animal material at various stages of decomposition. Both inorganic and organic components carry pH-dependent charges that play a crucial role in controlling the availability of trace elements, such as Pb, in the soil. The pH-dependent charges influence the interactions and mobility of these elements within the soil environment (Neina, 2019). Lead sorption is a significant process in soils and mostly controlled by pH. The pH influences Pb speciation and the charge of soil surface groups, impacting the sorption capacity of soil components. Variations in pH play a significant role in determining the fate and mobility of Pb in the soil environment. This pH-dependent relationship underscores the importance of soil chemistry in understanding the behavior of lead in soils (Martı´nez-Villegas et al., 2004).

In acidic conditions, adsorption is an important process for reducing metal concentration in soil solution while precipitation of solid phases is favorable in alkaline conditions. Specific adsorption exhibits strong pH dependence, and complexation may occur at near-neutral pH. Cation adsorption and exchange are sometimes pH-dependent. The pH-dependent surface charges are associated with oxide and hydroxide minerals, as well as specific functional groups within humic substances. Sorption of metals is directly proportional to soil pH, and it may be permanent in some cases. The intricate interplay of these processes emphasizes the pH sensitivity of metal interactions in the soil environment (Apple and Ma, 2002; Guanxing et al., 2011; Uchimiya et al., 2020)

At low pH, metal adsorption decreases because H^+ (and Al^{3+}) ions compete for adsorption sites. Higher pH conditions promote metal hydrolysis and may facilitate the formation of strong, preferential adsorption in the form of metal hydroxy complexes. Beyond pH 6, nearly all Pb ions are removed from the solution, likely as adsorbed hydroxy species. According to Guanxing et al., (2011) Pb undergoes multiple hydrolysis reactions at low pH. Below pH 5, the predominant speciation is Pb^{2+}, while between pH 6 and 9 $Pb(OH)^+$ is predominant, and the formation of $Pb(OH)_2$ is predominant between pH 9 and 11 (Guanxing et al., 2011). The reduced adsorption at low pH may be attributed to acid-catalyzed dissolution of oxides and their adsorption sites. Critical pH values are approximately 3–5 for Pb when considering adsorption on Fe oxides. Low pH around 4.2, requires 70–200 years to leach out 10% of the Pb in an organic A horizon compared with 17–20 years at pH 2.8 (Rieuwerts et al., 1998; Apple and Ma, 2002; Guanxing et al., 2011).

Many researchers have shown that Pb adsorption follows the Langmuir relation, exhibiting an increase with increasing pH above 3.0, particularly in the near-neutral to alkaline pH range. The extent of this relationship depends on other soil properties (Lee et al., 1998; Rieuwerts et al., 1998; Martı´nez-Villegas et al., 2004; Weng, 2004; Basta et al., 2005; Sipos et al., 2005; Shi et al., 2013). Martı´nez-Villegas et al., 2004, revealed that the sorption of lead must be inherently linked to charges of soil surface groups according to pH. As the concentration of H^+ decreases, Pb sorption increases. This is attributed to the deprotonation of hydroxyl groups, resulting in an increase in negative charges on the soil surface. The lowered competition between H^+ and metallic cations for sorption sites contributes to the enhanced sorption of Pb. In a study, conducted on a regosol eutric soil from Mexico, isotherms indicate that the soils have a limited capacity for sorption of Pb and the maximum was observed at pH = 5.5 (Martı´nez-Villegas et al., 2004). Sipos et al., 2005 reported that above pH ~6 the relative adsorption of Pb is nearly 100%. However, below this pH, the adsorption decreases quickly on the genetic horizon of a natural brown forest soil profile used in the study. In addition, the presence of high carbonate content hindered likely adsorption due to common precipitation and adsorption of lead (Sipos et al., 2005). Weng, 2004 has shown that the surface acidity of hydrous sandy loam soil used in their model development, pH is the key factor affecting the characteristics of Pb(II) adsorption onto hydrous soil particles. The amount of Pb(II) adsorbed increased with increasing pH and decreasing surface loading (Wang, 2004). Lee et al, (1998) studied the adsorption characteristics of fifteen Taiwan soils and found that adsorption of Pb(II) followed the Langmuir relation and increased with increasing pH between pH 3.0 and 8.5. Basta et al, (2005) explained the possible chemical reaction pathways behind the higher Pb and other metal cation adsorption capacity with the increasing soil pH.

pH effect on the solubility of lead-bearing minerals

Bioavailability, determined by the release of Pb from the solid phase to the aqueous phase, plays a crucial role in determining its potential impact on living organisms. Hettiarachchi and Pierzynski, (2004) reported the equilibrium reactions of thirty-four different Pb-containing minerals and complexes found in soil, sediments and mining materials. Pyromorphites are the most stable forms of Pb under a wide range of pH conditions. The solubility of Pb^{2+} is highest at low pH ($pH < 5$) and gradually decreases with increasing pH. In contrast, the total dissolved Pb follows a different pattern, being high at low pH, decreasing until a pH of approximately 6, and then increasing again at higher pH (>8.5). These observations highlight the strong Pb-complexing capacity of the soil. At near neutral or higher soil pH (pH >6.5,) soil lead is held more strongly, and its solubility is very low (Sauvé et al., 1998). Therefore, in general, lead has low solubility in the slightly acidic to alkaline pH range ensuring low plant uptake, and less impact on soil organisms. The low solubility is further enhanced by the presence of adequate amounts of phosphorus, carbonates, and organic matter. In summary, the adsorption of Pb becomes significant at approximately pH 3–5, while precipitation of insoluble solids appears to become important at approximately pH 6–7 for Pb. These pH ranges indicate Pb interactions with the environment undergo notable changes, impacting its behavior and fate in the soil. (Rieuwerts et al., 1998; Yang et al., 2006; Yu et al., 2023). Several studies have reported that Pb bioavailability and toxicity are related to the concentration of dissolved Pb in soil solution regulated by soil pH. Raising the pH of a silty clay loam spiked with 1,000 mg Pb kg^{-1} from 3.8 to 5.2, and the reported lettuce tissue Pb concentration was reduced from 233.6 to 54.3 mg kg^{-1}. Liming a clay loam soil from pH 4.75 to pH 6.85 reduced the uptake of Pb in five plant species by 34% and Pb content of lettuce grown on 29 Pb contaminated soils was related to soil pH and not to the OM content of those soils (Dayton et al., 2006).

Soil textural effect on lead adsorption and solubility

Soil particles serve as the fundamental structural unit of soil, and their size determines the soil texture. There are three primary size classes based on the nature of soil particles: sand (>50-2000 μm), silt (2–50 μm), and clay (<2 μm). Soil texture, influenced by the proportions of these particles, is a master variable that significantly affects various soil physical and chemical properties. These properties include bulk density and porosity, aeration, water holding capacity, surface area, cation exchange capacity, and others. The composition and arrangement of soil particles play a crucial role in shaping the overall characteristics and behavior of the soil.

Researchers have observed that particle size has a substantial impact on the stability of lead and other trace elements in soil. The size of soil particles influences the surface area, reactivity, and interactions with contaminants like Pb. The stability of Pb in soil can vary depending on the specific particle size distribution, with finer particles often having a greater capacity to retain and stabilize contaminants than coarser particles. Understanding the role of particle size is crucial for assessing the fate and transport of Pb in soil environments (Yu et al., 2023). The significant attraction of Pb and other metals to soil particles ranked by clay > silt > sand in terms of adsorption (Rieuwerts et al., 1998). Clay particles in soil possess the largest surface area among soil particles, contributing to increased CEC, total porosity, micropores, WHC, and OM content. The accumulation of Pb in the clay fraction is attributed to its extensive surface area, providing ample space for adsorption. Additionally, the presence of clay minerals, organic matter, Fe–Mn oxides, sulfides, and negative surface charges on clay particles enhances the ability of clay particles to retain and adsorb metals, such as lead. These complex characteristics make the clay fraction a significant factor in influencing the fate and behavior of metals in soil environments (Apple and Ma, 2002). Therefore, they have more affinity in binding positively charged cations and have a higher capacity to carry trace metals than coarse particles (Uddin, 2017). Clays also contain exchangeable cations and anions held to the surface. Clay fraction consists of a variety of fine-grained secondary minerals such as layered silicate minerals, crystalline oxides, and amorphous oxides (Li et al., 2022a). Aluminosilicates (hydrous) are common clay minerals and are co-associated with a variety of Fe and Mn oxide and hydroxide minerals, as well as carbonate, sulfate, sulfide, and chloride minerals, depending on the particular soil environment (Schulze, 2023). These clay minerals are the most active components of soils that significantly affect the fixation and migration of Pb in soil.

Lead, when compared to other metals, exhibits a high attraction and adsorption affinity for various types of clay. The unique properties of Pb and the specific characteristics of clay minerals contribute to these strong interactions (Kayode et al., 2019). The adsorption of Pb on clay particles consists of two stages: an initial fast adsorption phase followed by a secondary slow sorption phase. The fast adsorption stage, known as physisorption, involves instantaneous retention to the surfaces of solid particles. This phase is initiated by forming outer sphere complexes, driven by electrostatic or physical interactions. Cations (Pb^{2+}) undergo movement across a concentration gradient, transitioning from the solution phase to the solid phase, where they are subsequently attracted by negatively charged clay particles. The secondary slow sorption, also known as chemisorption, occurs as these cations gradually transform into inner-sphere complexes located at the edges of the soil minerals. This process involves a progressive conversion, contributing to the formation of stable bonds between the cations and the soil matrix. (e.g., Si-OH and Al-OH sites) (Uddin, 2017). The mode and rate of retention of Pb among various Pb species in clay minerals can change over time (Fendorf et al., 2004). In summary, clay minerals involve a series of complex adsorption mechanisms through direct bonding between metal cations with the surface of clay minerals, surface complexation, and ion exchange (Uddin, 2017).

Clay minerals can be categorized into major groups, including layer and chain silicates, sesquioxides, and other inorganic minerals. The layer silicates can be classified as 1:1, 2:1, and 2:1:1 based on silica tetrahedral and alumina octahedral sheets involved in their structures. The 1:1 (e.g., kaolinite) and 2:1 (e.g., smectite, illite) clay minerals occur predominantly in all soils, but 2:1:1 (e.g., chlorite) are found in temperate climates. The chain silicates (e.g., palygorskite), are found in a few soils, and the sesquioxides occur universally in all soils with varying degrees of crystallinity, such as oxides/oxyhydroxides of Fe and Al, and they can account for 50% of the total soil mass. The key members of the crystalline sesquioxides include goethite, hematite, and magnetite, whereas ferrihydrite is the most common non-crystalline mineral (Sarkar et al., 2018).

The average particle size of montmorillonite is generally much smaller than that of kaolinite giving a specific surface area of montmorillonite (15–160 m^2 g^{-1}) than kaolinite (6–40 m^2 g^{-1}). Smectite (montmorillonite and vermiculite) produces a much larger layer charge than kaolinite due to higher isomorphous substitution, is subsequently creating more active sites. A large proportion of the adsorption capacity is due to permanent, pH-independent charges. Cation adsorption by expandable clays such as vermiculite and smectite occurs largely in inter-layer spaces compared to the inactive surfaces. As a result, CEC in kaolinite varies between 0 and 10 cmol (p+) kg^{-1}, and for smectite can be as high as 160 cmol (p+) kg^{-1}. However, illite (mica) contains a higher specific surface area (55–195 m^2 g^{-1}) but a medium-range CEC (10–40 cmol (p+) kg^{-1}). Both sesquioxide groups with unique physicochemical characteristics and hydroxide/oxide minerals have a very high specific surface area (700–1500 m^2 g^{-1}). As a result, these materials have been reported to retain remarkably greater amounts of metals than the layered silicate clay minerals. These minerals are also able to take part in specific chemical complexation (e.g., ligand exchange) with soil organic matter (Rieuwerts et al., 1998; Li and Li, 2000; Sarkar et al., 2018).

The maximum adsorption capacity of Pb in kaolinite (1:1) is 7.75–13.32 mg g^{-1}, montmorillonite is 31.10–75.35 mg g^{-1} (Dayton et al., 2006). Many studies have shown that Pb^{2+} can form inner-sphere complexes with Al hydroxyl groups on the surfaces of kaolinite (Li et al., 2022a). Clay minerals, particularly swelling clay minerals, exhibit notable adsorption capacity for Pb.

According to Sipos et al. (2008), vermiculite, smectite, and Fe-smectite showed Pb adsorption in the range of 0.0–0.5 at.% along with copper (0.3–0.7 at.%) and zinc (0.0–0.8 at.%). Interestingly, higher Pb adsorption was observed on iron-containing illite particles. Clay minerals are commonly found in soils with mixed structures and phases. The study revealed that illite/smectite and illite/vermiculite/smectite phases adsorbed similar amounts of metals to the iron-free illite particles (Sipos et al., 2008). Covelo et al., 2007 reported that Pb has higher sorption than Zn, Cd, Ni, and Cu but less than Cr in kaolinite and mica, and higher than Cd, Cr, Ni but less than Cu and Zn in vermiculite in competitive sorption retention study. The highest Pb retention was recorded in mica and vermiculite (Covelo et al., 2007). Lead has the ability to replace K, Ba, Sr, and even Ca, both in minerals and in sorption sites of clay. Martínez-Villegas et al., (2004) reported that Pb sorption is highly probable where secondary minerals such as kaolinite, halloysite, and metahalloysite; have broken edges and/or permanent charge sites facilitated by isomorphous substitutions (Martínez-Villegas et al., 2004).

Effect of Fe and Mn oxides

Hydrous Fe and Mn oxides occur in clays as coatings on phyllosilicates and as free gels and crystals (Post, 1999). Lead is a redox-stable divalent cation found in soil with a high affinity for soil solids in clay minerals, Fe and Mn oxides, and organic matter (Fendorf et al., 2004; Sipos et al., 2005). Low concentrations of these oxides are usually found in reducing conditions and hydrous Fe and Mn oxides may have an impact on metal solubility under relatively oxidizing conditions. Galena (PbS) is usually found in ore deposits and is highly stable under reduced conditions. When galena is exposed to an O_2 environment it readily transforms into an oxidized form of Pb, such as $PbSO_4$, $PbCO_3$ and $Pb(PO_4)X$ (X=Cl, F, OH). They also can be incorporated in clay minerals, in Fe, Al and Mn oxides, and in organic matter. Iron and Mn oxides showed both the oxidation performance of manganese oxide and the high adsorption capacity of iron oxide (González-Costa et al., 2017; Li et al., 2022b).

In aqueous solutions, metals can act as a Lewis acid (i.e., an electron acceptor). An electron-pair donating surface functional group (such as -OH, -SH, and -COOH) and an electron-pair acceptor metal ion (such as Pb^{2+}) form Lewis salt-type compounds. For an oxide (e.g., ferric oxide) the functional surface hydroxo groups -Fe-OH may act as Lewis base in deprotonated form (-Fe-O−) to bind a Lewis acid metal ion Pb^{2+} (Bradl, 2004).

Freshly formed Fe and Mn precipitates bear highly active adsorption sites for metal ions (Hettiarachchi and Pierzynski, 2004) and surface charge, rich pore structure, surface-active functional groups, and strong adsorption ability (Li et al., 2022b). They have a high capacity to adsorb lead and contribute to reducing soluble concentration in soils (Sauvé et al., 2000). Specific adsorption and coprecipitation are the main mechanisms that allow adsorption of Pb into these surfaces other than oxide-metal complexes. With increasing pH and surface area of Fe and Mn oxides Pb adsorption increases (Hettiarachchi and Pierzynski 2004). Besides, Fe oxides represent specific sorption sites at low pH values and negative charge sites at high pH as phyllosilicates do (Martìnez-Villegas et al., 2004). Lead adsorption by Mn oxides was observed to be up to 40 times greater than that by Fe oxides and compared to other metal cations Pb has the highest affinity. It was found that with mine waste-impacted soil ferromanganese oxides and phosphates under neutral pH conditions and thereby with soluble Pb-bearing phases such as Pb oxides, they continue to alter to less soluble phosphate and ferromanganese solid phases. Therefore, the presence of Pb phosphates and ferromanganese oxides reduces the bioavailability of Pb (Hettiarachchi and Pierzynski 2004). Iron oxides adsorb 3–4 wt.% Pb according to a study conducted by Sipos et al., (2005) (Sipos et al., 2005).

Organic Matter

Clay fraction and organic matter association were the dominant soil constituents influencing the adsorption of Pb. Because the majority of the organic materials are associated with inorganic colloids via adsorption by clays (Rieuwerts et al., 1998). Organic matter represents the decomposing carbon-based biomass of plant, animals and microorganisms. Although the organic matter content of soils is highly variable and often small (1-10 %) (Li et al., 2022a) compared to that of clay (20%) it can affect many soil functions that are either directly or indirectly, due to its capacity to retain water and nutrients and other elements. Soil organic matter is comprised of humic substances, or humus, and non-humic substances. Non-humic substances are labile compounds readily utilized as substrates by soil microorganisms. Humic substances are more stable organic matter compounds that have the ability to bind metals, alleviating both heavy metal toxicity and nutrient deficiency in soils (MacCarthy, 2001). Humic substances represent approximately 40–60% of the soil organic matter and include three different fractions, such as humin (insoluble fraction), humic acid (soluble under alkaline conditions) and fulvic acid (soluble under both alkaline and acidic conditions) (Guimarães et al., 2013). The humic fraction has been extensively decomposed and is resistant to further alteration. The most significant surface functional groups of soil organic matter are the negatively charged carboxyl (-COOH), carbonyl, phenolic, thiol, and amino groups (Li et al., 2022a).

Organic matter can involve in the complexation and adsorption of metal cations. Complexation with OM can be a major sink for soil Pb, either by adsorption of Pb to the CEC of OM or in more stable Pb complexes with OM ligands such as thiol (e.g., –SH) and carboxylate (e.g., –COO) functional groups which have varying affinities for adsorption onto the soil surface. Metals may be complex with low molecular weight organic components such as fulvic acids in solution. The organic matter fraction is rich in pH-dependent cation-exchange sites, and has an effect on CEC (Li et al., 2022a; Lwin et al., 2018). Lead has shown the highest affinities towards organic matter compared to other heavy metals. A study that used 14 non-calcareous New Jersey soils showed that OM was a dominant adsorbent of Pb at pH 3.0 to 5.0, compared with Pb adsorption to Fe and Al oxides and clay minerals (Shi et al., 2013). Dayton et al., (2006) found that OM was important in modifying Pb bioaccumulation and Pb was associated strongly to OM and CEC. They have suggested that soil pH increased the negative surface charge of OM and clay, thereby increasing CEC indirectly. The direct effect of organic carbon (OC) can be attributed to the formation of surface Pb complexes by OM functional group ligands, thus increased OC and/or CEC which reduced Pb solubility and bioavailability in the 21 soils used in their study (Dayton et al., 2006). Wu et al., (2003) indicate that based on Langmuir adsorption isotherm, the adsorption capacity for Pb at pH 6 humic acid (22.7 mg g^{-1}) were the highest compared to goethite (11.04 mg g^{-1}), montmorillonite (10.4 mg g^{-1}), kaolinite (0.91 mg g^{-1}), feldspar (0.503 mg g^{-1}) and quartz (0.148 mg g^{-1}). Further, the addition of citric acid increased the amount of Pb adsorbed onto humic acid (Wu et al., 2003). Sipos, (2009) reported Pb and Cu showed higher affinity of A horizon than other horizons which have relatively higher TOC content (6.74%). This study explained the behavior of metals during their competitive sorption onto humic substances based on the Hard-Soft Acid-Base concept. The Pb^{2+} and Cu^{2+} are borderline acids that can behave both as hard and as soft acids depending on the environment solution. The Pb showed relatively more hardness than Cu at the studied conditions resulting in higher relative sorption capacity on samples rich in organic matter (Sipos, 2009).

Other cations and anions

Total phosphorus (P) content in typical soils ranges from 500-800 mg kg^{-1} and occurs in the form of orthophosphate. Soil phosphorus can be associated with organic matter (20- 80 %) and mineral soils. Only a smaller fraction of the total soil P is available in the soil solution (labile) and >90% of total P is present as insoluble and fixed forms (non-labile) (Mengel et al., 2001). Soluble Pb can be immobilized as Pb-P compounds (pyromorphite) by reacting with P. These Pb-P compounds form highly insoluble, highly stable Pb precipitates that are not biologically available. With the presence of an adequate labile pool of P, Pb salts and inorganic Pb-bearing minerals, such as anglesite, cerussite, and galena, spontaneously transform into pyromorphite ($Pb_5(PO_4)_2Cl$, OH) (Karna et al., 2018; Zeng et al., 2017; Li et al., 2021). Therefore, as a remediation method for Pb-contaminated soil including the formation of pyromorphite b adding P-containing materials is highly recommended.

Together with P, iron (Fe) and aluminum (hydr)oxides have been found to show greater Pb adsorption than any other metal (hydr)oxide. The source of P, pH, and P: Pb molar ratio are the rate-limiting factors that affect the formation of pyromorphite effectively. Under low pH conditions apatite (P-containing hardly soluble mineral) can easily dissolve and at the same time, Pb in the solution phase creates an environment for pyromorphite formation (Park et al., 2011). Therefore, depending on soil pH reduction, the P sources have a greater effect on pyromorphite formation (NASEM, 2017). The use of P for in-situ stabilization of soil Pb is found to be highly effective in long-term reductions in Pb bioavailability. Pyromorphite is highly stable even in low pH and will not dissolve appreciably in the human digestive system with low gastric pH (Miretzky and Fernandez-Cirelli, 2008).

Mitigation measures of soil Pb

Ex-situ and in-situ remediation methods are available for treating Pb-contaminated soil. These techniques involve employing physical or chemical methods to control trace element contamination in soils. Whether through the removal and replacement of contaminated soil, the installation of physical barriers, the use of soil leaching with specific reagents or environmentally friendly approaches like in-situ stabilization, the goal is to control and mitigate the presence of Pb in the soil environment. These comprehensive approaches aim to reduce the health risks associated with exposure to Pb contamination.

Soil management is different from remediation of contaminated soil. Soil management incorporates the application of different operations, practices, and treatments to protect soil and enhance its performance via changing or enhancing existing soil properties.

Ex-situ approaches

Ex-situ remediation involves the removal of contaminated soil from its original location for treatment or disposal. This approach encompasses various methods, including digging and hauling, the contaminated soil is excavated and transported to a designated area for further management, which may include treatment, containment, or disposal. Soil washing is a technology used to clean up excavated material by separating contaminants from the soil through washing or leaching processes. Ex-situ remediation techniques are employed when on-site treatment or in-situ remediation methods are not feasible or effective. These approaches aim to reduce the environmental impact of contaminated soil and mitigate potential risks to human health and ecosystems (Hussain et al., 2022).

In-situ approaches

In-situ remediation refers to the application of remediation techniques directly at the location where the contamination is present, without moving the affected soils. This approach is conducted in the natural environment where the contamination exists, and the remediation methods are applied without significant disturbance to the soil or surrounding ecosystem. In the context of Pb-contaminated soil, in-situ remediation methods may include techniques such as engineering control, soil amendments, phytoremediation, and immobilization (Hettiarachchi et al., 2024). Overall, in-situ remediation strategies are designed to address contamination issues at the site itself, providing a more environmentally sustainable and minimally disruptive approach compared to ex-situ methods.

Engineering controls

The use of physical barriers or structures on site to prevent or minimize the spread and exposure to Pb-contaminated soil is known as engineering control methods. These control methods are designed to manage and contain the contamination rather than removing or treating the soil. The application of different engineering controls has different purposes, advantages, and disadvantages.

1. Cover Systems: Installing impermeable cover systems such as geotextiles, geomembranes, or mulch can act as a barrier, preventing direct contact with Pb-contaminated soil. These covers also help reduce the potential for wind or water erosion that can disperse Pb particles.

2. Encapsulation: Encapsulation involves applying a protective layer over the contaminated soil. This layer could be a cement-based material or a sealant like an asphalt that encapsulates the Pb, reducing its mobility and accessibility.

3. Fencing and Barriers: Erecting physical barriers, such as fences or walls, can restrict access to areas with Pb-contaminated soil, preventing exposure and reducing the risk of ingestion or inhalation.

4. Capping: Capping involves placing a layer of clean soil or another material over the contaminated soil, creating a cap or cover that isolates the Pb and prevents its movement or exposure.

5. Grading and Landscaping: Modifying the topography of the site through grading and landscaping can help control the movement of water over the contaminated soil, minimizing erosion and runoff.

Engineering controls are often employed in situations where excavation or removal of contaminated soil is impractical or too disruptive (Oh et al., 2023). While these measures can effectively contain Pb contamination, it is essential to regularly monitor and maintain the controls to ensure their continued effectiveness. The major advantage of engineering controls is containment, preventing its spread to surrounding areas and reducing the risk of exposure. Additionally, these controls contribute to reducing soil destruction and have cost savings compared to excavation or removal of contaminated soil. The flexibility and adaptability of engineering controls allow them to accommodate various site conditions while ensuring long-term stability, effectively managing Pb contamination for extended periods (Padhye et al., 2023).

The primary drawback of engineering controls is that they do not eliminate Pb from the site. Furthermore, regular maintenance and monitoring are necessary for sustained effectiveness, and there is a potential for accidental disturbance. Additionally, these controls may have aesthetic impacts on the site, affecting its visual appearance.

Soil amendments

Soil amendments involve adding substances to the soil to modify its chemical properties and enhance the retention or immobilization of lead. The primary role of soil amendments is reducing its environmental risk by chemically altering soil Pb to less bioavailable forms (Hettiarachchi and Pierzynski 2004). Therefore, soil amendments can influence soil properties, serving as a feasible soil management strategy for controlling Pb-contaminated soil.

The application of soil amendments to Pb-contaminated soil typically enhances and stabilizes the fraction of total Pb in the soil solid phase, as opposed to the soil solution phase. This increase in metal adsorption, complexation, precipitation, and redox reactions leads to an overall reduction in soil metal bioavailability. Additionally, the use of organic and inorganic soil amendments can concurrently enhance soil biological, chemical, and physical properties. By enhancing soil organic matter content and releasing nutrients like N, P, and K, soil amendments contribute to increased CEC and improved soil microbial activities, thereby directly and indirectly reducing Pb bioaccessibility in contaminated soil (Lwin et al., 2018).

Organic soil amendments that contain humic acids have been widely used to immobilize soil Pb by changing speciation from initially highly bioavailable forms to the much less bioavailable fractions associated with organic matter (OM), metal oxides, or carbonates. The most commonly used organic soil amendments are composts, manures, biosolids (sewage sludge), and biochar

(Lwin et al., 2018). They are low-cost and help to alter some physical-chemical properties of soil. Application of biosolids, composts, or manures facilitates better plant growth in mine waste-impacted soil with poor soil conditions. Initial OM content, nature and stability of OM, the soil type, and the rate of transformation of OM over time are important considerations of OM addition, because of OM decomposition over time. Organic matter amendments have a long-term impact on the partitioning of metals between the solution and the solid phase due to the release of organic acids, an increase in dissolved organic carbon, and changing the surface area, pH and CEC in soil (Lwin et al., 2018; Palansooriya et al., 2020).

Based on a pot experiment it was found that the application of farm yard manure (FYM) significantly reduced Cd and Pb concentrations in the shoots and roots of Amaranth in sandy loam soil with the increase in the level of FYM application (Alamgir et al., 2011). The availability and plant uptake of Pb were reduced by the organic amendments observed in the study conducted by Walker et al., (2004). The study revealed that immobilization of Pb by humified OM, in the compost-treated soil increased soil pH facilitated the formation of insoluble carbonates and/or phosphates due to CO_2 and soluble phosphates, released during mineralization of the OM by manure (Walker et al., 2004).

Biosolids are a product of the wastewater treatment process; the solid is separated from the liquid during the wastewater treatment process (US EPA, 2023). The addition of biosolids composts has been shown to reduce Pb bioaccessibility using in vitro and in vivo measures. A compost with Fe added to wastewater treatment residuals (Fe WTR) added to soils at 100 g kg^{-1} was reported to decrease Pb bioaccessibility in a laboratory incubation study (Brown et al., 2012). The Fe WTR amended soil showed Pb present as Fe-sorbed Pb (88 and 100%) and pyromorphite

(12 and 53%) and the addition of high-Fe biosolids compost is an effective means to reduce Pb accessibility (Brown et al., 2012; Attanayake et al., 2017).

Biochars are the sloid product of pyrolyzed organic materials (manures, green wastes, etc.), which can be amended to soils to address Pb contamination. Biochar effectiveness varies based on the feedstock material and pyrolysis temperature used with lower pyrolysis temperatures associated with higher Pb immobilization. The porous structure, charge surface area, different surface functional groups (carboxyl, hydroxyl, phenolic hydroxyl, and carbonyl groups), alkalinity (high pH), and the release of available P, K, and Ca (resulting from high CEC) are properties that commonly contribute to its immobilization of heavy metals and with metal stabilization in soil (Bian et al., 2014; Yang et al., 2019).

The addition of organic amendments increases soil pH due to the specific adsorption of organic anions on hydrous Fe and Al surfaces and the corresponding release of hydroxyl. Organic soil amendments improve soil physical characteristics such as particle size distribution, aeration, and porosity, and promote the formation of water-stable aggregates. In addition, organic amendments can release significant amounts of N, P, and other essential elements such as Ca, Mg, and Fe. Moreover, the build-up of soil OM through the addition of organic amendments results in significant improvements in overall soil quality (Lwin et al., 2018; Palansooriya et al., 2020).

The most common and popular inorganic soil amendment is P-containing materials for managing Pb-contaminated soil. Phosphate treatment has been proposed as a Best Management Practice for firing ranges where Pb occurs in its metallic forms and several other phases (Dermatas et al., 2008). Different natural and synthetic P sources with different solubilities may impact the effectiveness of Pb immobilization. Apatite, hydroxyl apatite, rock phosphate, phosphate-based salts, synthesized P fertilizers (diammonium phosphate, mono ammonium phosphate, triple super phosphate, ammonium polyphosphate, struvite), and phosphoric acid; have all been successfully used for the remediation of Pb contaminated soils aiming to reduce Pb bioavailability and bioaccessibility. Phosphate amendments reduce Pb mobility by ligand exchange as well as ionic exchange and mainly through the precipitation of pyromorphite minerals (Park et al., 2011).

Since phosphate is an essential macronutrient for plant growth phosphate amendments tend to promote revegetation in metals contaminated soils. Mayer et al., (2022) reported that among different sources of P, soluble P-amendments reduce bioaccessible Pb more than insoluble amendments and phosphoric acid is more effective than other phosphate amendments. Further, initial low soil pH and high total Pb showed greater reductions in bioaccessible Pb (Mayer et al., 2022). The redistribution of Pb from more chemically labile forms to non-labile phases resulted in less bioavailability and less phytotoxicity, an indication of the efficacy of P amendments in Pb-contaminated soil.

Phytoremediation techniques

Phytoremediation is an environmentally friendly approach that utilizes plants to absorb, accumulate, or stabilize contaminants, effectively reducing their concentration in the soil. Phytoextraction utilizes plants to mitigate and remediate Pb-contaminated soil. The process involves the ability of certain plants, known as hyperaccumulators, to absorb, accumulate, and sometimes translocate Pb, from the soil into their tissues. While phytoextraction offers a sustainable and cost-effective solution for Pb-contaminated sites, its effectiveness can vary depending on the plant species selected, site conditions, and the extent of Pb contamination. Further, these plants are not effectively transfer Pb from root to above ground parts, instead it accumulates in roots tissues. Phytostabilization utilizes plants to keep the contaminated soil in place, reducing erosion or covering the soil surface. Perennial plant species with a lower ability to accumulate metals and well-established root systems are ideal for phytostabilization (Egendorf et al., 2020; Lan et al., 2020). This is ideal for sites where complete removal of contaminants is not feasible. The emphasis is on reducing the mobility and bioavailability of Pb in the soil. Phytodegradation is another method that plants are used to facilitate the breakdown or transformation of contaminants through the release of root exudates that enhance microbial activity which can use to reduce bioavailability. However, the choice of phytoremediation technique depends on factors such as the site-specific conditions, the type and extent of contamination, and the selected plant species. Successful implementation requires careful consideration of these factors to achieve effective and sustainable remediation. Additionally, to successfully remediate heavily contaminated sites like chat or mine tailing disposal areas, soil amendment is often necessary to enhance the growing conditions of the soil or contaminated material. This involves addressing physical, chemical, and/or biological aspects before planting, as the initial conditions may be too harsh to support plant growth. Many of these highly contaminated sites pose challenges for plant establishment due to properties that suppress plant growth (Mendez and Maier, 2008).

Techniques for identifying sources and potential for transfer of Pb in soil

Lead concentrations, potential sources, and the fate and transport in the environment have been reviewed within the scientific community for a few decades. In these studies, the following techniques are utilized: sequential extraction, Pb isotope relative abundance, X-ray absorption spectroscopy (XAS), microscopy, and bioavailability measurements. The suitability of these measurement techniques for providing information on target questions about Pb fate and transport varies widely (Hettiarachchi et al., 2024). Therefore, employing a combination of methods simultaneously yields more robust results to address specific objectives or provide answers regarding environmental Pb.

Pb Isotope relative abundance

The measurement of the relative abundance of Pb isotopes in environmental media is considered the most effective technique for tracing the source of origin of Pb. The identification of the root of contamination through the measurement of Pb isotopes is of significant interest to regulators and researchers, guiding future actions and strategies. Environment Pb is a mixture of four main isotopes: ^{208}Pb (52%), ^{206}Pb (24%), ^{207}Pb (23%) and ^{204}Pb (1%) with varying abundances (Weis, 2017). Radioactive decay of ^{238}U, ^{235}U and ^{232}Th produces radiogenic isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb respectively, while ^{204}Pb is the only non-radiogenic (primordial) stable isotope with a constant abundance on Earth over time (USGS, 2004; Komárek, 2008). The isotopic composition of Pb is not significantly affected by changes in soil properties or laboratory extraction process. Therefore, Pb isotopes serve as an efficient tool for determining the sources and pathways of Pb contamination. The isotopic composition of Pb is commonly expressed as ratios because it can be determined precisely analytically and the abundances of these isotopes are relatively important (Rodríguez–Salazar et al., 2010). The isotopic ratios for most natural materials range from 14.0-30.0 for ^{206}Pb/^{204}Pb, 15.0-17.0 for ^{207}Pb/^{204}Pb and 35.0-50.0 for ^{208}Pb/^{204}Pb. Potential complications to the use of Pb isotopes can be overcome with a better understanding of distinct compositions of materials such as anthropogenic contamination, soil formation, and natural minerals phases. Isotope ^{210}Pb, is a useful radiogenic (via ^{238}U and ^{222}Rn) and radioactive isotope with a half-life of 22.1 years that is used to age-date materials formed in the last 100 or so years. Furthermore, ^{210}Pb is widely used for dating of glacial age, recent sediments and peat deposits and catchment research (USGS, 2004; Komarek et al., 2008).

In uncontaminated soils, Pb typically exhibits a more radiogenic isotopic composition ($^{206}Pb/^{204}Pb \sim 18.5–19.5$) as most Pb in such soils originates from weathered bedrocks, and the isotopic composition is primarily influenced by the decay of ^{238}U to ^{206}Pb. Conversely, Pb ores, characterized by higher Pb/U ratios resulting from the separation of Pb from U, demonstrate a less radiogenic composition ($^{206}Pb/^{204}Pb \sim 16.0–18.5$). Similarly, gasoline, coal, waste, ore and smelter incineration showed different isotopes ratios ($^{206}Pb/^{204}Pb$ and $^{206}Pb/^{207}Pb$) (Hansmann and Köppel, 2000; Komarek et al., 2008).

Different types of mass spectrometry, either with thermal ionization (TIMS) or with inductively coupled plasma as the ion source (ICP-MS) can be used for Pb isotopic ratio measurements. The ICPMS instruments with quadrupole-based (ICPQMS), sector-based (or sector field) (ICP-SFMS) and time-offlight-based mass analysers (ICP-TOF-MS) and the sector-based techniques equipped with single (SC) or multiple collector (MC) detections can also be used (Komarek et al., 2008).

X-ray absorption spectroscopy

Synchrotron radiation (SR) covers a large spectrum of electromagnetic waves, from infrared to hard X-rays (in wavelength, tens of micrometers to less than 0.01 nm). Synchrotron radiation is a versatile X-ray source that can be used in many different fields of research, it is used as an excitation source for higher-intensity X-rays (Yagi, 2014). In combination with a monochromator, the exciting radiation can be tuned to the energy with the optimum value of the photoelectric cross section for the investigated sample. It is also possible to tune the energy below the absorption edge of a main element in the sample to excite an element at trace levels with $Z<Z_{main}$ element thus, offers several advantages in trace element analysis (Streli et al., 2017).

X-ray absorption spectroscopy (XAS) and X-ray diffraction (XRD) are complementary techniques that have been extensively used to study crystalline and non-crystalline materials on the short-range and mainly long-range scales, respectively. As XRD has limitations in providing details of the local structure due to its Bragg scattering component, Extended X-ray Absorption Fine Structure (EXAFS) has become increasingly utilized. EXAFS offers insights into the local atomic and electronic structure, overcoming the constraints of traditional XRD techniques (Castellano, 2022). The best technique to measuring Pb speciation is XAS which utilizes X-rays to move below and above the Pb L3-edge energy (13,035eV). This process excites a core electron and one can measure the perturbations in the electronic structure. XAS provides valuable information about the speciation of Pb by identifying the bond types, bond distances, bonding elements and oxidation state of the central Pb atom. These parameters obtained from XAS can be compared to known Pb species standards. to identify the combination of species in an environmental sample. This comparison helps identify the specific combination of Pb species present in an environmental sample to predict the fate and transport of Pb (Hettiarachchi et al., 2024).

Bulk XAFS and XANES

The X-ray absorption spectrum is commonly divided into two categories as X-ray absorption near-edge spectroscopy (XANES) and extended X-ray absorption fine-structure spectroscopy (EXAFS). XANES is highly sensitive to the formal oxidation state and coordination chemistry of the absorbing atom, such as octahedral or tetrahedral coordination. On the other hand, EXAFS is employed to ascertain the distances, coordination number, and species of neighboring atoms to the absorbing atom (Newville, 2004). XAS data generated by EXAFS and XANES are easily usable tools for the study of nondestructive samples, unrestrained concerning type and concentration range, but depends very much on the chosen technique (Streli et al., 2017). Bulk environmental samples EXAFS measurements provide structural information for each type of atom by simply tuning the X-ray energy to coincide step by step with an absorption edge of each of the elements in the sample. Information about the chemical environment such as the number and type of neighboring atoms and the interatomic distances (one-dimensional (1D) radial distribution function (RDF)) of 2–3 next neighbor atoms of the absorbing element. Hence, EXAFS is highly specific and element-selective, focusing on the immediate environment around each absorbing species (up to 5–6 A˚, corresponding to about five coordination shells) (Castelleno, 2022; Welter et al., 1999). All atoms present in the sample that differ from the absorbing species just provide a featureless background. In addition, every chemical compound of an element yields a unique structure of a core electron absorption edge (“fingerprint”). The XANES region of the XAFS spectra can provide additional detailed information. Linear combinations (LC) of reference spectra from single, pure reference compounds, can be used for identification of Pb species using XAFS and XANES spectra. Prior to this, a polynomial function can be used to fit and subtract the pre-edge background from the raw spectrum. The energy calibration of the background-corrected spectrum needs to be adjusted using the reference spectrum of the Pb foil. Following this, the spectra can be normalized and used for LC fitting (Welter et al., 1999).

Micro XRF, XFAS and XANES

Micro X-ray fluorescence (µXRF) is an elemental analysis technique that allows for the examination of very small sample areas even with inhomogeneous samples with areas of interest nondestructively. Micro XRF uses direct X-ray excitation to induce characteristic X-ray fluorescence emission from the sample for elemental analysis similar to conventional XRF. Micro XRF has a typical spatial resolution ranging in diameter from several hundred micrometers up to several millimeters, µXRF uses X-ray optics to restrict the excitation beam size or focus the excitation beam to a small spot on the sample surface so that small features on the sample can be analyzed quickly. Micro-XRF with X-ray optics has been successfully used for applications including small feature evaluation, elemental mapping, thickness measurement, detection of micro-contamination, and evaluation of collected information on XRD and XAFS (Baker et al., 2014; Haschke and Boehm, 2017; XOS 2024).

Electron microscopy and EDX

Energy Dispersive X-ray Spectrometry (EDX) is an advanced microanalytical technique used for determining the elemental makeup and chemical composition of materials. EDX is associated with electron microscopy either scanning or transmission (SEM/TEM) (Agrawal, 2022, Quadri et al., 2023). It provides both quantitative and semi-qualitative information on the elements present on the sample surface by relying on the generation of characteristic X-rays (Torres-Rivero et al., 2021). The emission of X-rays is stimulated by irradiating the specimen with a high-energy beam of charged particles such as electrons or protons, or a beam of X-rays, focused into the sample being study (Mehrban & Bowen, 2017; Telegdi et al., 2018). In the SEM instrument, the atoms on the surface are excited by the electron beam, resulting in the emission of specific wavelengths of X-rays that are characteristic of the atomic structure of the elements, revealing the identity of the elements in the sample. The data obtained from EDX analyses are presented as a plot of energy vs intensity to have a characteristic identity having the same wavelength and energy with the same position in the EDX spectrum. This technique also quantifies the elemental composition of the sample (Torres-Rivero et al., 2021; Hussain et al., 2021).

Sequential extraction

Chemical fractionation or sequential extraction methods in soils and sediments provide an approach to determine the speciation/forms of elements, as well as their fate and transport. Sequential extractions are simple procedures commonly employed in chemical laboratories for many studies (Hettiarachchi et al., 2024). The increased interest in sequential extraction methods can be traced back to the work of Tessier, Bison, and Campbell in 1979. They developed a five-stage extraction method to fractionate Cd, Co, Cu, Fe, Pb, Mn, Ni, and Zn in river sediments, which is still used with modifications (Bacon and Davidson, 2008). The extraction procedure involves a number of extractions (mostly 3–8), which are sequentially applied to in order of increasing reactivity so that the successive fractions obtained correspond to trace elements associated with lesser mobility fractions. The extractants more commonly used in sequential extraction include water, unbuffered salts, weak acids, reducing agents, oxidizing agents, and strong acids (Laing, 2011). Sequential extraction methods offer detailed information about the origin, mode of occurrence, mobility, and biological and physicochemical availability of trace elements in solid samples. They provide a convenient means to determine the trace elements associated with principal accumulative phases in soils and sediments.

The partitioning of trace elements obtained by sequential extraction procedures is operationally defined, meaning that the extractants lack selectivity in the chemical separation of trace elements from the solid (Silveira et al., 2006; Laing, 2011). Tessier et al. 1979 named these operationally defined fractions as exchangeable, carbonate bound, Fe and Mn oxide bound, organic matter bound, and residual (Tessier et al., 1979). They range from water-soluble to recalcitrant forms immobilized in mineral lattices. These are also often referred to in the literature as exchangeable, weakly absorbed, hydrous-oxide bound, organic bound, and lattice material components. Typically metals of anthropogenic inputs tend to reside in the first four fractions and metals found in the residual fraction are of natural occurrence in the parent rock (Maiz et al., 2000; Zimmerman and Weindorf, 2010).

Metals in the water-soluble, exchangeable, and/or carbonate fractions are expected to be become available or more accessible with an increase in environmental salinity or acidity. The exchangeable fraction comprises weakly adsorbed elements held on the solid surface through relatively weak electrostatic interactions, making them easily removable by altering the ionic composition of water, such as using a salt solution. To remove elements released through ion-exchange processes and those co-precipitated with carbonates, particularly the carbonate-bound fraction susceptible to pH changes that may cause remobilization of metals, an acid solution is used to extract this fraction. Metals bound to Fe and Mn oxides or reducible fractions are particularly susceptible to anoxic (reducing) conditions so a solution capable of dissolving insoluble sulfide salts is used for this fraction. The removal of metals bound in the organic phase requires the oxidation of the organic material. Metals precipitated as sulfides or bound to sulfides are typically included in this fraction, and an oxidation process is commonly applied to leach them. The most common oxidant is H_2O_2 in an acidic medium. The residual fraction consists of metals incorporated into the silicate/crystalline-bound trace elements of primary and secondary minerals. This fraction is the hardest to remove, not available under normal environmental conditions, and requires the use of strong acids to break down silicate structures to release all remaining trace elements (Zimmerman and Weindorf, 2010; Laing, 2011).

Many sequential extraction procedures designed for metal fractionation in soils and sediments cover a broad range of metals, although some are developed for specific elements or groups of elements, considering their unique chemical properties (Laing, 2011). The procedure proposed by Tessier et al. (1979) has been widely used for trace element analysis with some modifications (Zwonitzer et al., 2002). Sequential extraction methods, when compared to direct determination methods, may exhibit inconsistencies due to the redistribution of analytes among phases during extraction, non-selectivity of reagents for target phases, incomplete extraction, and precipitation of 'new' mineral phases during extraction. However, with an improved understanding of the metal speciation within the matrix and the solid phases attacked by the extractants, sequential extraction can be used to determine the bioavailability of elements, as well as their fate and transport in environmental media (Bacon and Davidson, 2008).

Urban and residential soil Pb contamination

In 2019 WHO estimated that more than 800 million children globally encounter blood lead levels (BLL) above 5 µg/dL, the former Center for Disease Control and Prevention (CDC) reference value (Rees and Puller, 2019). After revision of, BLL to 3.5 µg/dL this number of children would be much higher. Furthermore, the current EPA soil screening guideline level for residential properties was revised to 200 mg kg^{-1} from 400 mg kg^{-1} Pb at the beginning of 2024 (US EPA, 2024b). Today, much of modern Pb exposure is thought to result from exposure to soil and dust. Similarly, a significant portion of Pb exposure among children is believed to originate from interactions with soil and dust. Soil serves as a crucial source of Pb exposure, primarily occurring through ingestion (hand-to-mouth contact) and the inhalation of fine soil particles that are resuspended in the air. Thus, urban and residential soil Pb sources remain a critical public health challenge.

Urban and residential areas can encompass all the anthropogenic Pb sources mentioned elsewhere, representing both point and non-point sources. These areas often exhibit multiple Pb sources within a specific areas. Numerous studies have investigated and measured substantial Pb contamination in cities, explored potential sources and linked soil contamination to Pb exposure in those cities. One of the major findings of those studies was the highly variable levels of contamination, even at the same lot, ranging from background level to few thousands' ppm of Pb (Wade et al., 2021). The risk associated with Pb is very common among Black and Hispanic urban communities with low levels of income (Aelion et al., 2013). Further, the age of the housing, which is associated with lead-based paint and Pb-contaminated house dust, contributes to the risk of Pb exposure, particularly among children under 5 years. Historically, racial segregation and low-income demographics have led to the occupancy of older housing options, often situated in proximity to lead (Pb) industrial sources and heavily trafficked roads where Pb from gasoline has accumulated on various components. Consequently, individuals in these communities are exposed to multiple Pb sources simultaneously.

Remediating urban and residential sites affected by Pb contamination is challenging due to significant questions that need answers before identifying potential remediation methods. The major concerns for applying potential remediation measures include cost, feasibility, adaptability, and sustainability. The arising questions focus on determining the extent of Pb contamination, identifying the most widely distributed sources of Pb contamination, and examining the relationship between potential sources and the speciation of Pb in soils to determine appropriate remediation methods. Hence, there is a growing need for additional research to investigate urban and residential soil Pb contamination, particularly to comprehend the chemistry of Pb in urban soils. The content of Pb in urban soil exhibits significant variability and is often associated with mild contamination from multiple sources. Furthermore, Pb is frequently found alongside other co-contaminants such as As, Cd, Zn, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), etc. Furthermore, urban soils are highly heterogenous i.e. unique and poor soil characteristics (compaction, underlying concrete layers, filled with sub-soil, debris, and wastes), poor soil quality (lack of A horizon/ OM rich soil surface, mixed with building materials and industrial and urban waste materials) and presence of different co-contaminant. In addition, studies have shown that urban gardening is becoming an increasingly interesting practice due to the demand for fresh, locally-grown food, and as a sustainable practice. Therefore, low-cost and effective in-situ mitigation strategies are needed to minimize the effect of soil contaminants in urban soils. This is attributed to considering both human health and protecting land to produce quality fresh food from safer community gardening. Therefore, this is high time to understand the urban soil Pb chemistry and find affordable, safer, and environmentally sound sustainable soil management/ remediation strategies to remediate mildly Pb-contaminated urban soils.

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Chapter 3 - Reduction of Lead Bioaccessibility in Pb/As/Cd/Zn/Cu Co-contaminated Alkaline Smelter Impacted Soil by Soil Amendments: Assessing Pb in vitro bioaccessibilty, sequential extraction and synchrotron speciation

Abstract

Globally, trace element contamination in soil, particularly lead (Pb), due to human activities, such as mining and smelting poses a significant challenge. Inactive smelters have spread potentially toxic metal-laden waste to nearby soil, a health hazard primarily through direct soil ingestion and inhalation. Current remediation methods are costly and have limited usability in some environments. Soil amendments, promising means for in-situ remediation, involve adding materials to transform Pb into stable forms, reducing Pb bioaccessibility. The bioaccessible Pb portion in soil is affected by its speciation and their stability in the gastrointestinal environment and other soil properties. Pyromorphites formation and subsequent reduction in Pb bioaccessibility, has been researched, but little is known about their formation in alkaline soils, following treatment with P sources, particularly in multi-element contaminated soils. This study investigated the correlation between Pb species formation and bioaccessibility in alkaline, smelter-impacted soil co-contaminated with As, Cd, and other potentially toxic elements using phosphorus-containing amendments.

The soil was collected from near ASARCO smelter, El Paso, Texas, containing a total concentration of: Pb= 3200±142, As=254±14, and Cd=110±8 mg kg^{-1}. Soil was mixed with two organic (biosolids and compost; 5 and 10%) and two inorganic P fertilizers, triple super phosphate and monoammonium phosphate (TSP and MAP; Pb:P molar ratio 1:4 and 1:6) and incubated for up to 24 weeks. Bioaccessible Pb concentration was measured periodically using a modified physiologically based extraction test (PBET). Speciation of Pb was determined using sequential chemical extraction and synchrotron techniques, including X-ray absorption spectroscopy (XAS) and spatially-resolved micro X-ray diffraction (μ-XRD) and μ-XAS aided by micro X-ray fluorescence (μ-XRF), after 24 weeks.

Results indicated a significant decrease in bioaccessible Pb levels and pH over time in treated soil samples compared to untreated soil, with biosolids showing the greatest reduction in bioaccessible Pb. Sequential extraction and synchrotron analysis confirmed the formation of Pb-associated P and Fe minerals, including pyromorphite and Fe (hydro)oxides sorbed Pb. Class A Fe-rich biosolids outperformed all other treatments in terms of reduction of bioaccessible Pb and transforming more Pb to residual fractions. The reduced bioaccessible Pb was mainly associated with pyromorphite, Pb bound to Fe (hydro)oxides, and Pb bound to organic complexes. Overall, applications of biosolids, TSP, and MAP proved to be effective in diminishing exchangeable Pb fractions and forming stable fractions in the studied alkaline soil.

Introduction

Trace element contamination in soil is one of the critical issues faced globally due to the excessive loading of trace elements in the soil caused by human activities (Wuana and Okieimen, 2011; Zhao et al., 2022; Priya et al., 2023; Rashid et al., 2023; Sánchez-Castro et al., 2023). In most countries, soil Pb contamination increased from the start of the industrial revolution, due to the vast application of Pb in many industries (Su et al., 2014). Mining and smelting of Pb ores are the known point sources that contaminate nearby soils with Pb and other contaminants. Many of the former smelters are currently inactive; however, the potentially toxic trace element - containing waste generated by these smelters is a prime human health hazard (US EPA 2021; Adnan et al., 2022; Schindler et al., 2022). The ASARCO smelter in El Paso, Texas processed metals including lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn), and antimony (Sb) during its > 110-year history (Darby, 2012; Shekhter, 2013). Generally, depending on the type and operational history of the smelter, and the specific local environmental conditions, total Pb concentration in the soil close to the smelter sites varies from a few hundred to several thousand parts per million (ppm). The impact of ASARCO smelter operations on the region has already occurred, affecting it significantly. With the absence of established natural vegetation and the prevalence of dry, loose soil, dust storms and sandstorms easily form due to dry winds (El-Hage and Moulton, 1998). This resulted in soil contamination with several thousand parts per million (ppm) of Pb, even extending several miles away from the El Paso smelter, and these areas are currently densely populated human settlements. The main routes of Pb exposure are recognized to be the direct ingestion and inhalation of Pb-contaminated soils and dust (Aschengrau et al., 1994; Laidlaw et al., 2015).

The current approaches to mitigate Pb-contaminated soil can be broadly classified into two categories: in-situ and ex-situ remediation techniques (Nejad et al., 2018; Liao et al., 2022). Ex-situ remediation involves excavating contaminated soils and replacing them with clean soils, while certain in-situ methods include the installation of permanent physical barriers (such as concrete or asphalt) and phytoremediation. However, these traditional approaches are characterized by high costs, environmental damage, limited effectiveness, and societal unacceptability (Hettiarachchi et al., 2024).

Thus, there is a pressing need to explore alternative remediation strategies that not only safeguard human health and the environment from Pb contamination but are also economically viable and applicable on a large scale. Soil amendments emerge as a promising in-situ remediation technique that is both practical and environmentally sustainable for addressing widespread soil Pb contamination (US EPA, 2007; Henry et al., 2015; Mahar et al., 2015; Lwin et al., 2018; Palansooriya et al., 2020). Soil amendments entail the addition of specific materials to contaminated soil to form stable compounds with reduced toxicity, thereby lowering Pb bioaccessibility (Hettiarachchi and Pierzynski 2004). These reactions contribute to safeguarding human health by minimizing the risk of inadvertent ingestion of Pb-contaminated soil.

The health risks associated with soil Pb are directly linked to its bioavailability, defined as the capacity of ingested soil Pb to enter the systemic circulation (Ruby et al, 1996; Hettiarachchi and Pierzynski, 2004). The dissolution and subsequent absorption or excretion of Pb in the gastrointestinal tract are largely influenced by the mineral species in which soil Pb is incorporated and their stability in the gastrointestinal environment (Deshommes et al., 2012). It is a well-established fact that the conversion of soil Pb into species that bind Pb in stable forms can reduce Pb solubility and bioavailability, a process achieved through the addition of various organic and inorganic amendments (Rizwan et al., 2021; Mayer et al., 2022; Kastury et al., 2023). In the past two decades, many researchers have found that the conversion of soil Pb to pyromorphite (lead phosphate [$Pb_5(PO_4)_3(OH, Cl, F, I)$]), can immobilize soil Pb and reduce its bioavailability (Basta et al., 2001; Hettiarachchi and Pierzynski, 2004; Zhang et al., 2017; Li et al., 2021). Pyromorphite is formed through the dissolution of Pb solids and reaction with soil solution P. This process is controlled by the availability of soluble Pb and P in the soil alongside various other soil properties (Karna et al., 2018). Pyromorphite demonstrates distinctly lower solubility in the lower pH conditions of the gastrointestinal tract when compared to various other lead minerals. Furthermore, it exhibits both chemical and biological stability across a range of surface soil conditions (Hettiarachchi and Pierzynski, 2004; Koptsik, 2014; Zeng et al., 2017).

Both theoretical and research studies have consistently shown that the dissolution of soil Pb increases as soil pH decreases (Lindsay, 1979; Yang et al., 2001; Sanderson et al., 2016). This pattern is evident in various research endeavors utilizing acidic soil and amendments that lower soil pH, such as phosphoric acid and triple superphosphate (TSP) (Yang et al., 2001; Cao et al., 2009; Mayer et al., 2022). In contrast, calcareous and high-pH soils have demonstrated limited efficacy in P treatments. This limitation is attributed to reduced solubility and increased precipitation of Ca-P products (Mavropoulos et al., 2002; Chrysochoou et al., 2007; Li et al., 2013), as well as the constrained availability of free P and Pb for reaction and promote formation of Pb oxides and carbonates (Miretzky and Fernandez-Cirelli, 2008).

Experimental data regarding the influence of alkaline pH on Pb behavior following treatment with P sources are scarce, particularly in multi-element contaminated soils. Therefore, the primary objective of this laboratory incubation study was to explore the correlation between the formation of Pb species and their impact on Pb bioaccessibility in alkaline smelter-impacted soil co-contaminated with As and Cd. This was achieved by amending the soil with phosphorus-containing organic and inorganic amendments.

Materials and Methods

Test Soil Collection and Location Characteristics

The soil was collected from El Paso, TX specifically the area near the Rio Grande and adjacent to ASARCO smelter (Figure 3.1 A and B). A preliminary investigation was conducted using a portable X-ray fluorescence device to identify the sites with elevated concentrations of Pb. This site was named as EP4 and found to have very high total Pb concentration during the preliminary investigation (~2000 mg kg^{-1}).

The arid to semi-arid desert climate is inherent to El Paso. The area receives plenty of sunlight throughout the year, characterized by low humidity, very low annual precipitation (8 -10 inches per year), and a very high potential evaporation rate (150 inches per year) (El-Hage & Moulton, 1998). El Paso soil consists of a mix of colluvial sediments from the surrounding mountains and fluvial sediments from the Rio Grande. Typical fluvial sediments can be classified into fine to coarse grain, gravel and sand with a mixture of silt and clay (Texas Commission on Environmental Quality, 2023).

Preliminary Soil Analysis

The collected soil was air-dried and sieved to 2-mm size using a stainless-steel sieve. For in vitro bioaccessibility extractions, a subsample of the <2-mm size fraction was further sieved to obtain the <250-mm size fraction, which resembles particle size that can adhere in children's hands (Duggan and Inskip, 1985; Kelley et al., 2002).

Total Pb in the <250-mm and <2-mm fractions of the soil samples were analyzed using EPA method 3051A (USEPA, 2007). In addition to the total Pb, some selected elements (As, Cd, Fe, Mn, K, Ca, Mg, Al, Si, Se, Ni, Cu, Na and Zn) were analyzed using the aqua regia digestion method followed by subsequent Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Varian 720-ES, Santa Clara, CA) analysis. For the method 3051A, 10 mL of trace metal-grade concentrated HNO_3 was added to 0.5 g of soil (n=5) in Teflon tubes and the content was digested in a microwave digestion unit (MARS Xpress, CEM Corp. Matthews, NC). The temperature of the soil-acid mixture was increased to 165°C within the first 5.5 minutes in the CEM MARS microwave digestion unit. The temperature was further increased to 175°C in the second step within the next 4.5 minutes, and then a 175°C temperature held for 5.5 minutes to complete the digestion. After digestion, the tubes were transferred from the microwave digestion unit and left to cool in the fume hood until the contents reached room temperature. The solution was then filtered, using a Whatman No. 42 filter paper, into screwed plastic scintillation vials under the fume hood and stored in 4 ° C. Then diluted samples were analyzed for Pb using an ICP-OES. Aqua regia digestion used 10 mL of trace-metal grade 1:3 HNO_3 to HCL mixture added into 1 g of soil and digested at continuous increase in temperature in 30-minute intervals (75, 100, 110, 140 ° C) in a hot plate block digestion unit. The final step in digestion with 140 ° C is completed when the solution is evaporated yielding about 1 mL solution. The remaining 1 mL solution was diluted with 0.1% HNO_3 and filtered using a Whatman No. 42 filter paper, into screwed plastic scintillation vials and the solution was then analyzed for total elemental concentration using ICP-OES. In addition, total organic carbon (TOC), total N, CEC, soil carbonate and soil texture were measured following the standard procedures at soil testing laboratory at Kansas State University. Soil pH was measured using 1: 2 and 1: 5 soil-water ratio (Orion Star A111, Thermo Scientific), and soil texture was analyzed by using the pipette method.

Experimental Design

To investigate the reduction of Pb bioaccessibility, a laboratory incubation study was conducted in a completely randomized design (CRD). Two inorganic and organic soil amendments representing four treatments were selected at two different rates and included one control treatment. Inorganic soil amendments were commercial fertilizers, triple superphosphate (TSP) and mono ammonium phosphate (MAP). Their application rates were 1:4 and 1:6 based on Pb: P molar ratio to be acheived in the soil. Organic soil amendments were the biosolids (BS) and compost (CO) which were applied as 5 and 10 percent of Mehlich-III extractable P availability in these materials. Biosolids were obtained from Blue Plains wastewater treatment facility, Washington DC, and characterized as Class A biosolids. Another distinctive characteristic is its elevated iron content, approximately 10% higher than that of typical biosolids derived from numerous wastewater treatment plants. This increase is due to the addition of iron salts such as ferric chloride and ferrous sulfate for phosphorus removal (US EPA, 2018). The compost used in this study was from Agronomy North Farm, Kansas State University, Manhattan, KS, and produced from food and crop waste materials. Before beginning the experiments selected physiochemical properties such as pH, moisture percentage, Mehlich-III extractable P, and total elemental concentrations of (P, Fe, Ca, K, Mn, Na, Pb, Cd, As, Zn, Ni, Cu) in biosolids and compost were measured.

For each treatment <2 mm sieved 100 g of dry soil was weighed into 250 mL high density polyethlene (HDPE) bottles in three replicates. The soil was then wetted into 40% maximum water holding capacity (MWHC) by mass using milli-Q water. The MWHC of soil was determined using the protocol from Jenkinson and Powlson (1976). They were ground into fine powders to ensure proper mixing of TSP and MAP granules. For biosolids and compost treatments moisture content was considered before wetting the soil, and a balanced amount of water was added, after the treatment addition, to bring the MWHC to 40%. Then treated soil in HDPE bottles was mixed using the ball mill for about one hour. After mixing, bottle openings were covered with parafilm and incubated at 25 ° C temperature for 24 weeks. Moisture adjustments were done frequently on a weight loss basis during the incubation period.

Samples with 20 g treated soil were collected at 2, 4, 12, and 24 weeks of incubation and dried at 40 ° C for about 24-48 hours. A subsample was used for pH measurement and < 250 µm sieved fraction was used for bioaccessibility measurements.

In vitro Soil Pb, As, and Cd Bioaccessibility Tests

An in vitro measure of the physiological solubility of the metal such as Pb that may be available for absorption into the body is defined as bioaccessibility (US EPA, 2012). The physiologically based extraction method (PBET) (Attanayake et al., 2015) methods were used to assess Pb, As and Cd bioaccessibility in soil samples. In-vitro bioaccessibilty Pb determination methods were developed to mimic the human gastric or gastrointestinal dissolution process. Based on the applied in vitro bioaccessibile Pb determination method, bioaccessibile Pb amount can vary because of differences in extraction solutions and pH (Kelly et al., 2002). In the current study gastric phase was used in both methods with pH 2.5. The percentage of bioaccessible Pb, As and Cd was calculated to assess the effects of treatments (soil amendments) on reduced bioaccessibility, following Equation 1.

$$\%\, Bioaccessible\ Pb = \frac{Bioaccessible\ (Pb\ mgkg^{-1})}{Total\ Pb\ (mgkg^{-1})} \times 100 \quad \text{(Equation 1)}$$

Physiologically based extraction method (PBET)

The physiologically based extraction test originally developed by Ruby et al.1996 modified by Brown and Chaney (1997) and Medlin, (1997) was used in this study (Attanayake at al., 2015). The gastric solution was prepared by dissolving 1.25 g of pepsin, 0.5 g of sodium L-malate, 0.5 g of sodium citrate dihydrate, 420 µL of L(+)- lactic acid, and 500 µL of trace metal-grade acetic acid in 1 L of Milli-Q water. The pH of the solution was subsequently adjusted to ~2.5 by drop-wise addition of trace-metal grade HCl and then final volume brought to 1L. The extraction procedure was started by weighing 1.00±0.05 g of soil (sieved to < 250 µm) into a 250 mL wide mouth HDPE bottle and adding 100 mL (soil: solution ratio 1:100) 37°C heated gastric solution. The soil solution mixture was mixed properly and then the pH of the soil solution was measured. Different volumes of trace metal-grade concentrated HCl were added to samples adjusting pH 2.5 ± 0.5 before extraction. The bottles containing the mixture were then placed in a Queue incubator orbital shaker (Parkersburg, WV) with temperature maintained at 37°C, and were shaken for one hour at 150 rpm. After one hour of extraction, the pH of the soil solution was assessed to confirm the pH is 2.5 ± 0.5. If the pH has deviated from 2.5 ± 0.5 the samples were re-extracted. The supernatant solution was filtered using 0.45-µm syringe filters into plastic scintillation vials and analyzed for Pb by ICP-OES. A soil standard reference material (Montana II), duplicate samples, samples spiked with known amounts of Pb concentration, and blanks were routinely included in the PBET extraction and analysis as QA/QC samples.

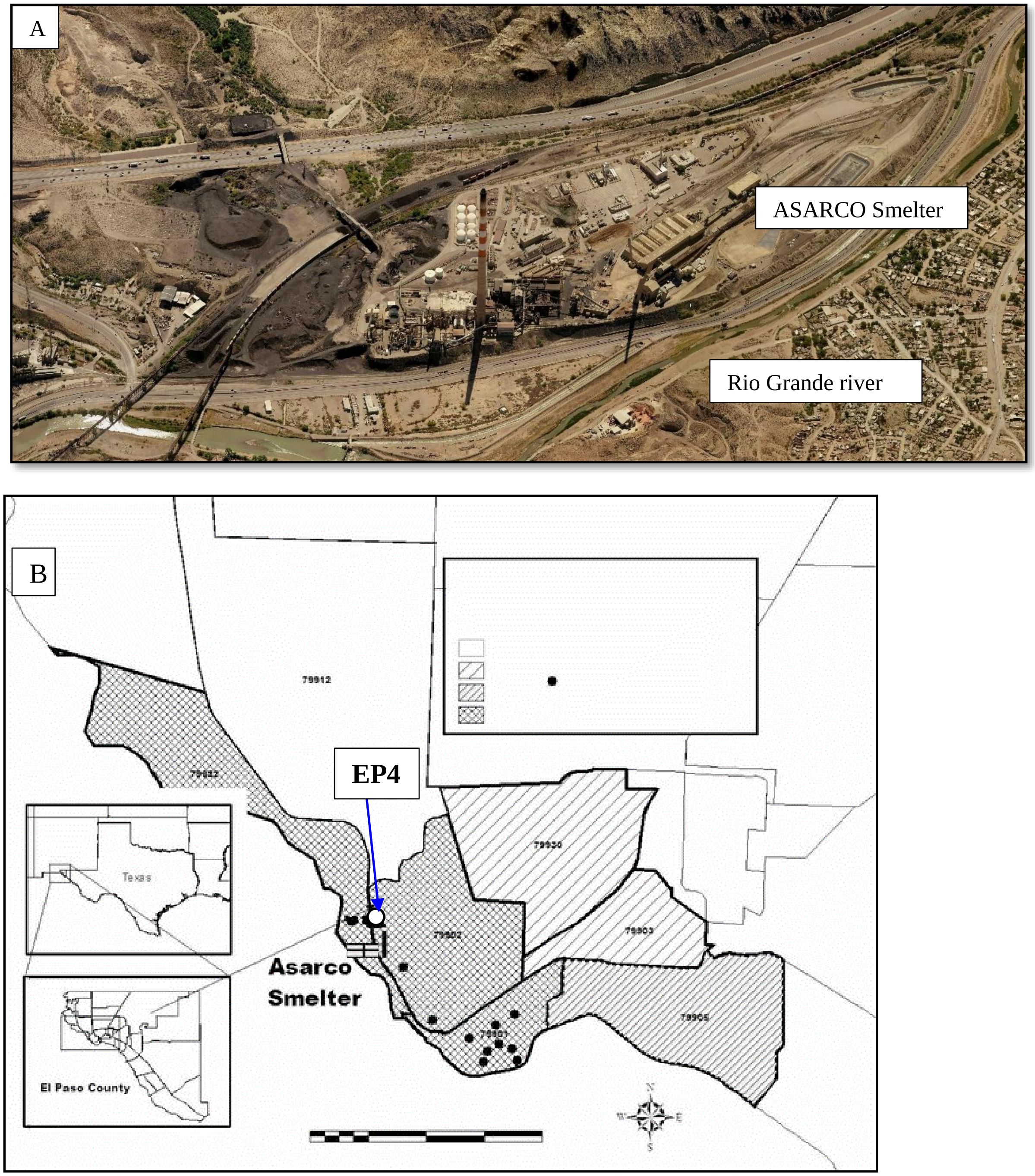


Figure 3.1: A- The location of the Asarco smelter and Rio Grande river (Source: https://www.recastingthesmelter.com). B- the location of the Asarco smelter northwest of the downtown zip code area (79901) (Source: Cunningham and Tiefenbacher, 2008)

Sequential Extraction

A sequential extraction procedure, originally developed by Tessier et al. (1979), serves as an analytical method involving sequential chemical extractions to separate various trace metals (Cd, Co, Cu, Ni, Pb, Zn, Fe, and Mn) into five fractions. These fractions include exchangeable, bound to carbonates, bound to Fe-oxides, bound to organic matter, and residual (Tessier et al., 1979). The original procedure was later used by Zwonitzer et al. (2003) and Scheckel et al. (2003), with small alterations, and was used in this study to estimate the amount of Pb and some other trace metals bound to various soil constituents such as Fe/Al oxides, carbonates, and organic matter, etc. after addition of P containing soil amendments (Scheckel et al., 2003; Zwonitzer et al., 2003). The soil used in the sequential extraction was <2 mm sieved fraction from treated soil after 24-week incubation period.

Briefly, 2.00±0.05 g was weighted into pre-weighed 50 mL centrifuge tubes. The soils were then sequentially exposed to a series of extraction solutions intended to target specific Pb-bound fractions. The targeted soil Pb fractions were extracted as five operationally defined fractions: exchangeable, bound to carbonates, bound to Fe and Mn oxides, bound to organic matter, and residual. After each extraction step samples were centrifuged at 1000 rpm for 30 minutes; the supernatants were carefully decanted and filtered through Whatman No. 42 filter paper into screw capped plastic scintillation vials, and stored at 4°C in the refrigerator until analysis using ICP-OES and graphite furnace atomic absorption spectrometry (GF AAS) (Zwonitzer et al., 2003). After each extraction step, the weight of the residue remaining in the centrifuge tube was recorded to account for metals remaining in the entrained solution. In between each extraction step 8 mL of milli-Q water was added, shaken for 10 minutes, and then centrifuged for 1000 rpm in 30 minutes. The supernatant was carefully discarded and the remaining solid residue was used for the next extraction step. The five extraction steps followed in the sequential extraction procedure were as follows:

Step 1: Exchangeable fraction (F1). Twenty-five mL of 1 M $MgCl_2$ was added to 2 g of soil in a 50 mL centrifuge tube and sealed properly. The soil and $MgCl_2$ mixture was shaken for one hour in a reciprocal shaker at room temperature. The mixture was centrifuged at 1000 rpm for 30 minutes, and the supernatant was filtered through Whatman No. 42 filter paper into screwed plastic scintillation vials, and stored at 4°C in the refrigerator.

Step 2: Carbonates-bound fraction (F2). One molar $NaCOOCH_3$ (Sodium acetate) was prepared and adjusted to pH 5.0 with trace-metal grade CH_3COOH (acetic acid (HOAc)). Then of 25 mL of this buffered acetate was added to each of the residual treatments in step 1, and sealed tubes were shaken for five hours at room temperature. After centrifugation at 1000 rpm for 30 minutes filtration procedures were followed as in step 1.

Step 3: Iron and manganese oxides-bound fraction (F3). The residue of step 2 was extracted by 40 mL of 0.04 M $NH_2OH\text{-}HCl$ (hydroxylamine hydrochloride) in 25% (v/v) HOAc (acetic acid) solution. The soil solution mixture was heated for 6 hours at 96°C with occasional agitation, and then centrifugation and filtration procedures were followed as in step 1.

Step 4: Organic matter-bound fraction (F4). First, 6 mL of 0.02 M HNO_3 and 10 mL of 30% H_2O_2 were added to the residue of step 3 and heated at 85°C for 2 hours followed by adding another 6 mL of 30% H_2O_2 and continued heating for an additional three hours. After cooling, 10 mL of 3.2 M NH_4COOCH_3 (ammonium acetate) in 20% (v/v) HNO_3 and 10 mL of milli-Q water was added the samples was mixed; centrifugation and filtration procedures were followed as in step 1.

Step 5: Residual fractions (F5). The remaining residue from step 4 was treated with 20 mL of 4 M HNO_3 and the mixture was heated for two hours at 80°C. Filtration procedures were followed as in step 1.

Synchrotron-based Lead Speciation

Soil sample preparation

A composite soil sample of three replicates from each treatment, after 24 weeks of treatment addition, was prepared for the Pb speciation study. Equivalent quantities of the <250 µm fraction of soil from each replicate of the treatments were ground to a fine powder with an agate mortar and pestle to facilitate improved homogenization. Soil samples were packed in 2 mm thickness Plexiglass sample holders with the aid of Kapton® tape to study bulk soil Pb speciation. First, one side of the sample holder slit was sealed with Kapton® tape and filled and packed with the soil. Second, the other side was sealed using Kapton® tape. The labelled sample holders were then mounted on the sample stage (45° angle to the beam) to allow the X-ray beam to pass through the soil sample, which was positioned between the two pieces of Kapton in the holder slit. To study spatially resolved micro X-ray fluorescence (µ-XRF) analyses, soil-thin films were prepared by sprinkling the soil onto a Kapton® tape and sealed with another Kapton® tape and then mounted on the sample stage.

X-ray absorption measurements

Both bulk and micro X-ray fluorescence analyses were done at the Advanced Photon Source

(APS), Argonne National Laboratory in Argonne, IL. Bulk Pb X-ray absorption near-edge spectroscopy (XANES) analysis was performed at the 5 BM-D beam line of DND-CAT. Data were collected at Pb L_{III}-edge (13,035 eV) in fluorescence mode with the Canberra Inc.13-element Ge solid-state detector operated in an energy range between 4.5-25 keV. The energy was calibrated using the pyromorphite standard. This standard spectrum was used to distinguish any energy drifts induced by the monochromator drifts during the sample run. The number of spectra collected per sample was 6 to 8 (mostly 6).

Bulk XANES and X-ray absorption fine structure (XAFS) spectroscopy data processing and analysis were done using Athena-Demeter software version 0.9.26 in IFEFFIT software (Ravel, 2005). In the data analysis process first, the 3-4 scans of a sample were merged to reduce noise. Then the background was corrected using pre-edge subtraction, and the spectrum was normalized. Using the normalized spectra of reran standards, previously run standard spectra were aligned for any energy drifts. For XAFS data analysis, the data were converted to k-space, ranging from 2-10 $\acute{\text{Å}}^{-1}$, k^2 weighted to compensate for the dampening of the XAFS amplitude with increasing k. The linear combination fitting (LCF) was conducted to obtain the best combination of Pb species using standard Pb compounds.

The following 23 standard Pb reference spectra were used for linear combination fitting of both bulk and micro XAFS: pyromorphite, chloro-pyromorphite, Pb-montmorillonite, Pb-goethite, Pb-calcite, Pb-kaolinite, $Pb(OH)_2$, hydro-cerussite, cerussite, Pb-citrate, Plumboferrite, Pb-adsorbed hydroxyapatite, hydroxy-pyromorphite, sorbed-Pb, Pb bound to humic acid, Pb bound to fulvic acid, anglesite, galena, plumboferrite, plumbogummite, litharge, plattenerite, and plumbocarnite. The XANES spectra of selected Pb reference standards are shown in Figure S6.

Spatially resolved micro XRF, XAFS, and X-ray diffraction (XRD) analyses were performed to determine elemental distribution (Pb, Fe, As, Ca, Mn, Cd, and As) association and speciation at the GSC CARS 13 ID E at the Advanced Photon Source for selected treatments. These treatments were the control, TSP1:6, MAP1:6, and biosolids 10% together with the original (untreated) soil. X-ray fluorescence (μ-XRF) mapping for elements was done for a 2 x 2 mm area in soil thin film using X-ray emission energies at 16 keV. Multiple elements were analyzed simultaneously. Approximately 20 hot spots were selected to perform μ-XRD for mineral identification with spatial resolutions of <1 μm using the Eiger 1M area detector. For, XRD analysis data were then transformed into Cu- K-alpha wavelength. In addition, μ-XANES/XAFS spectra were collected at each spot. Micro-XANES/XAFS data were analyzed following the same procedure mentioned in bulk XAFS analysis using Athena-Demeter software. Geo soil enviro (GSE) XRM map viewer (Larch: Newville, 2013) was used to read ROI maps and develop elemental correlation maps to visualize elemental distribution and associations in soil samples. Match software version 3 (Crystal Impact GbR, 2003) was used for μ-XRD data analysis.

Statistical Analysis

Collected data in pH, bioaccessible Pb, As, and Cd were entered into an Excel database, and double-checked for accuracy. Percent bioaccessible Pb, As and Cd was used to evaluate the treatment (soil amendments) effects on reducing bioaccessibilty.

All analyses were conducted using SAS 9.4 software (2016 by SAS Institute Inc., Cary, NC, USA). Changes in bioaccessible Pb were evaluated as a function of time, pH, and treatment. Comparisons by treatment and time were tested with 9 x 4 ANOVAs ($p < 0.05$) with post hoc comparison of means for percent bioaccessible Pb and pH. For main effects, post hoc comparisons of LSM differences were calculated using the Tukey–Kramer adjustment for multiple comparisons.

The sequential Pb fractionation data was first converted into percentages of each fraction relative to the total Pb. Subsequently, the percentage of Pb, As, and Cd fractions at each step was analyzed using the PROC GLM procedure in SAS, employing least square means to compare all treatments at a significance level of 0.05.

Results and Discussion

Soil characterization

Selected total elemental concentrations of the EP4 soil are presented in Table 3.1. According to the natural resources conservation service (NRCS), the Delnorte soil series (Taxonomic Class: Loamy-skeletal, mixed, superactive, thermic, shallow Calcic Petrocalcids) was identified in the region where EP4 soil samples were collected. The EP4 soil texture is characterized as loamy sand (70% sand, 18% silt, and 12% clay) typical to the El Paso area and Delnorte soil series (Elkekli, 2013; NRCS, 2024). Soil pH was slightly alkaline having a 7.63 pH value when measured in a 1:2 soil: water ratio and 8.09 in a 1:5 soil: water ratio. The climatic conditions promote the formation of alkaline soil, which is typical for arid climates. The arid to semi-arid desert climate in El Paso is marked by the combination of abundant sunlight, low humidity, minimal precipitation, and high evaporation rates contributing to the development of alkaline soil conditions in the region (El-Hage and Moulton, 1998; Elkekli, 2013; Paz et al., 2017; NRCS, 2024). The electrical conductivity was 414 µS cm^{-1} and CEC was low (8.39 $cmol_c$ kg^{-1}). The percentage of MWHC was found 40% and the TOC was about 1.2%. The total Pb concentration of soil was 3199 ± 192 mg kg^{-1} in < 2 mm fraction, indicative of its proximity to the nearby smelter. In addition to that considerably higher concentrations of As, Cd, Zn, Cu, Se and Mo were found. The total Pb concentration in < 250 µm fraction was 3417±156 mg kg^{-1} and the bioaccessible Pb concentration was 1541±15 mg kg^{-1}, representing 48% of total Pb in the fraction. The higher total Pb was observed in the fine fraction (< 250 µm) of soil. Additionally, the bioaccessible As and extractable Cd percentages were 53 and 90%, respectively, based on the PBET extraction method. Many studies have reported that concentrations of trace metals in smelter-impacted soil vary, and the amounts of these metals tend to change with different factors. Some of them are the distance from the smelter, wind direction, primary minerals, history of operation, and soil characteristics (Du et al., 2008; Fritsch et al., 2010; Ettler, 2016; Ono et al., 2016; Adnan et al., 2022; Smieja-Król et al., 2024). Maximum contaminant concentrations in EP4 soil surpass the regional screening levels (RSLs) for certain trace elements (As, Pb, Cd, Se) for industrial soils, as proposed by the US EPA by several folds (US EPA, 2024). Contamination by multiple trace elements, including As, Pb, Zn, Cd, Cu, and Ni commonly found in mineral deposits, is prevalent in mining and smelting areas. The variability in both total and bioavailable concentrations of trace elements among different sites has been evident in numerous studies (Schaider et al., 2007; Du et al., 2008; Ono et al., 2016). Their mobility and availability in the environment are controlled by specific physicochemical parameters in the soil (Borgese *et al.*, 2013; Kicińska et al., 2022).

Class A biosolids had a pH of 7.47±0.02 and a 62% moisture content. Both Pb and As concentrations in Class A biosolids were far below in the pollutant limit requirement specified by the US EPA, (2024) (< 8 mg kg^{-1}) and Cd was not detected. Approximately 10% of total Fe and 3.5% of total P were found in Class A biosolids, while Mehlich-III P was 744 mg kg^{-1}. Compost exhibited a slightly acidic pH of 6.62±0.06 and a moisture content of 26%. Despite having a total phosphorus concentration of 1467 mg kg^{-1}, the Mehlich-III P concentration in the compost (669 mg kg^{-1}) was notably higher, compared to the biosolids. Lead and As concentrations were low, and Cd was not detected in the compost. Kim and Owens, (2011) reported that toxic trace elements such as Pb, Cd, and Cr were not detected in five sources of biosolids from all over the world (Kim and Owens, 2011).

Changes in pH with time

The pH decreased over time in all treatments, including the control, as illustrated in Figure 3.2. The acidulation effect resulting from the high rate (1:6) of TSP and MAP addition was the most pronounced, with the pH of the high biosolids treatment (10%) being significantly higher than all other treatments, including the control. Throughout the incubation period, the measured pH values in the 10% biosolids and 5% compost treatments exhibited the smallest decline (about 0.1). In contrast, the control treatment exhibited a decline in pH, at 7.60 after 4 weeks and reaching 7.09 by the conclusion of the study. The pH in all inorganic P-treated soils continued to decrease from 2 weeks after and reduced to ~ 6.78 to 6.15 by the end of the study. The pH of MAP and TSP 1:4 treatment showed a significant decrease by 2 weeks to 4 weeks. However, at the end of the study, the pH of the MAP and TSP 1:6 treatment was significantly ($p=0.05$) lower (6.15 and 6.25 respectively) than all other treatments.

Table 3.1: Selected total elemental concentrations of the EP4 soil, Class A biosolids, and composts

Element	**EP4 soil**	**Class A biosolids***	**Compost***
Pb mg/kg	3199 ± 192	20.7	4.14
As mg/kg	254 ±14	7.8	1.3
Cd mg/kg	110 ± 8	< DL	< DL
Ni mg/kg	21.1 ± 3.6	17.4	5.8
Mo mg/kg	16.9 ± 2	-	-
Se mg/kg	11.9 ± 1.7	-	-
Mn mg/kg	266 ± 14	434	432
P mg/kg	1138 ± 102	34603	1467
K mg/kg	3237 ± 129	1365	2803
Zn mg/kg	1982 ± 88	700	93
Cu mg/kg	2708 ± 129	369	9
Na mg/kg	638 ± 19	481	756
Al mg/kg	8317 ± 828	-	-
Ca mg/kg	17065 ± 419	28377	16247
Fe mg/Kg	16604 ± 764	97519	11705

***** Corrected values for moisture

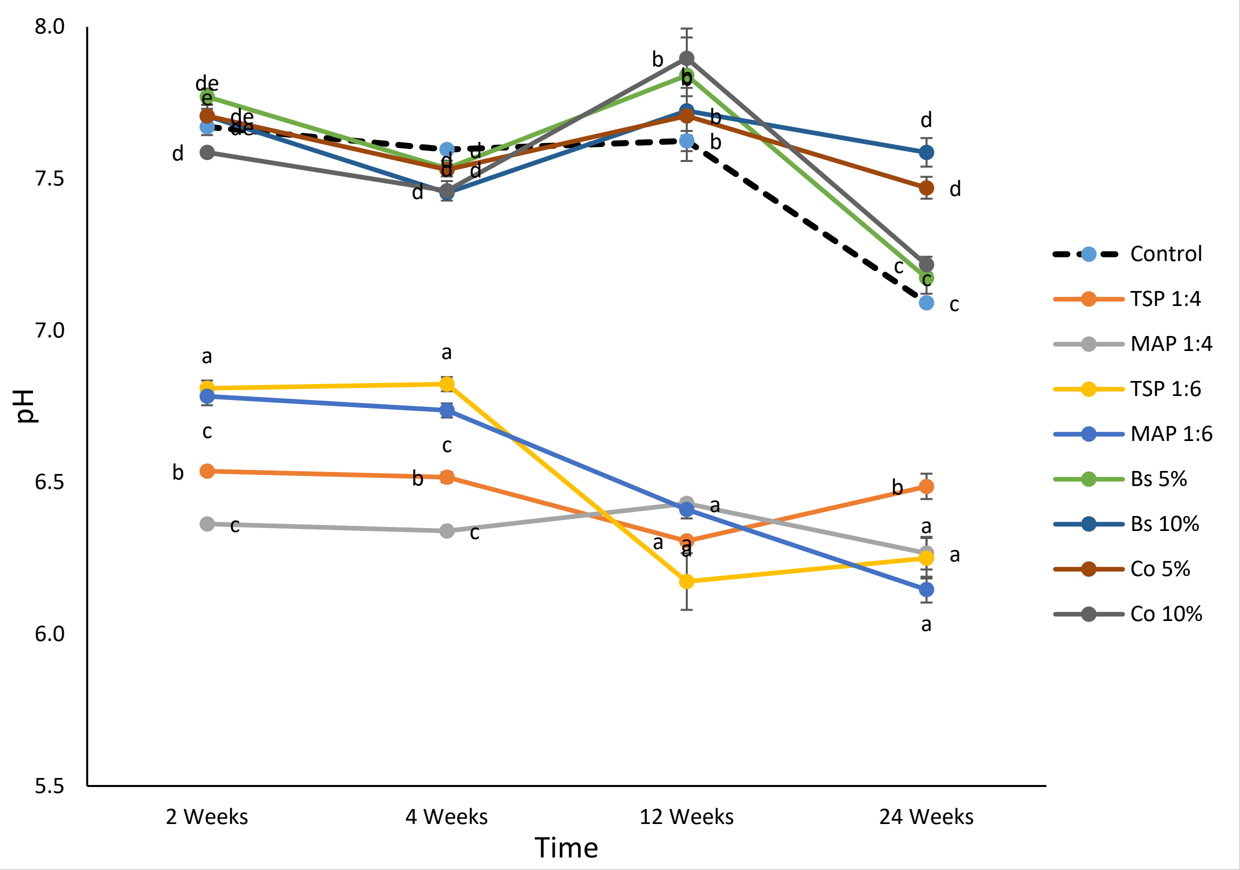


Figure 3.2: Changes in pH with time during incubation study sampled at 2, 4, 12 and 24 weeks. TSP, triple superphosphate; MAP, mono ammonium phosphate; Bs, biosolids; Co, compost. (Means within each time containing the same letter are not significantly different at P = 0.05).

In addition to the direct impact of soil amendment treatments on soil acidity, the retention of moisture (MWHC 40%) is attributed to enhancing soil chemical reactions. These reactions facilitate the release of H^+ into the soil solution, leading to an increase in soil acidity. The chemical processes contributing to soil acidity include but are not limited to the dissolution, degradation, oxidation, and mineralization of minerals and organic matter in the soil (Bolan et al., 2005; Zhang, 2017). Over the 2 to 24-week time period, inorganic soil amendments demonstrated a more significant reduction in pH (Figure 3.2) compared to all organic soil amendments and the control. Both MAP and TSP introduce phosphorus to the soil in the form of the $H_2PO_4^-$ (orthophosphoric) ion, contributing to soil acidification through the dissociation of H^+ (equation 1 and 2). The acidulating reaction is prominent within a pH range of 7.2 – 7.7 but has no impact on decreasing soil pH in acidic soils (McLaughlin, 2009) based on pKa of the transition between diprotonated and monoprotonated P anion. With an initial pH of 7.63, the EP4 soil experiences a significant reduction in pH after treatment with both TSP (P is in monocalcium phosphate form, $[Ca(H_2PO_4)_2]$ and MAP $[NH_4(H_2PO_4)]$ in both rates.

$$\mathrm{Ca(H_2PO_4)_2 + H_2O \rightarrow CaHP}O_4 + \mathrm{H_2PO_{4^-} + H^+} \quad \text{Equation 1}$$

$$\mathrm{NH_4H_2PO_4 \rightarrow NH_4^+ + H_2PO_4^- \rightarrow NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O} \quad \text{Equation 2}$$

However, compared to the control, both organic treatments (biosolids and compost) showed higher pH after 12- and 24-week incubation periods, indicating biosolids and compost addition was effective against soil acidification. The alkaline nature of biosolids and compost can elevate soil pH, while organic fertilizer supplies base ions to counteract soil acidification (Chen et al., 2022). Dai et al. (2021) observed a pH increase of 0.18 units with organic fertilizer (made from cattle manure) and Chen et al, (2022) observed increased soil pH values of the topsoil 0.49–0.75 units with manure compost (Dai et al., 2021; Chen et al., 2022). Additionally, organic fertilizer application decreased soil exchangeable acid content and increased total exchangeable base compared to chemical fertilizer (Dai et al., 2021). Xiao et al. (2021) found that continuous chemical fertilizer usage decreased soil pH over 28 years, whereas manure application alone or combined with chemical fertilizer raised soil pH. Codling et al, (2020) studied biosolids application in different locations in the USA reported that increased pH with the biosolids treatments in slightly acidic soil (Codling et al., 2021). A study discovered that aging biosolids with soils of varying pH levels, including both acidic and neutral soils, led to a significant reduction in the concentrations of Zn and Cu in the solution over time. Additionally, there were simultaneous increases in the pH of the solution as the aging period progressed (Murtaza et al., 2012). Further, the soil had a loamy sand texture with limited clay and organic matter content, enabling opportunities for soil improvement through the addition of organic amendments.

Soil pH is a critical factor that serves as a master variable influencing various soil properties, bioavailability, and the mobility of soil contaminants, including trace elements (Apple and Ma, 2002; Uchimiya *et al.*, 2020). Many studies have reported that Pb bioavailability tends to decrease with increasing soil pH due to the precipitation of hydroxides, carbonates, or the formation of insoluble organic complexes (Rieuwerts *et al.*, 1998; Basta *et al.*, 2005; Sipos *et al.*, 2005; Yang *et al.*, 2006; Yu *et al.*, 2023). However, the current study contradicts this trend, revealing a decrease in bioaccessibility with decreasing pH in TSP and MAP treated soil. This may be due to the promoted immobilization of Pb upon enhanced dissolution of Pb-associated minerals/ complexes under acidic conditions. At low pH, multiple hydrolysis reactions are promoted and the Pb^{2+} becomes available for reactions. Interestingly, the biosolids 10% treatment, despite having a pH above 7.5, also exhibited reduced bioaccessibility. Hence, the bioavailability of Pb in this soil is not solely determined by pH but is also influenced by the properties of Pb and other co-contaminants, as well as many other soil and treatment characteristics.

Changes in bioaccessible Pb with time

Percent Pb bioaccessibility is shown in Figure 3.3 and total bioaccessible Pb is shown in Figure 3.4 measured using a modified PBET procedure (Attanayake et al., 2017) at pH 2.5 in < 250 µm soil fraction. Total bioaccessible Pb concentrations ranged from 883 (MAP 1:4) to 2165 (control) mg kg^{-1} (26 to 46% of total Pb in soil) (Figure 3.3 and 3.4) after 24 weeks incubation period. The PBET extraction results showed a significant reduction in bioaccessibility ($p<0.05$) with all the soil amendments, except compost 5% treatment after 24 weeks. The percentage of bioaccessible Pb reduction compared to the control range from 3% (compost 5%) to 44% in MAP 1:4 treatment after 24 weeks. The 5% and 10% biosolids treatments demonstrated a notable 40% reduction in bioaccessible Pb compared to the control, whereas the compost treatment exhibited the lowest reduction ranging from 3% to 14%. The reduction range for both MAP and TSP treatments ranged from 29% to 44%, with MAP showing a relatively higher reduction than TSP. The percent Pb bioaccessibility after 2 and 24 weeks ranging between 63-46% in control, 70-33% and 52-29% in TSP 1:4 and 1:6 respectively, 64-26 and 48-28% in MAP 1:4 and 1:6, respectively, 41-28% and 40-27% in biosolids 5 and 10%, respectively and 56-45% and 46-40% in compost 5 and 10%, respectively.

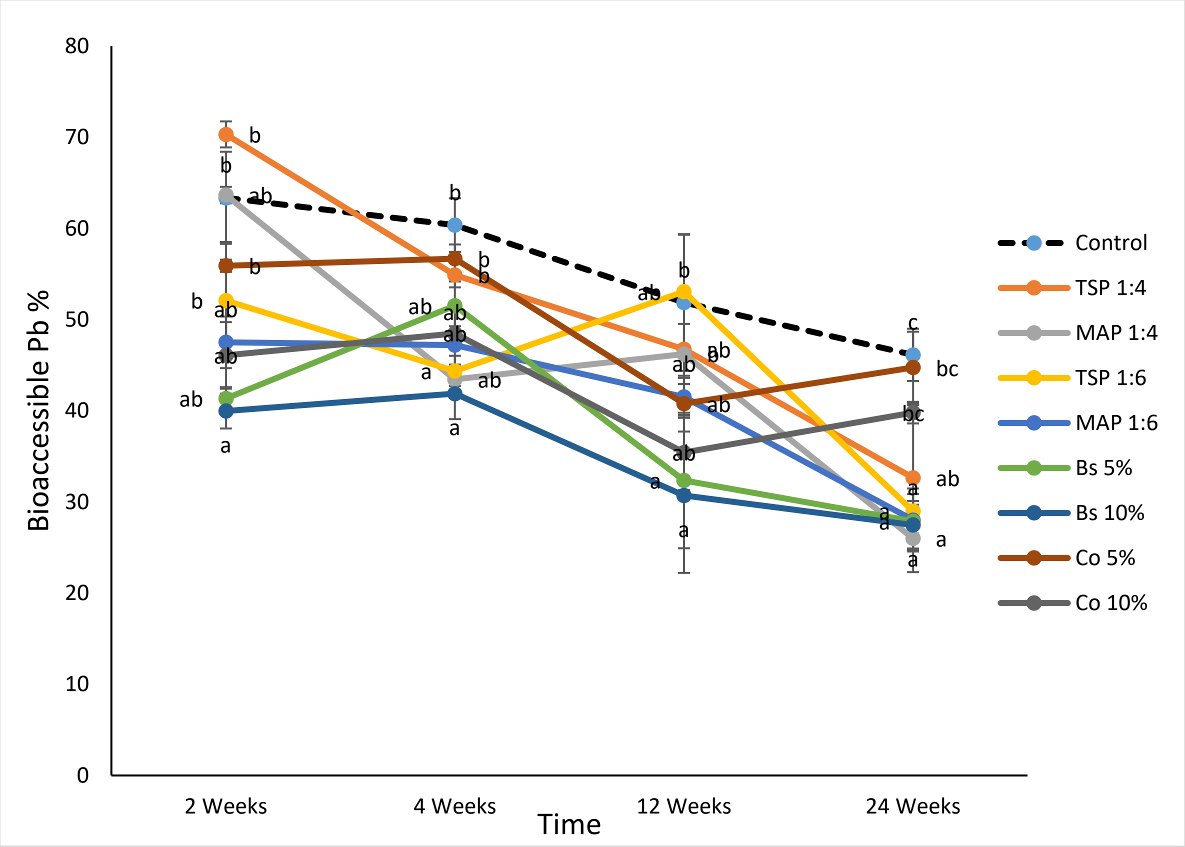


Figure 3.3: Percent bioaccessible Pb (PBET) changes with time during the incubation study sampled at 2, 4, 12 and 24 weeks. TSP, triple superphosphate; MAP, mono ammonium phosphate; Bs, biosolids; Co, compost. Means within each time containing the same letter are not significantly different at P = 0.05.

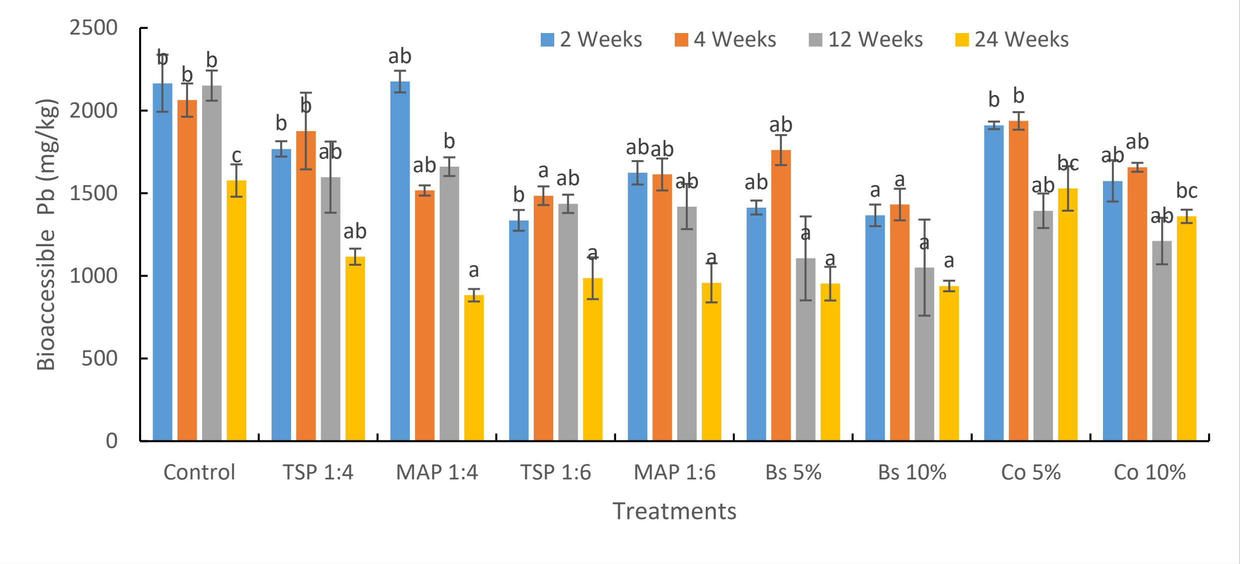


Figure 3.4: Bioaccessible Pb (mg kg^{-1}) (PBET) concentrations of soils during the incubation study in 2, 4, 12 and 24 weeks. TSP, triple superphosphate; MAP, mono ammonium phosphate; Bs, biosolids; Co, compost. (Means within each time with the same letter are not significantly different at P = 0.05).

The percent bioaccessible Pb results were influenced by both treatment and time. There was a slight increment in percent bioaccessibility for some treatments after 4 and 12 weeks compared to 2 weeks or 4 weeks. However, after 24 weeks, the reduction remained significant for all treatments. Soluble P amendments such as TSP and MAP were found to lower soil pH, leading to a greater reduction in Pb bioaccessibility, as observed in a meta-analysis by Mayer et al. (2022). The decrease in soil pH is known to enhance the dissolution of Pb minerals, potentially explaining the inverse relationship between soil pH and bioaccessibility (Sauvé et al., 1998). Miranda, (2021) reported that soluble P (SP) was the most effective in reducing the percent bioaccessible Pb, with both SP and biosolids, either applied individually or combined, resulting in reductions ranging from 25% to 50%. Additionally, they found that compost application contributed to the reduction of bioaccessible Pb in strongly acidic soils (Miranda, 2021).

Similar findings were reported by Attanayake et al. (2015), who observed a decrease of approximately 38% in the bioaccessibility of soil Pb across all treatments, including composted biosolids, non-composted biosolids, mushroom compost, leaf compost, and a non-amended control. They noted that the absolute bioaccessibilities of soil Pb were notably lower in soils amended with composted biosolids, likely due to the significant dilution of total Pb with their application (Attanayake et al., 2015). Brown et al. (2003) found that the addition of high-iron biosolids compost reduced the bioavailability of soil Pb by 37% and 43% in in vivo and in vitro studies, respectively. In their study, among the four compost materials tested, three resulted in reductions in Pb bioavailability exceeding 20% (Brown et al., 2003). Similarly, Farfel et al. (2005) observed reductions of 64% and 67% in bioaccessible Pb concentrations one year after the application of biosolids compost rich in Fe and P at two locations in their field study (Farfel et al., 2005).

The relationship between the percentage of bioaccessible Pb and various fractions obtained through sequential extraction

The sequential extraction results after 24 weeks revealed a significant influence of soil amendments on the distribution of Pb across different fractions (Figure 3.5). Each extraction step in this sequential process targets a specific Pb fraction, while each progressing step targets more resistant Pb species. It is important to note that these operationally defined fractions may represent various Pb mineral species.

The treatment with 10% biosolids exhibited the lowest percentages of exchangeable (1%), carbonate-bound (9%), and organic-bound (3%) Pb, whereas the original (untreated) soil, control, and compost treatments showed significantly higher percentages of exchangeable Pb

(5%), carbonate-bound (11-21%), and organic-bound (6-13%) Pb. Additionally, the 10% biosolids treatment demonstrated the highest percentage of residual Pb, accounting for approximately 61%. A statistically significant ($p < 0.05$) negative correlation ($R^2 = 0.62$) was observed between bioaccessible Pb and the residual fraction. Additionally, exchangeable, carbonate-, and Fe-Mn oxide-bound Pb fractions exhibited statistically significant ($p < 0.05$) positive correlations, while the organic-bound fraction did not show a significant relationship with bioaccessible Pb in this study.

Similar results were reported by Elkhatib and Moharem (2015), who found that the application of biosolids to calcareous and sandy soils significantly reduced exchangeable, carbonate, and organic Pb fractions while increasing oxide and residual Pb fractions. Li et al. (2021) observed a significant increase in the residual fraction of Pb (59%) with composted sewage sludge compared to the control, surpassing other organic treatments. The confirmation of pyromorphite or pyromorphite-like minerals formation after treatment was provided by solid-phase microprobe analysis in this study (Li et al., 2021). The residual Pb fraction, representing the least soluble or bioavailable form, may include pyromorphite or pyromorphite-like minerals, indicating enhanced Pb transformation into relatively stable species by amendment treatments (Miretzky and Fernandez-Cirelli, 2008).

However, Gomes et al. (2022) reported contrasting findings, noting that biosolids were the only amendments to increase Pb in organic Pb fractions while decreasing iron-oxide Pb contents and residual fraction. Lime and phosphate amendments increased Pb in the residual fraction, while biosolids had the opposite effect (Gomes et al., 2022). The composted and stabilized biosolids, produced by the water treatment plant was used in this study. Further, they have observed Pb-citrate complexes based on XANES analysis indicating a potential binding of Pb with organic groups present in the biosolids. They have suggested that the complexation of Pb by soluble organic ligands biosolids could have contributed to the enhanced desorption of Pb in their study (Gomes et al., 2022).

The results of sequential extraction and percentage of bioaccessible Pb give some useful insight into the immobilization of Pb in the soil and confirm the effectiveness of the amendments (Figures 3.5 and 3.6). The data of sequential extraction of the treatments, biosolids, TSP, and MAP, demonstrated that the Pb extracted in the exchangeable and carbonate fractions were decreased and was transferred to the residual fraction indicating the presence of more recalcitrant species that is an evidence of the pyromorphite formation. Notably, the biosolids treatment exhibited exceptional performance in reducing bioaccessible Pb, along with reductions in exchangeable, carbonate-bound, and organic-bound fractions of Pb. The superior performance of MAP and TSP underscores the crucial role of P fertilizer, highlighting their ability to readily transform Pb into a residual fraction in this soil indicating the presence of more recalcitrant species such as pyromorphite. In the same line decrease in the Pb extracted in the exchangeable, carbonate-bound, and organic-bound fractions of Pb which are more soluble.

The poor performance of reducing bioaccessible Pb observed in compost treatments in this study may be attributed to several factors. Firstly, the minimal change in pH associated with compost treatments could have played a role. Soil pH is a crucial factor influencing the mobility and availability of Pb in soil (Apple and Ma, 2002; Uchimiya *et al.*, 2020), and the smallest pH change in compost-treated soils compared to other treatments may have limited the effectiveness of Pb remediation. Additionally, the stability of Pb-bound fractions in compost treatments appeared to be similar to that of the control treatments (Figure 3.5). This similarity suggests that compost treatments did not significantly alter the stability of Pb-bound fractions compared to untreated soil, further contributing to their poor performance. These findings underscore the importance of considering pH changes and the stability of Pb-bound fractions when evaluating the efficacy of compost treatments for Pb remediation in soil. In our current study, we utilized fully decomposed food and crop residue compost, which likely contains a higher proportion of humin complexes compared to humic and fulvic acids (Wei et al., 2020). Humin, being macro-organic substances, are insoluble and undergo slow decomposition processes. In contrast, humic and fulvic acids are chemically active, facilitating ion exchange and retention processes more readily. The predominance of humin complexes suggests a slower release of active compounds, potentially impacting the rate and extent of remediation processes (Palanivell et al., 2013; Hu et al., 2022; Lanno et al., 2022).

The findings from sequential extraction methods strongly indicate a connection between the conversion of exchangeable, carbonate-bound, and organic-bound Pb fractions into highly stable compounds and the subsequent decrease in Pb bioaccessibility following these treatments such as lime-stabilized municipal biosolid, municipal biosolid–alkaline admixture blend marketed as N-Viro Soil, rock phosphate, anaerobically digested municipal biosolid, high Fe biosolids compost and high Fe + lime biosolids compost (Basta et al., 2001; Brown et al., 2003). Additionally, the fractions bound to Fe and Mn oxides were notably lower in biosolids compared to TSP and MAP treatments, while they exhibited higher levels in compost treatment, control, and the original soil.

Lead demonstrates a higher adsorption affinity than most other trace metal cations on Fe and Mn oxide minerals (McKenzie, 1989; Fendorf et al., 2004; Hettiarachchi and Pierzynski, 2004; Yang et al., 2023). However, sequential extraction results suggested that the reduction in bioaccessible Pb is more closely associated with the decrease in Fe and Mn-bound fractions, accompanied by an increase in the residual fraction. This may be due to the formation of mineral-organic associations accelerated by soil organic matter and Fe-Mn oxides. Treatments biosolids TSP and MAP were evident that reduction in Fe and Mn-bound Pb together with organically bound Pb fractions while increasing residual fraction (Figure 3.5). Hu et al. (2023) conducted a study investigating the influence of mineral-organic interactions on Pb speciation. Their findings indicate that metal oxides and soil organic matter primarily act as scavengers for Pb. The accumulation of the most stable residual Pb was attributed to the presence of Fe/Al oxides, facilitated by organic matter through the formation of amorphous Fe/Al oxides. Surprisingly, the addition of organic matter resulted in the activation of metal oxides, while competition from Fe/Al/Ca ions decreased the binding of Pb by soil organic matter (Hu et al., 2023).

Lead in soil exists in various forms, including dissolved, exchangeable, adsorbed, and precipitated with other soil components (Kabala and Singh, 2001; Qayyum et al., 2016). The majority of Pb found in the residual fraction is naturally occurring and exists in the form of adsorbed and precipitated compounds. The first two forms (dissolved and exchangeable) are highly bioavailable, while the other two (adsorbed and precipitated) are potentially bioavailable depending on the exposure conditions (Kabala and Singh, 2001). Understanding the mechanisms by which a heavy metal element transitions from one form to another, the rate of this transformation, and the stability of the formed compounds are crucial factors determining the effectiveness of treatments (Egendorf et al., 2020; Yu et al., 2023). The current study showed grater insights into the sequential extraction results suggesting that the transformation of exchangeable, carbonate-bound, and organic-bound fractions of Pb into stable compounds in residual fraction correlates with a reduction in Pb bioaccessibility. The distribution of Pb fractions among different treatments indicates variations in Fe and Mn oxide-bound fractions, with biosolids showing lower levels and compost treatment exhibiting higher levels. Understanding the forms of Pb in soil and their bioavailability is crucial for effective remediation strategies. Overall, the mechanisms governing Pb transformation and stability in soil play a pivotal role in determining the success of remediation treatments and mitigating Pb exposure risks.

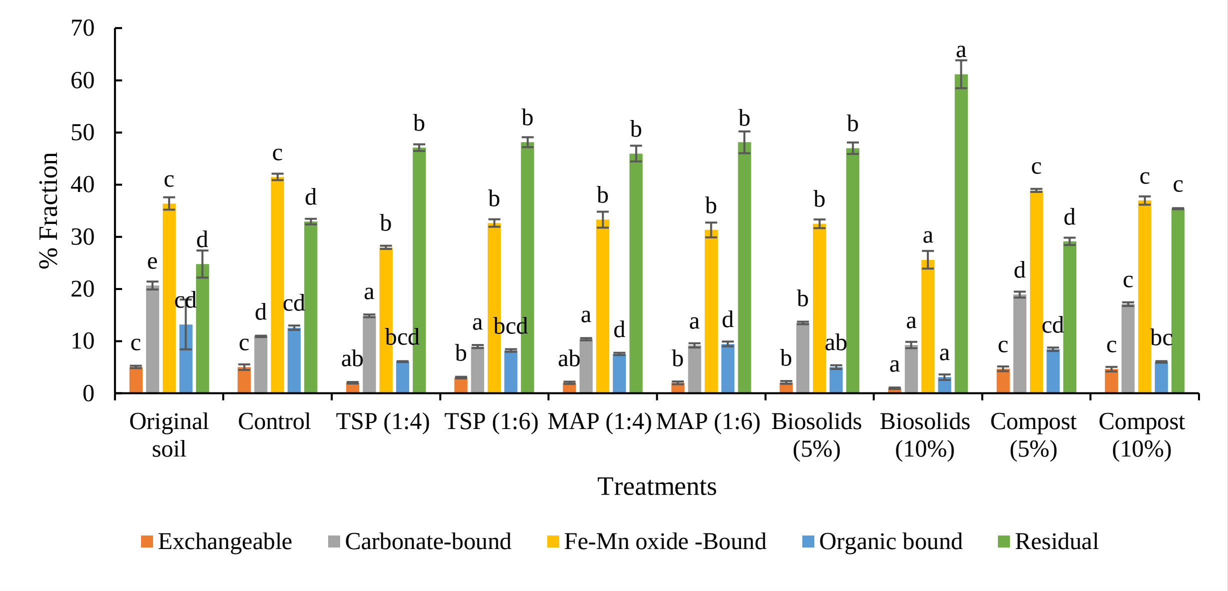


Figure 3.5: Percentage of total Pb measured in each fraction of sequential extraction performed on 24 weeks of incubation. TSP, triple superphosphate; MAP, mono ammonium phosphate. Means within each fraction containing the same letter are not significantly different at P = 0.05.

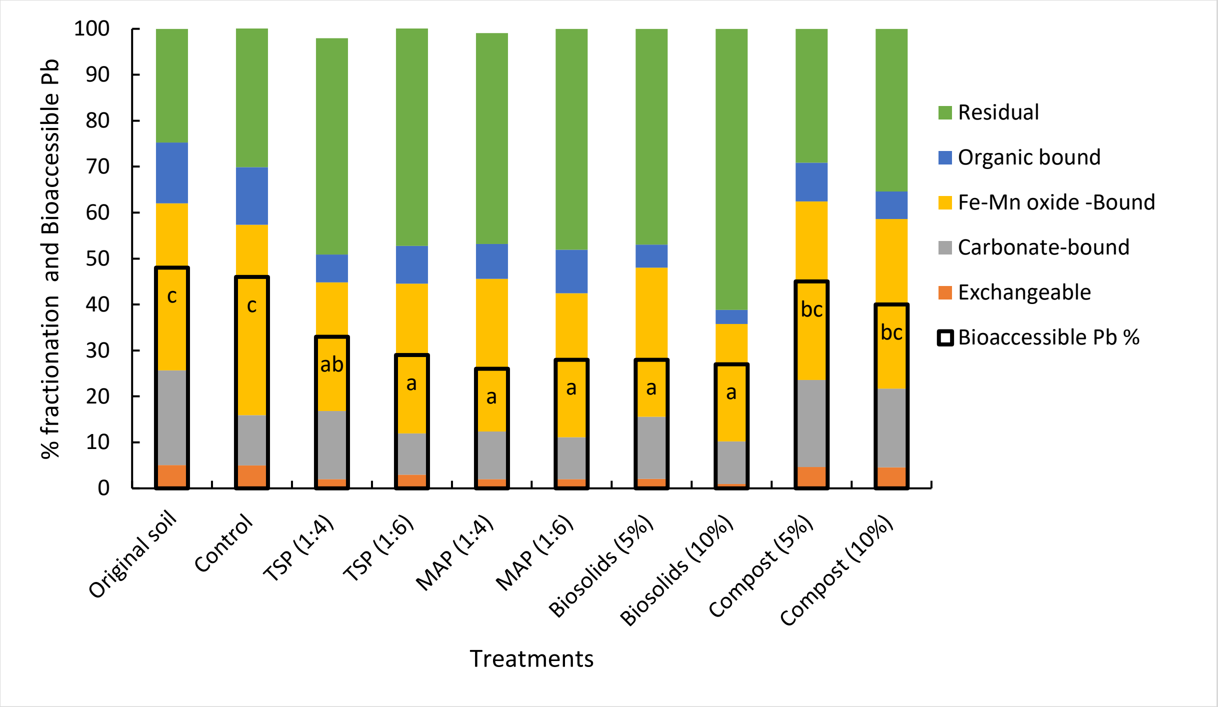


Figure 3.6: Percentage of bioaccessible Pb overlaid upon the % total Pb measured in each fraction of sequential extraction, performed on 24 weeks of incubation. TSP, triple superphosphate; MAP, mono ammonium phosphate. Means within percent bioaccessible Pb in each treatment containing the same letter are not significantly different at P = 0.05.

Synchrotron X-Ray Analysis

The synchrotron X-ray analysis of Pb speciation after 24 weeks of incubation indicated a significant influence of certain soil amendments on Pb speciation. Results from both bulk XANES/ XAFS and micro XANES analyses unveil the formation of pyromorphite compounds in treatments that lead to a notable reduction in Pb bioaccessibility. Despite the EP4 soil having a high total Pb concentration, during our bulk XANES analysis for Pb species determination, our findings indicated a lack of XANES spectral sensitivity in terms of different spectral features in accurately discerning the species. This limitation appears to be attributable to the specific adsorbed phases that are challenging to specifically identify using Pb XANES spectra in the reference standard and samples utilized in the LC fitting for different treatments (Sowers et al., 2021). In EP4 soil, bulk XAFS spectra exhibited a greater sensitivity in determining Pb species distribution than the XANES region, across each treatment (Figure 3.7). Accordingly, the synchrotron X-ray analysis revealed that specific treatments correlate strongly with reduced bioaccessibility of Pb species. This indicates that certain experimental conditions or interventions have a notable impact on altering the bioavailability of Pb in the soil, potentially through mechanisms such as precipitation or transformation of Pb species into less soluble or less accessible forms.

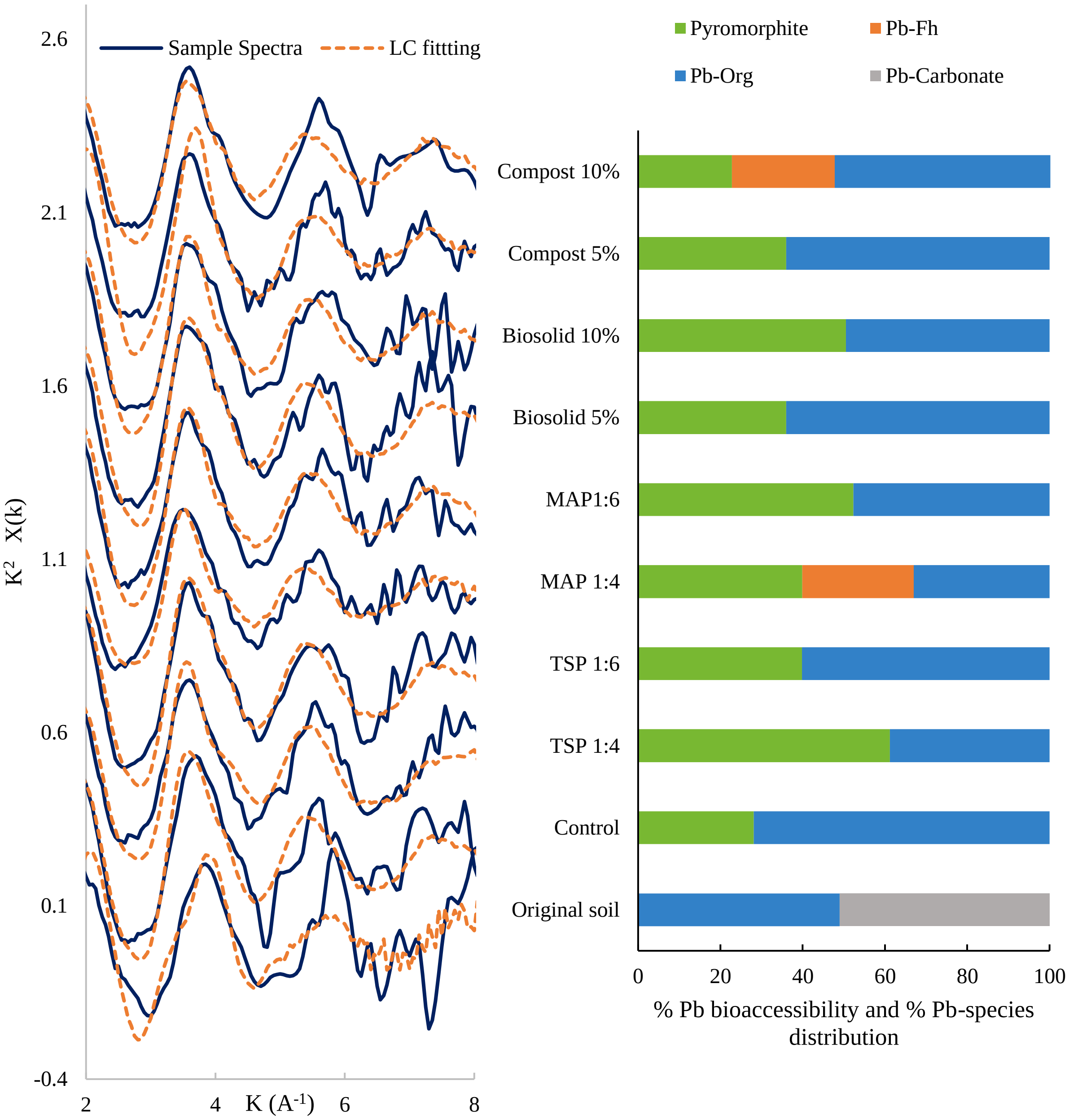


Figure 3.7: Normalized Pb K-edge XAFS spectra of each treatment with results of linear combination (LC) fitting as percentage of Pb-species distribution. (TSP: triple superphosphate; MAP: mono ammonium phosphate; Pb-Fh: Fe-Ferrihydrite sorbed Pb and Pb-Org: Pb bound to organic compounds)

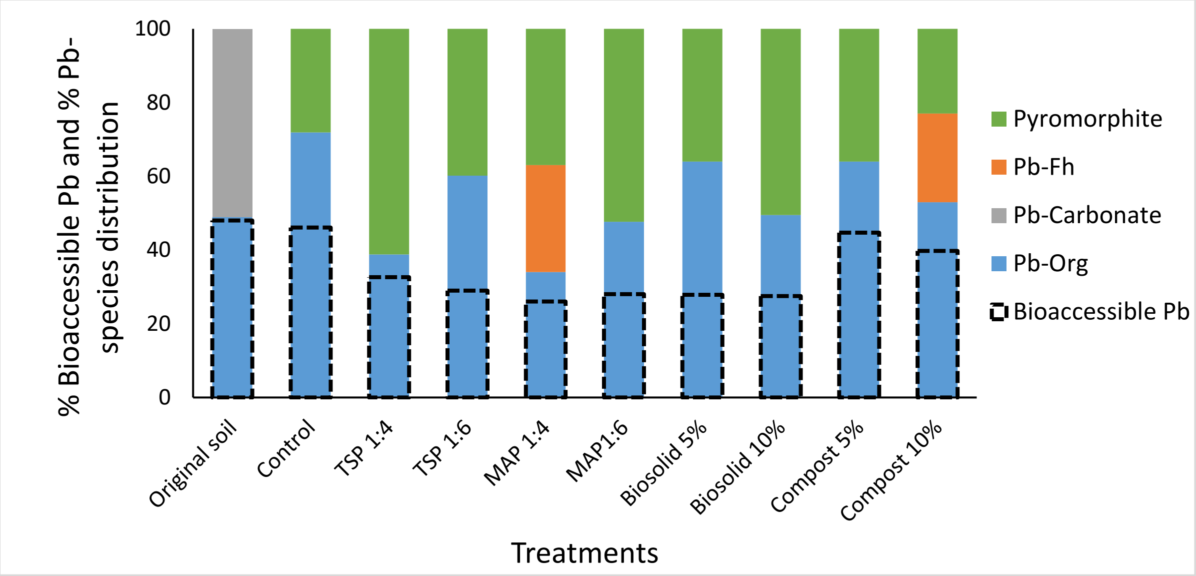
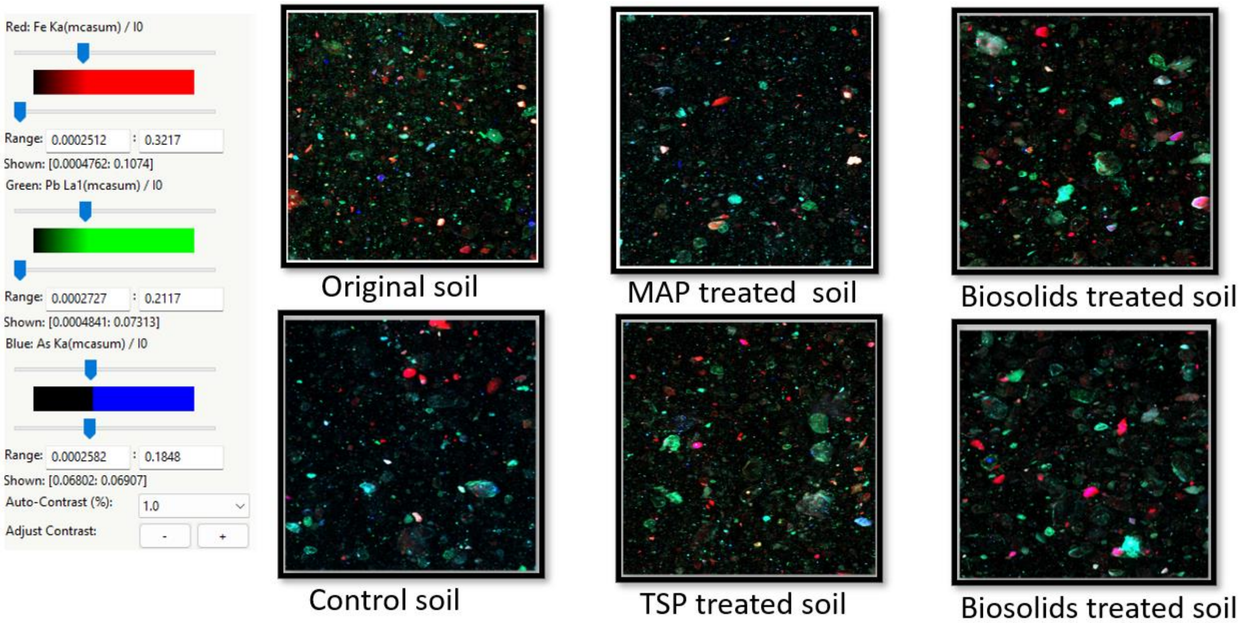


Figure 3.8: The relationship between percentage of bioaccessible Pb and Pb-species distribution resulted in linear combination fitting. TSP, triple superphosphate; MAP, mono ammonium phosphate; Pb-Fh, Fe-Ferrihydrite sorbed Pb and Pb-Org, Pb bound to organic compounds)

Both bulk XAFS (Figures 3.7 and 3.8) and micro XANES analyses (Table 3.2) revealed that the original (untreated) soil was primarily characterized by Pb bound to humic acid (proxy for Pb bound to organic matter), followed by Pb carbonate species. Plumbonacrite ($Pb_5O(OH)_2(CO_3)_3$), identified through bulk XAFS, showed a close association with hydrocerussite (Mindat, 2024), while cerussite was detected via micro XANES. Additionally, ferrihydrite bound Pb species (proxy for Fe-bound Pb) and Pyromorphite were identified in both bulk and micro XANES spectral analysis techniques. The presence of Pb carbonate species was further confirmed by the micro XANES results in the selected spots (Figures S4 and 3.12). We have extended our investigation by examining the correlation between Pb and elements such as P and Fe using micro-XRF mapping (Figure 3.9) and in selected areas (Figures S5, 3.10 and 3.11). This additional analysis allows us to gain a more detailed understanding of the spatial distribution and potential associations between Pb and these key elements within specific regions of interest. By elucidating these correlations, we aim to uncover potential mechanisms or processes influencing the behavior and fate of the formation of different Pb species in the studied soil with better certainty. The analysis of the original soil revealed a prevalent occurrence of Pb bound to Fe across the majority of selected spots examined in the micro-XRF map (ranging from 40% to 90%) (Figure 3.12). These results reinforced the findings from sequential extraction. Specifically, the fractions bound to Fe and manganese Mn oxides were significantly lower in biosolids treated soil than treatments involving TSP and MAP. Conversely, these fractions showed higher levels in the compost treatment, control, and the original soil. As depicted in Figures 3.10 and 3.11, a stronger correlation between Pb and Fe is observed compared to Pb and P. This observation indirectly suggests a lower prevalence of stable Pb minerals, such as pyromorphite, in the original soil. Instead, there appears to be a weak association between Pb and Fe species, which may be soluble in acidic environments, leading to heightened bioaccessibility. It should be noted that residual fraction could contain stable precipitates and strongly adsorbed Pb species via inner sphere and other mechanisms, such as organo mineral complexes.


Red: Fe Ka(mcasum) / I0

Range: 0.0002512 : 0.3217

Shown: [0.0004762: 0.1074]

Green: Pb La1(mcasum) / I0

Range: 0.0002727 : 0.2117

Shown: [0.0004841: 0.07313]

Blue: As Ka(mcasum) / I0

Range: 0.0002582 : 0.1848

Shown: [0.06802: 0.06907]

Auto-Contrast (%): 1.0

Original soil

MAP treated soil

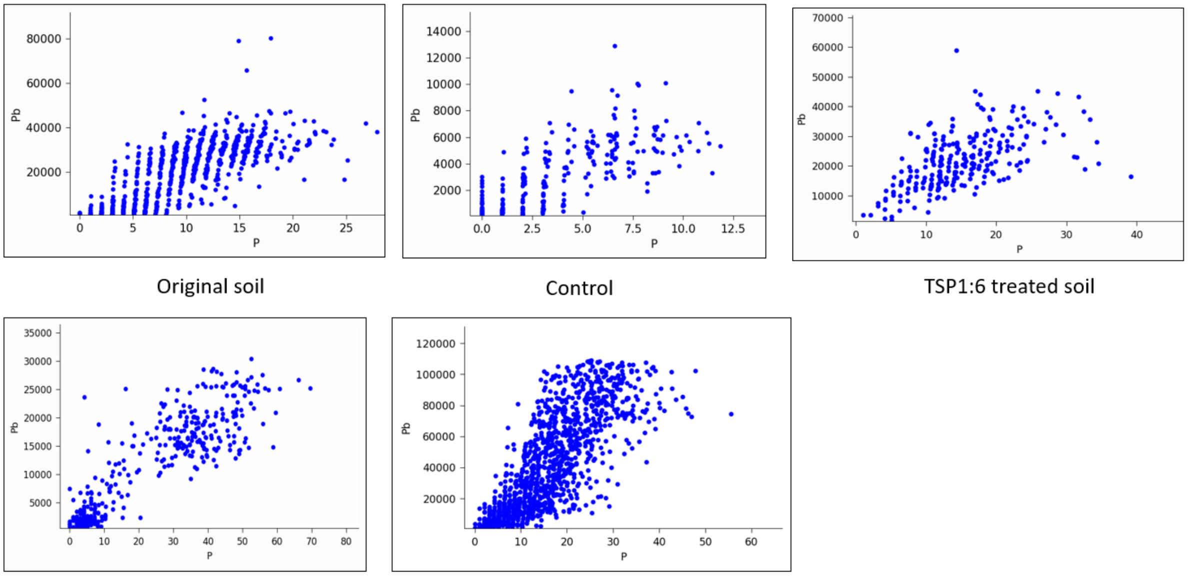
Biosolids treated soil

Control soil

TSP treated soil

Biosolids treated soil

Figure 3.9: Micro-XRF elemental maps (2 mm x 2 mm) for selected treatments. The color scheme used three elements were red for Fe, green for Pb and blue for As. Color combinations resulted in cyan (green+blue) for Pb and As, magenta (red+blue) for Fe and As and yellow (red+green) for Pb and Fe interactions/ combinations. Shading is relative across each map.



Original soil

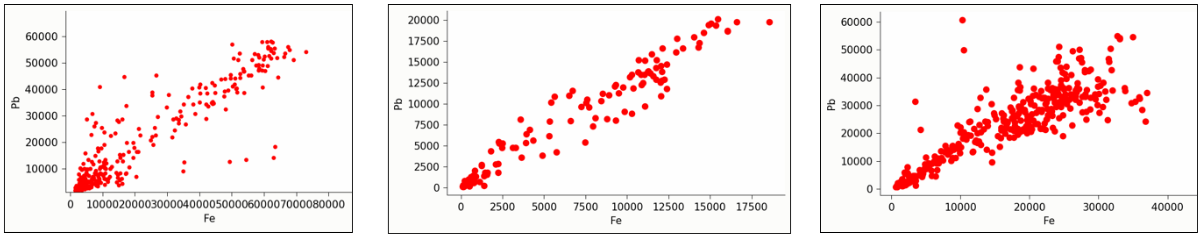
Control

TSP1:6 treated soil

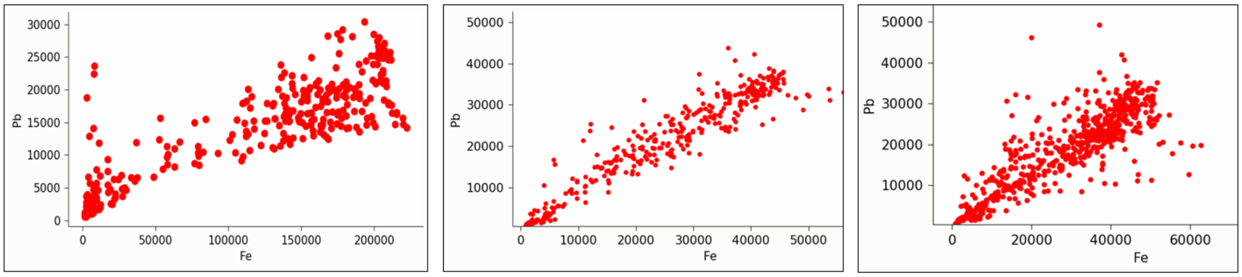
MAP1:6 treated soil

Biosolids treated soil

Figure 3.10: Pb and P correlation maps in selected areas in micro-XRF map of different treatments. TSP, triple superphosphate; MAP, monoammonium phosphate; Bs, biosolids; Co, compost.



Original Soil Control TSP1:6 treated soil



MAP1:6 Treated soil Biosolids treated soil Biosolids treated soil

Figure 3.11: Pb and Fe correlation maps in selected areas different treatments in micro-XRF map. TS, triple superphosphate; MAP, monoammonium phosphate; Bs, biosolids; Co, compost.

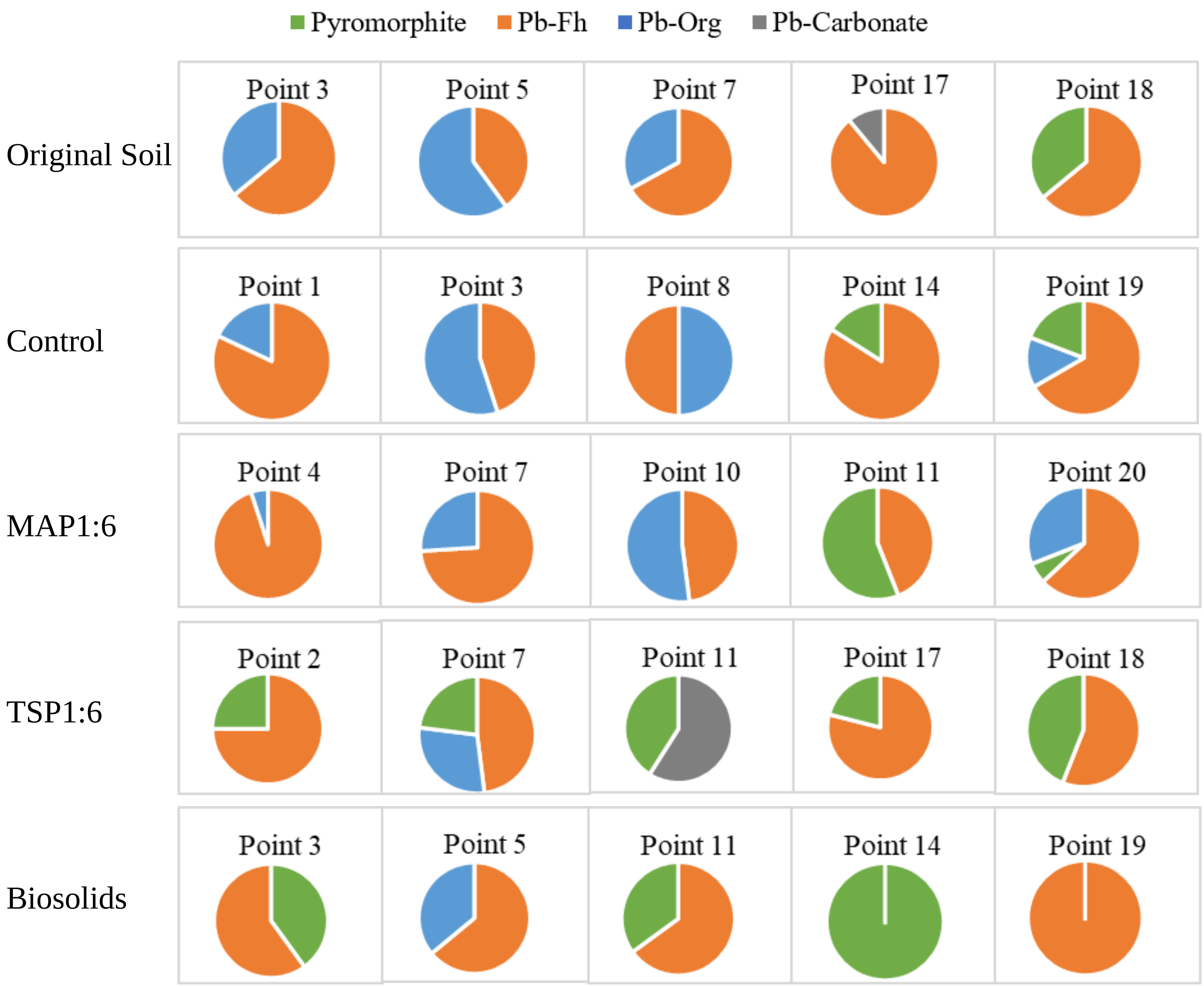


Figure 3.12: Relative distribution of Pb species in randomly selected five points in micro-XRF maps each represents different treatment maps. TSP, triple superphosphate; MAP, monoammonium phosphate.

Table 3.2: Summary of Pb species distribution in selected treatments with different synchrotron techniques. TSP, triple superphosphate; MAP, monoammonium phosphate.

Sample	Bulk XANES	Bulk XAFS	Micro XANES	Micro XRD
Original soil	Fe-ferrihydrite sorbed Pb	Pb bound to humic acid	Fe-ferrihydrite sorbed Pb	Feldspar $[Pb(Al_2O_8Si_2)]$
	Pyromorphite	Plumbonacrite	Pb_bound to humic acid	Mimetite $[Pb_5(AsO_4)_3Cl]$
	Pb bound to humic acid		Cerussite	Dadsonite $[Pb_{21}Sb_{26}S_{55}Cl]$
	Pb-adsorbed-hydroxyapatite		Pyromorphite	Beaverite $[PbFe_3(H_6O_{14}S_{2]})]$
				Barstowite $[Pb_4Cl_6(CH_2O_4)]$
Control	Fe-ferrihydrite sorbed Pb	Pb bound to humic acid	Fe-ferrihydrite sorbed Pb	(Did not perform)
	Pyromorphite	Chloropyromorphite	Pb bound to humic acid	
	Pb bound to humic acid		Pb-citrate	
	Pb-adsorbed-hydroxyapatite		Hydroxypyromorphite	
MAP	Fe-ferrihydrite sorbed Pb	Pyromorphite	Hydroxypyromorphite	Pb phosphate (different types)
	Pyromorphite	Fe-ferrihydrite sorbed Pb	Fe-ferrihydrite sorbed Pb	Bayldonite $[PbCu_3(As_2H_2O_{10})]$
	Pb bound to humic acid	Pb bound to humic acid	Pb bound to humic acid	

	Pb-adsorbed-hydroxyapatite	Pb-adsorbed-hydroxyapatite	Pb-adsorbed-hydroxyapatite	Pyromorphite [$Pb_5(PO_4)_3X$] X:Cl, F, OH
TSP	Fe-ferrihydrite sorbed Pb	Hydroxypyromorphite	Fe-ferrihydrite sorbed Pb	Pyromorphite
	Pyromorphite	Chloropyromorphite	Hydroxypyromorphite	Pb phosphate (different types)
	Pb bound to humic acid	Pb bound to humic acid	Pyromorphite	
	Pb-adsorbed-hydroxyapatite	Pyromorphite	Pb_bound to fulvic acid	
Biosolids	Fe-ferrihydrite sorbed Pb	Pyromorphite	Hydrocerussite	Pyromorphite
	Pb-adsorbed-hydroxyapatite	Pb bound to humic acid	Fe-ferrihydrite sorbed Pb	Barstowite
	Pb bound to humic acid	Chloropyromorphite	Pyromorphite	Feldspar
	Pyromorphite		Chloropyromorphite	Ludlockite [$PbFe_4(As_{10}O_{22})$]
			Pb-adsorbed-hydroxyapatite	Cerussite [$PbCO_3$]
				Lead Iron Phosphate Fluoride [$PbF_2Fe(PO_4)$]

Metal ions such as Pb can move into oxides, where they may integrate into the mineral lattice or occupy small surface pores after initial adsorption. In soils, hydrous oxides play a crucial role by the accumulation of organic matter on oxide surfaces, forming complexes that trap metal ions, and direct interactions between metal ions and the oxide surfaces, where the oxides serve as binding sites (Bradl, 2004). Iron oxide particles in metal-contaminated soils have been found to contain particularly high concentrations of Pb and Cd (Huang, 2005; Liu, et al., 2022). Hydrous Fe oxides are typically more abundant in soils which appear to be more important in the retention of metal cations (Alloway, 1995). Further, hydrous Fe oxides may be more readily associated with Zn than Pb in co-contaminated soil. Lead is preferred to precipitate with the presence of excess oxyanions such as phosphates, arsenate and sulfate in the soil solution. Pyromorphite or Pb-phosphate minerals could precipitate in a wide variety of soils and sediments. Lead is also adsorbed and coprecipitated with Fe and Mn oxides and hydrous oxides (Basta et al., 2005; Hettiarachchi & Pierzynski, 2004; Castaldi et al., 2014). Specific adsorption of metals by hydrous Fe oxides follows the preferential order: Pb > Cu >> Zn > Cd. Specific adsorption in soils is greatly influenced by pH and involves both organic and inorganic colloids, primarily hydrous oxides. This process occurs as ions form covalent bonds with charged mineral surfaces, selectively retaining certain ions based on soil conditions (Alloway, 1995).

After 24 weeks of incubation, the distribution of Pb species in the control treatment exhibited notable differences compared to the original (untreated) soil. Micro-XANES analysis conducted in specific areas revealed significant alterations, including a reduction in Pb-bound organic compounds and an increase in Pb sorption onto Fe-ferrihydrite and pyromorphite-like minerals.

Notably, Pb-associated carbonate minerals were absent in these selected areas. These findings suggest a decrease in bioaccessibility within the control treatment relative to the original soil. While the correlation between Pb and P in the control treatment resembled that of the original soils, a notably stronger correlation was observed between Pb and Fe. This increased correlation could be attributed to the declining pH over time and the presence of adequate moisture, both of which promote chemical reactions such as dissolution and subsequent precipitation and/or complexation. As a result of these reactions, Pb can form pyromorphite-like minerals and/or complexes with organic or inorganic ligands such as humic substances and hydroxide ions. These precipitates and complexes are robust and their stability are not significantly affected by changes in pH (Rieuwerts et al., 1998).

Both MAP and TSP at a ratio of 1:6 were subjected to bulk and micro XANES, XAFS and XRD analyses. These treatments exhibited consistent findings across all synchrotron-based X-ray analytical methods, revealing the presence of Pb bound to Fe (Fe-ferrihydrite), pyromorphite minerals, and Pb bound to organic complexes (humic acid) (Table 3.2). Moreover, similar reductions in pH and bioaccessibility were observed in both treatments. However, micro XRF mapping revealed a higher abundance of pyromorphite minerals in the TSP 1:6 treatment compared to MAP 1:6, along with a lower occurrence of organically bound Pb. Elemental correlation analysis using micro XRF data demonstrated a stronger association between Pb and P, as well as Pb and Fe in both treatments (Figures 3.10 and 3.11). Furthermore, the application of 10% biosolids exhibited analogous speciation results in bulk and micro XANES and XAFS analyses (Table 3.2), with a notably enhanced elemental correlation between Pb and P, and Pb and Fe compared to the other treatments (Figures 3.10 and 3.11). Interestingly, micro XANES spot analysis within micro XRF maps revealed exclusive occurrences of Fe-bound Pb or pyromorphite minerals in the biosolids 10% treatment (Figure 3.12) findings absent in the other treatments. The micro XRD analysis of the biosolids 10% treatment revealed distinct mineral compositions, analogous to those found in the original soil samples but with increased quantities of pyromorphite minerals. This similarity may be attributed to minimal pH alterations occurring in the biosolids 10% treatment, which likely facilitated the preservation of certain components of the natural mineral composition.

Compared to the original untreated soil, all treated soil samples exhibited a notable decrease in bioaccessible Pb levels. Notably, EP4 soil, characterized by elevated total Pb concentration (> 3000 mg kg-1), co-contamination with higher concentrations of competing metal cations (such as As, Cd, Zn, Cu), alkaline pH conditions (pH 7.63), and a low clay content (12%), presents a particularly unique and challenging environmental context of the studied soil.

The arid climate and low precipitation in the El Paso, TX region hinder soil chemical reactions. For approximately 100 years, the ASARCO smelter operation released significant quantities of Pb and other contaminants into the surrounding area (Elkekli, 2013; Paz et al., 2017). Soil serves as the primary sink for the accumulation of these toxic trace elements. However, the limited soil moisture necessary for facilitating chemical transformations prevents the efficient conversion of these contaminants into less toxic and immobile forms. Consequently, their bioavailability remains elevated. Many children in this region have been reported to have elevated blood Pb levels, likely due to exposure to contaminated soil and dust (Alvarez et al., 2018).

Phosphorus and Fe have been extensively investigated over several decades and proven to be efficient and effective in reducing the bioaccessibility of soil Pb (Zhang et al., 2017; Miretzky and Fernandez-Cirelli, 2008; Hettiarachchi & Pierzynski, 2004; Basta *et al.*, 2005; Henry et al., 2015). In this study, we examined the effectiveness of affordable and readily available soil amendments containing P and Fe in addressing high Pb bioaccessibility in challenging soil conditions. Including various other potentially toxic elements. Similarly to the decline observed in lead (Pb) bioaccessibility over time, the bioaccessibility of As and Cd also exhibited significant decreases in certain treatments compared to the control. The percentages of As and Cd bioaccessibility and sequential extraction results for As and cd fractionation are shown in Figures S1 and S2. Over the course of the study, it was evident that the bioaccessibility of As and Cd declined notably with the application of biosolids (at 5% and 10% rates), TSP, and MAP (at 1:4 and 1:6 ratio). Conversely, control and the compost treatment displayed minimal effects on the bioaccessibility of As and Cd over time. The sequential chemical extraction of As and Cd fractionation further emphasized the relationship between changes in their bioaccessibility and respective soil mineral associations or complexation patterns.

The control treatment, compared to untreated original soil, also exhibited notable chemical transformations resulting in pH reduction and decreased Pb bioaccessibility. The observed pH reduction in the control treatment was significantly influenced by microbial decomposition of organic matter, mineralization of C and N and oxidation of pyrite-like minerals (Bolan et al., 2003). Furthermore, carbonate mineral species like cerussite [$Pb_5O(OH)_2(CO_3)_3$] and hydro cerussite [$Pb_3(CO_3)_2(OH)_2$] release CO_2, potentially leading to a decrease in pH control treatment. The decrease in carbonate mineral species observed in the original soil, combined with favorable environmental conditions such as sufficient moisture (at 40% of MWHC), likely contributed to this phenomenon. This is further supported by the relatively higher organic matter content (TOC 1.2%) compared to soils found in arid climatic conditions (Husein et al., 2021; Naorem et al., 2023). This difference is likely attributable to the pronounced microbial decomposition of organic matter, mineralization of carbon and nitrogen, and oxidation of pyrite-like minerals, as elucidated by Bolan et al. (2003). Sequential extraction and synchrotron analysis confirmed the formation of Pb-associated P and Fe minerals, including pyromorphite and Fe (hydro)oxides sorbed Pb, through processes such as direct precipitation, adsorption, or ion exchange (Lee et al., 1998; Zeng et al., 2017). These compounds exhibit stability across a wide range of pH and environmental conditions, albeit their formation is pH-dependent. The slight acidity favorably promotes the release of H^+ ions into the soil solution, thereby enhancing the availability of reactive species and facilitating chemical reactions (Bolan et al., 2003). Consequently, the observed pH reduction during the incubation period signifies the formation of these stable compounds in the control treatment. However, the limited availability of P and Fe in the natural soil hindered the formation of stable Pb pyromorphite and Fe (hydr)oxides complexes. Supplementing additional P and Fe resulted in improved outcomes compared to the control treatment.

The reduction in pH facilitated by TSP and MAP promotes the transfer of solid-phase Pb into the solution phase via desorption and dissolution of soil solid fractions, resulting in the release of Pb^{2+}. This, in turn, together with $H_2PO_4^-$, enhances P-induced Pb adsorption, precipitation, ion exchange, and/or complexation reactions. Furthermore, alterations in surface charge and surface composition favor Pb immobilization in the presence of P treatments. The availability of ferrihydrite in the soil solution can further promote the formation of surface complexes such as ternary lead-phosphate-iron oxide, in addition to lead-phosphate precipitation (Xie and Giammar, 2007).

Observations from synchrotron analysis supported the presence of Pb sorbed onto Fe-ferrihydrite, indicating the influence of TSP and MAP treatments on Pb immobilization. Additionally, significantly higher amorphous Fe content was detected in both TSP (1:4 and 1:6; 2772 and 2803 mg kg^{-1}) and MAP (1:4 and 1:6; 2881 and 2971) treatments compared to the control treatment (2537 mg kg^{-1}), as determined by the method outlined by Loeppert and Inskeep (1996). Collectively, these findings suggest that TSP and MAP facilitate the formation of pyromorphite and Fe (hydr)oxides sorbed Pb, which exhibit reduced solubility in PBET solution at pH 2.5. Consequently, these treatments contribute to a significant reduction in Pb bioaccessibility and demonstrate long-term effectiveness in mitigating Pb contamination.

The effectiveness of Class A Fe-rich biosolids surpassed that of all other treatments in reducing bioaccessible Pb levels, while maintaining soil pH. Sequential and synchrotron analyses revealed a higher proportion of stable pyromorphite and Fe (hydr)oxides sorbed Pb compounds in biosolids-treated soil. The solubility, mobility, and bioavailability of Pb are influenced by its chemical form and its interaction with mineral surfaces. Lead incorporation into crystalline solids like pyromorphite renders it less soluble, mobile, and bioavailable compared to sorption onto mineral surfaces. The type of surface complex formed plays a pivotal role in Pb ion mobility. Strong, inner-sphere complexes confer relative immobility, while weakly bound outer-sphere complexes are more prone to desorption with pH changes. Lead typically forms strongly bound inner-sphere complexes, enhancing immobilization. Complex stability depends on the number of bonds with surface functional groups, with bidentate or tridentate complexes being more resistant to desorption. Multinuclear complex formation may lead to precipitation when the solution is supersaturated. Phosphate presence complicates sorption reactions by competing for surface sites or forming solid compounds. Metal ions may also be incorporated into solid coprecipitates formed by mineral surface dissolution, representing another potential sorption mechanism (Brown et al., 1999).

Conclusions

The reduction of bioaccessible Pb in the EP4 soil was notably achieved through amendment, likely due to adequate supplementation of P and Fe, facilitating the formation of stable Pb-P and Pb-Fe compounds such as pyromorphite and Pb sorbed onto Fe (hydr)oxides. Additionaly, the bioaccessibility of As and Cd also decreased significantly in some treatments compared to the control. Furthermore, the decrease in the percentage of bioaccessible Pb across treatments with reduced pH suggests mechanisms favoring Pb adsorption and precipitation over time, transitioning from initial outer-sphere to inner-sphere complexes or initially more soluble Pb-solid species to stable solid species. Among the treatments, the most effective in reducing bioaccessible Pb was the 10% biosolids amendment. Overall, applications of biosolids, TSP, and MAP proved more effective in diminishing exchangeable Pb fractions. In the untreated soil, Fe bound Pb and different Pb species were predominant. However, with the application of P treatments and over time, there was a notable increase in the formation of pyromorphite and Pb species exhibiting strong sorption characteristics. Considering environmental sustainability Class A, P, and Fe-rich biosolids are one of the prioritized P-based amendments when remediating soils contaminated with Pb in greater amounts.

Supporting Information

As and Cd bioaccessibility and relationship with different fractions obtained from sequential extraction

Similar to how Pb bioaccessibility decreased over time, the bioaccessibility of As and Cd also decreased significantly in some treatments compared to the control. The percentages of As and Cd bioaccessibility are shown in Figure S1, measured using a modified PBET procedure (Attanayake et al., 2017) at pH 2.5 in < 250 µm soil fraction together with Pb. Total bioaccessible As concentrations ranged from 78 (MAP 1:4) to 139 (compost 10%) mg kg^{-1} constituting 31% to 55% of the total As content in the soil, respectively (Figure S1). Correspondingly, total bioaccessible Cd concentrations ranged from 85 (biosolids 5%) to 109 (control) mg kg^{-1} representing 62% to 79% of the total Cd content in the soil, respectively (Figure S1), following a 24-week incubation period. Notably, the PBET extraction results delineated a substantial decrease in bioaccessibility ($p<0.05$) across all soil amendments over time. The control and compost treatments exhibited the least reduction in both As and Cd bioaccessibility. By the 24-week mark, a significant reduction in As was evident in the biosolids, MAP, and TSP treatments compared to the control and compost treatments (Figure S1). However, biosolids treatment exhibited significantly lower bioaccessible Cd compared to compost 5% and control treatments.

Sequential extraction results for As fractionation (Figure S2) indicated the absence of organically bound As in the soil. Additionally, approximately 1% of As was exchangeable, while around

10% was carbonate-bound, with over 50% being Fe-Mn bound and roughly 30% residual in the original soil. Minimal to no alterations were observed in the control and compost treatments across all fractionations similar to the original soil. Conversely, TSP and MAP treatments led to an increase in the exchangeable and residual As fractions by reducing the carbonate and Fe-Mn bound fractions. Biosolids treatment notably elevated the residual fraction by diminishing the exchangeable, carbonate, and Fe-Mn bound As fractions. Interestingly, the original soil displayed a significantly higher exchangeable Cd fraction (~16%) compared to the residual fraction (~4%) (Figure S2). Fractionation distribution was similar among the original soil, control, and compost treatments. However, biosolids, TSP, and MAP treatments exhibited slight increments in the residual and Fe-Mn bound fractions. While MAP and TSP treatments did not affect the exchangeable fraction but reduced the carbonate fraction, biosolids treatment reduced the exchangeable fraction and marginally affected the carbonate-bound fraction.

The comparatively lower As bioaccessibility in the original soil, coupled with the minimal reduction in bioaccessibility post-treatment, aligns with the lower availability in soluble fractions (exchangeable and carbonate bound) and subtle changes in stable (residual and Fe-Mn bound) As fractions. Conversely, the notably high Cd bioaccessibility (~90%) in this alkaline soil correlates well with the predominance of exchangeable and carbonate-bound Cd (>75%). Treatment-induced reductions in Cd bioaccessibility primarily due to the increased residual and Fe-Mn bound Cd fractions and decreased exchangeable and carbonate-bound Cd fractions. Overall, the bioaccessibility of As and Cd significantly diminished over time with biosolids (5% and 10%), TSP, and MAP (1:4 and 1:6) treatments, while control and compost treatments exhibited the least effect on As and Cd bioaccessibility over time. Sequential chemical extraction of As and Cd underscored the correlation between changes in bioaccessibility and their respective soil mineral associations/complexation.

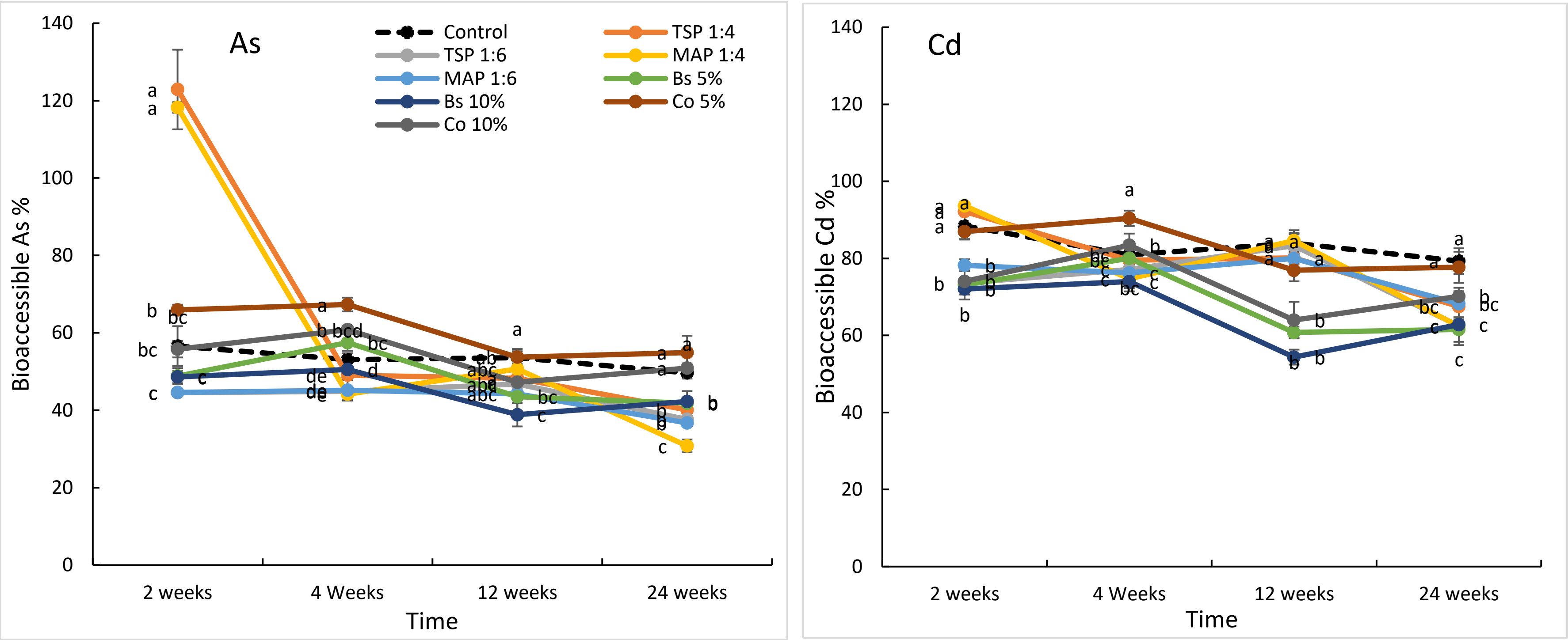
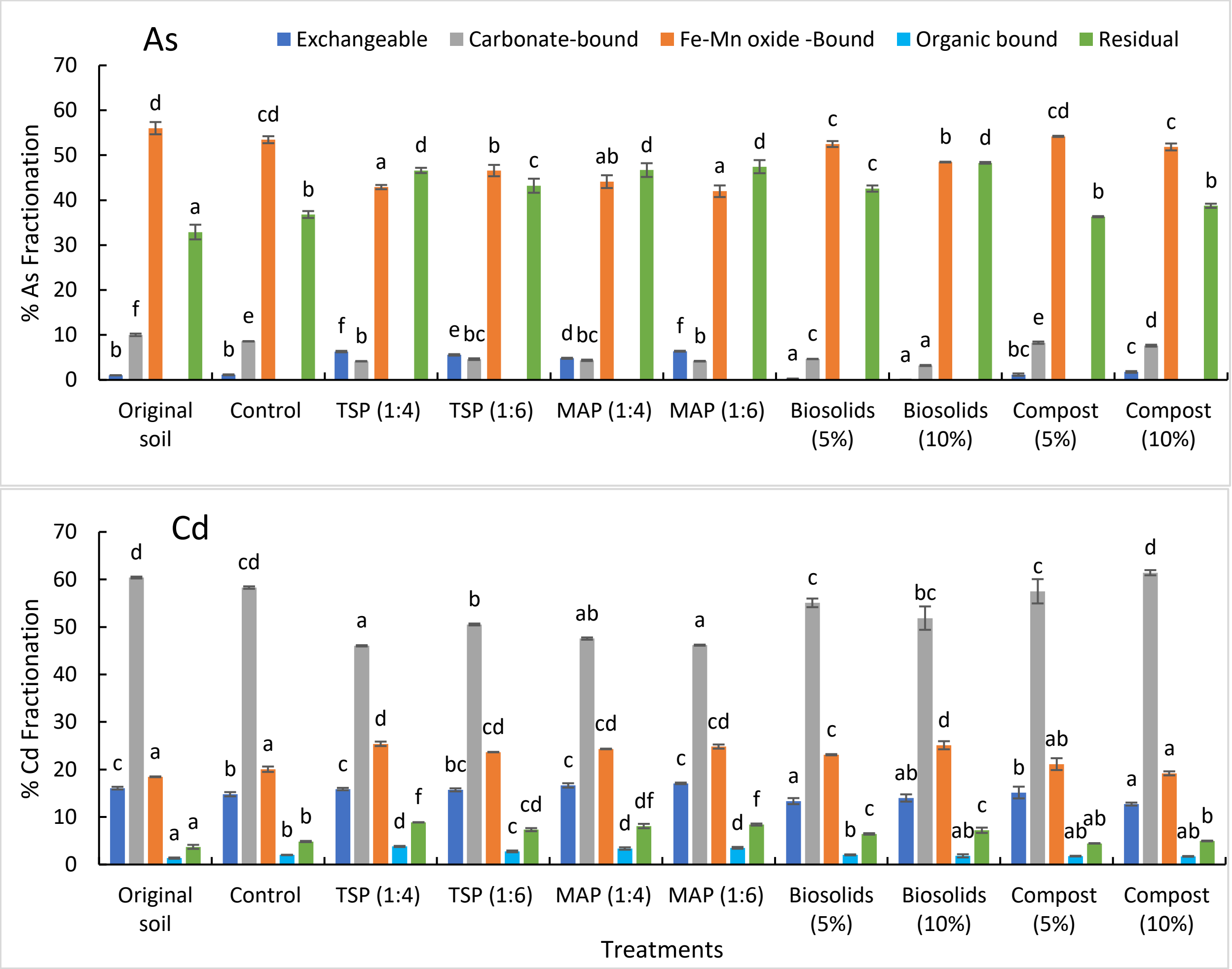


Figure S1: Changes in percent As and Cd bioaccessibility (PBET procedure) with time during the incubation study sampled at 2, 4, 12 and 24 weeks. TSP, triple superphosphate; MAP, mono ammonium phosphate; Bs, biosolids; Co, compost. Means within each time containing the same letter are not significantly different at P = 0.05.



FiFigure S2: Changes in percent As and Cd fractions extracted in sequential chemical extraction performed on 24 weeks of incubation. TSP, triple superphosphate; MAP, mono ammonium phosphate. Means within each fraction containing the same letter are not significantly different at P = 0.05.

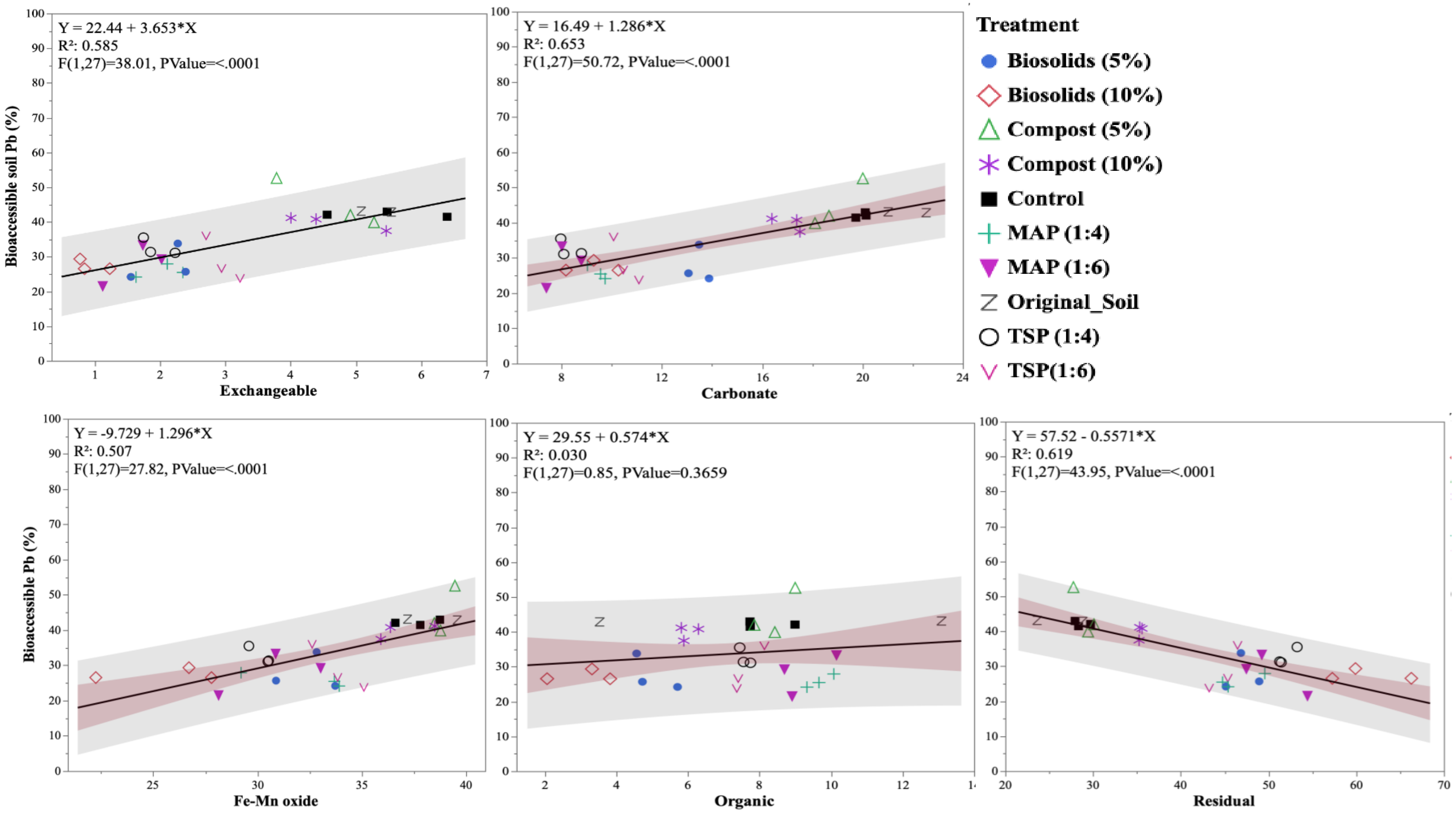
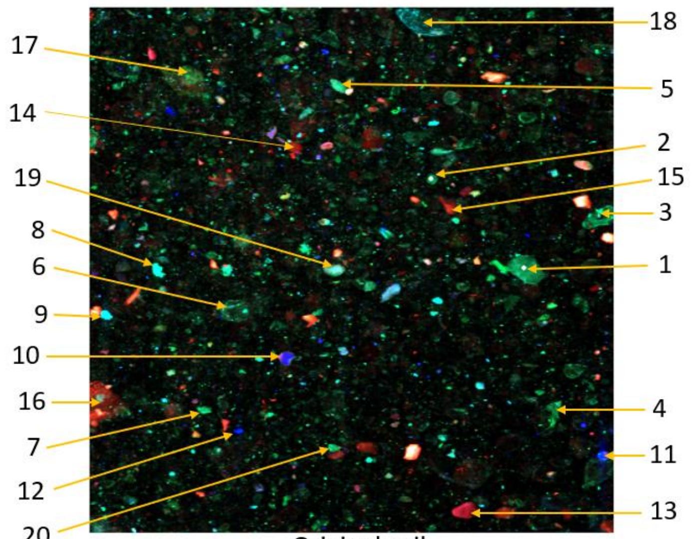
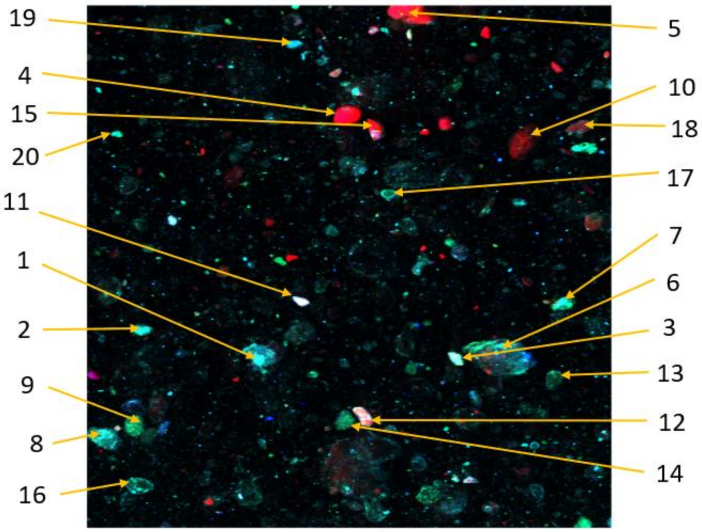


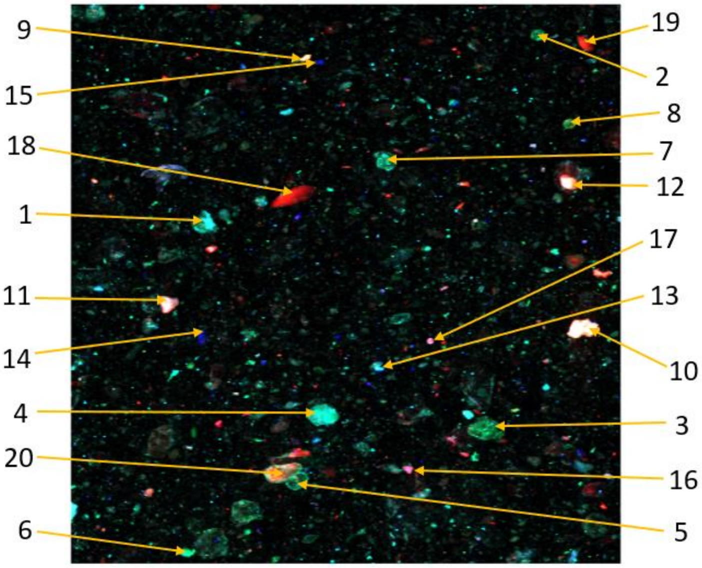
Figure S3: Correlation between percent bioaccessible Pb and percent total Pb in each fraction of sequential extractionAt 24 weeks of incubation. TSP, triple superphosphate; MAP, mono ammonium phosphate. Graphs were created using SAS JMP software version 17.2.



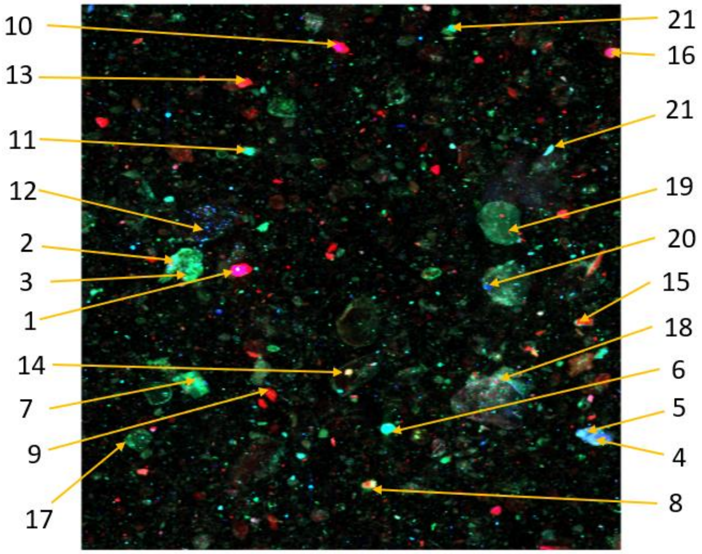
Original soil



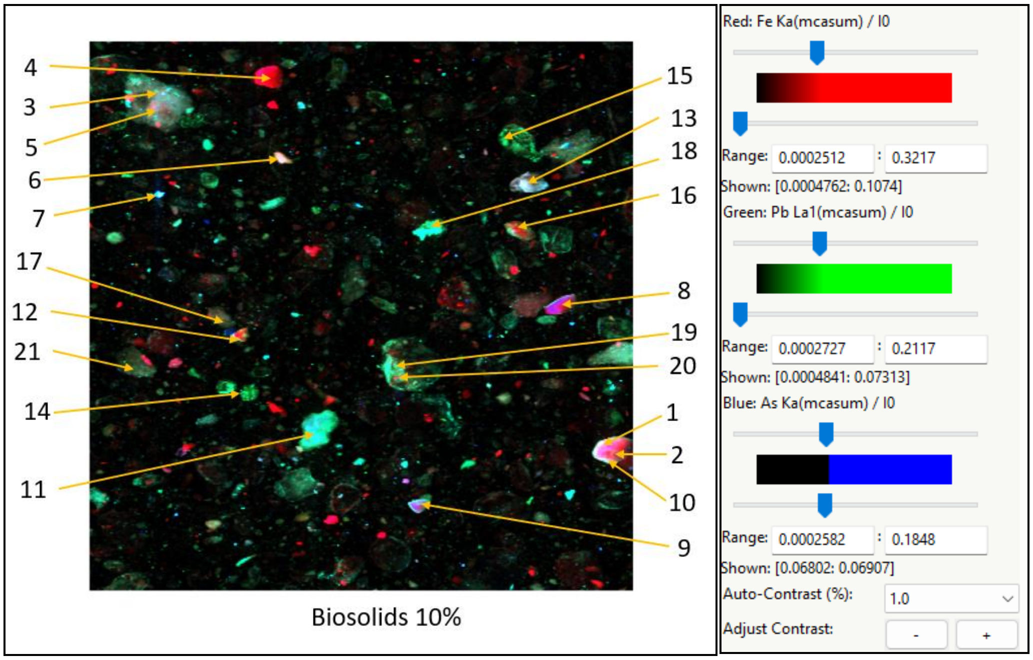
Control



MAP 1:6



TSP 1:6



Red: Fe Ka(mcasum) / I0

Range: 0.0002512 : 0.3217

Shown: [0.0004762: 0.1074]

Green: Pb La1(mcasum) / I0

Range: 0.0002727 : 0.2117

Shown: [0.0004841: 0.07313]

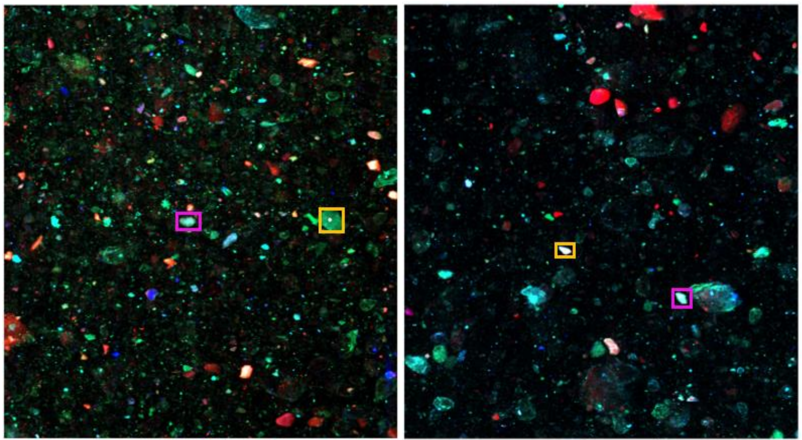
Blue: As Ka(mcasum) / I0

Range: 0.0002582 : 0.1848

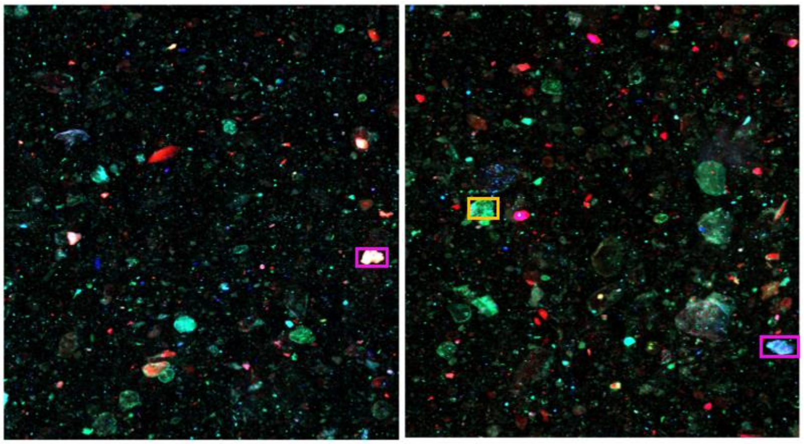
Shown: [0.06802: 0.06907]

Auto-Contrast (%): 1.0

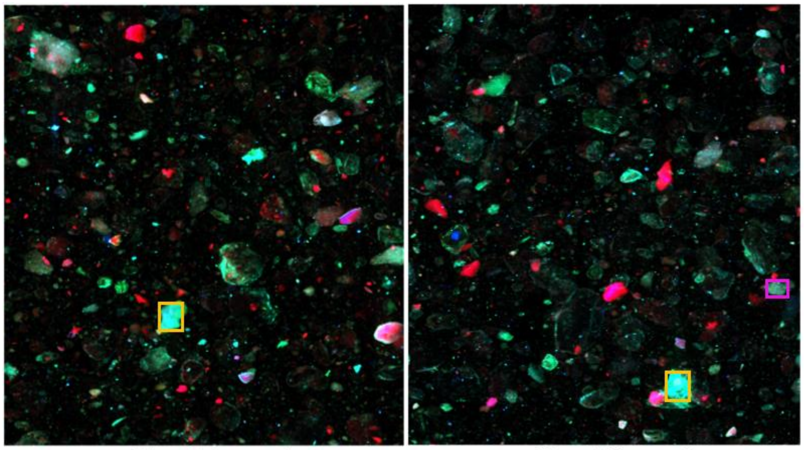
Figure S4: Selected spots in the micro XRF maps for micro XAFS analysis for Pb speciation in the original soil, control, TSP 1:6- triple superphosphate, MAP 1:6- mono ammonium phosphate and biosolids 10% treatments.



Original Soil Control



MAP 1:6 TSP 1:6



Biosolids map1 Biosolids map2

Figure S5: Selected area in the micro XRF maps for elemental correlations in the original soil, control, TSP 1:6- triple superphosphate, MAP 1:6- mono ammonium phosphate and biosolids 10% treatments. Purple color area for Fe and Pb association and yellow color for P and Pb association.

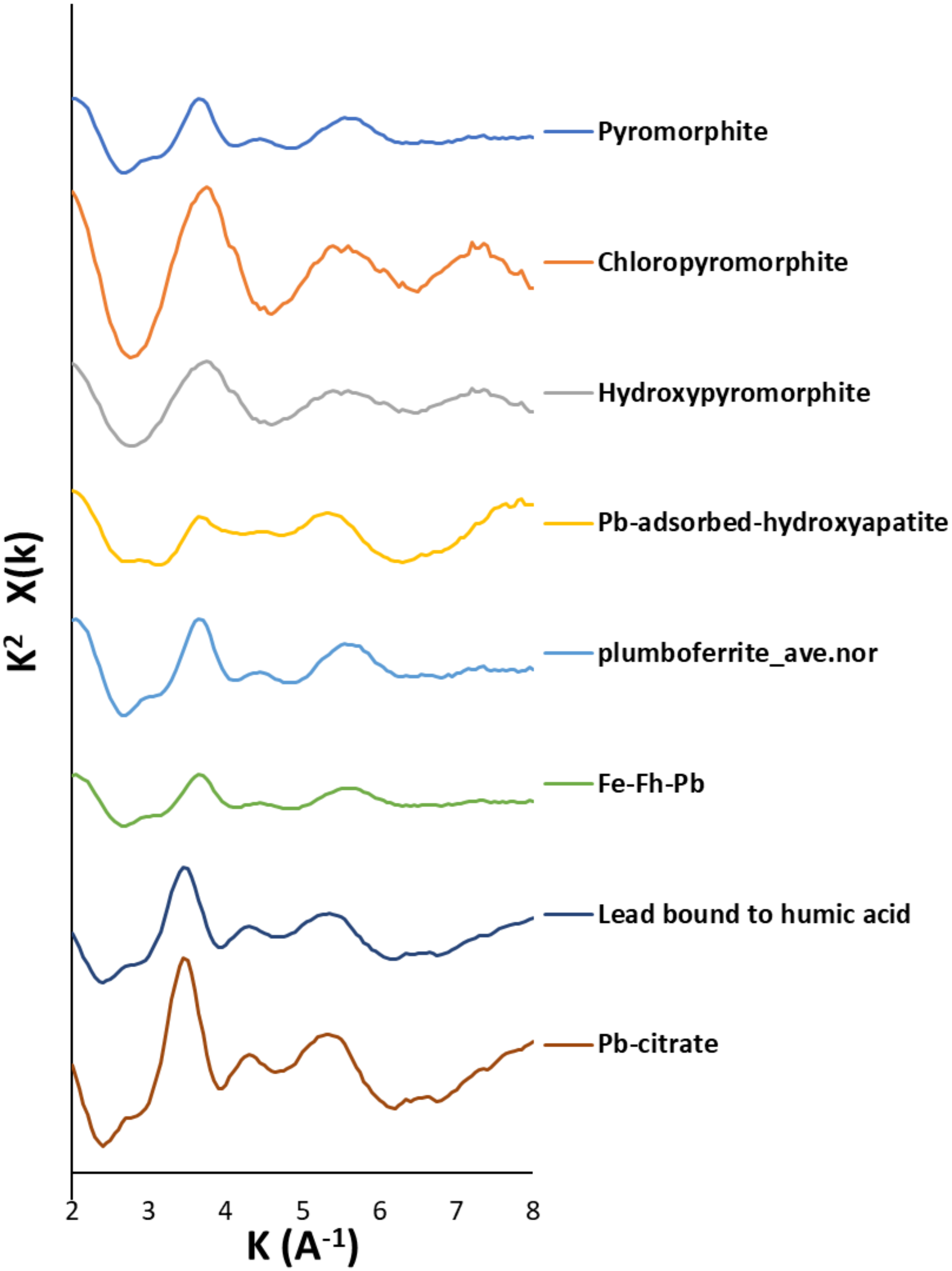


Figure S6: Selected Pb XANES spectra for standard reference samples.

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Chapter 4 - Impact of phosphorus-containing organic and inorganic soil amendments on reducing Pb bioaccessibility in mildly to moderately contaminated three residential soils

Abstract

Exposure to lead (Pb) through direct ingestion and inhalation of soil particles poses significant neurological and developmental risks, especially for children with high blood lead levels. Soil remediation methods, such as using organic or inorganic soil amendments to stabilize Pb chemically, aim to reduce its harmful effects by changing its chemical properties. Urban and residential soils, with diverse Pb sources and co-contaminants, present challenges for effective remediation, highlighting the need for a better understanding of soil Pb chemistry and remediation methods. This study aimed to investigate changes in bioaccessible Pb concentration in Pb-contaminated soils from El Paso, TX, using affordable soil amendments together with changes in Pb speciation. A laboratory experiment was conducted to evaluate the effectiveness of phosphorus-containing organic and inorganic soil amendments in reducing the bioaccessibility of Pb. Three soil samples from residential areas near El Paso, Texas, with different levels of Pb, named EP1, EP2, and EP3, were used. Eight treatments, including two rates of inorganic fertilizers (triple superphosphate (TSP) and monoammonium phosphate (MAP)) and two rates of organic materials (biosolids and compost), were tested over a 24-week period at a temperature of 25°C. A modified physiologically based extraction technique was employed to simulate the human stomach to determine Pb bioaccessibility, and the speciation of Pb was using X-ray absorption spectroscopy and sequential chemical extraction.

The total Pb concentrations in EP1, EP2, and EP3 soils ranged from mild to moderate levels of soil Pb contamination (157, 327, and 727 mg kg^{-1}, respectively). Over time, all treatments, including the control, showed gradual decreases in pH and the amount of bioaccessible Pb. Adding biosolids (at a high rate; 10%) consistently and significantly reduced the amount of bioaccessible Pb compared to other treatments in all soils. EP1 and EP2 had higher concentrations of Pb bound to carbonates, while EP3 had higher concentrations of Pb bound to Fe-Mn oxides, followed by organic-bound Pb. X-ray fluorescence analysis revealed significant transformations of soluble Pb fractions into stable or residual forms across all soils, including Pb sorbed to Fe-ferrihydrite and the formation of pyromorphite, particularly using biosolids, TSP, and MAP. Maintaining soil moisture also effectively reduced the levels of bioaccessible Pb in the control soil. Control and compost-amended soils showed similar levels of bioaccessible Pb and speciation profiles. This study underscores the importance of soil moisture in transforming soluble Pb into stable compounds, particularly in arid climates where the original forms of Pb are preserved and higher efficiency of inorganic P sources and biosolids for transforming stable Pb compounds.

Introduction

Soil Pb contamination has emerged as a substantial environmental challenge, prompting widespread public concern. Soil serves as the primary repository for Pb accumulation, resulting from both natural processes and human activities (Shi et al., 2018; Abedi et al., 2022; CDC, 2022). Human exposure to Pb occurs through direct ingestion and inhalation of soil particles, leading to a range of harmful neurological and developmental consequences. Of particular concern are children, who are especially vulnerable to the adverse effects of elevated blood Pb concentrations (Mielke et al., 2011; CDC, 2022; Mielke et al., 2022, WHO, 2023). Consequently, soil remediation has been extensively studied, especially in the last few decades, where ex-situ and in-situ remediation methods are available for treating Pb-contaminated soil.

Remediation strategies for trace element contamination in soils involve diverse physical and chemical approaches. These methods include soil replacement, isolation, leaching, phytostabilization, and chemical stabilization (Egendorf et al., 2020; Lan et al., 2020). Chemical stabilization or in-situ stabilization of Pb relies on the addition of immobilizing agents, either organic and/or inorganic soil amendments. The primary role of soil amendments is reducing its environmental risk by chemically altering soil Pb to less bioavailable forms (Henry et al., 2015; Karna et al., 2017; Lwin et al., 2018; Kastury et al., 2023). In particular, phosphate amendments have shown promise in immobilizing Pb, mainly through ligand and ionic exchange and the precipitation as pyromorphite-like minerals (Hettiarachchi & Pierzynski 2004; Dermatas *et al.,* 2008; Park *et al.,* 2011; Palansooriya et al., 2020). Various natural and synthetic P sources, each with distinct solubilities and characteristics, may influence the efficacy of Pb immobilization. These sources have been effectively employed in remediating Pb-contaminated soils to reduce Pb bioavailability and bioaccessibility (Schekel et al., 2013; Zeng et al., 2017; Mayers et al., 2022). However, past evaluations have primarily focused on acidic soils or pre-acidified alkaline soils, indicating a gap in understanding soil Pb chemistry, especially in urban soils with prevailing alkaline characteristics (Biasioli, et al., 2006; Gu et al., 2020; Mayers et al., 2022)

Soil remediation methods may encounter challenges leading to their failure, often due to inadequate understanding of site characteristics, contamination sources, and environmental conditions, which can result in ineffective treatment strategies. Regulatory and financial constraints, along with community resistance, also impact the success of remediation efforts (ITRC, 2017; Paltseva et al., 2022; Kastury et al., 2023; Sánchez-Castro et al., 2023). Therefore, addressing the Pb bioaccessibility in mildly to moderately contaminated residential soil requires careful consideration.

Bioavailability and bioaccessibility of Pb in soil, crucial factors influencing its toxicity, are determined by its speciation or fractionation and subsequent solubility (Tessier et al., 1979, Palansooriya et al., 2020), particularly in the gastrointestinal tract after ingestion (Hettiarachchi & Pierzynski, 2004; Scheckel et al., 2009). Soil properties such as pH, clay content and mineralogy, cation exchange capacity, and organic matter significantly influence Pb bioavailability and bioaccessibility in soil (Rieuwerts et al., 1998; Yan et al., 2017; Zhang et al., 2019; Lake et al., 2021; Kumar *et al.,* 2022; Lu et al., 2023). Urban and residential soils, characterized by multiple Pb sources and co-contaminants, exhibit high heterogeneity and variability, posing challenges for effective remediation (Sharp and Brabander, 2017; Paltseva et al., 2020; Haque et al., 2021; Wade et al., 2021). While elevated lead contamination is common in urban residential soils, a significant gap exists in understanding how to effectively use and interpret soil Pb chemistry for soil Pb risk mitigation, particularly when employing soil amendments as remediation methods. This challenge is further complicated by inherent soil properties such as alkalinity (Asabere et al., 2018; Yang and Zhang, 2015; Biasioli et al., 2006; Jim, 1998). Additionally, the effects of amendments on soil remediation vary with soil heterogeneity and fluctuations in environmental conditions such as temperature, precipitation, soil composition, and microbial activity (Kim et al., 2016; Lwin et al., 2018; Palansooriya et al., 2020)

Considering the continuous reporting of low levels of Pb exposure among children throughout the world (Burki, 2020; Ruckart et al., 2021) and interest in urban gardening for fresh, locally-grown food (Clark et al., 2006; Sharp and Brabander, 2017; Paltseva et al., 2022), affordable and sustainable in-situ mitigation strategies are crucial to mitigate soil contaminants' impact on urban soils (Attanayake et al., 2021). This necessitates a deeper understanding of urban soil Pb chemistry and the development of environmentally sound remediation strategies to address mildly Pb-contaminated urban soils, ensuring both human health protection and sustainable land use for safe community gardening practices. Therefore, this study investigated the changes in bioaccessible Pb concentration in Pb-contaminated residential soils from El Paso, TX using readily accessible and/or economical soil amendments. Our objectives were to assess the changes in Pb speciation after incorporation and aging of organic and inorganic soil amendments; and to relate speciation and in vitro bioaccessibility of Pb in soil. These findings can provide insights into the role of soil amendments and offer a scientific basis for the successful remediation of mildly to moderately Pb-contaminated urban residential soil.

Materials and Methods

Soil Sample Collection

A laboratory incubation study was carried out to evaluate the impact of phosphorus-containing organic and inorganic soil amendments on the reduction of Pb bioaccessibility. Three distinct soil samples, namely EP1, EP2, and EP3, were obtained from three locations in El Paso, Texas. The samples EP1 and EP2 were collected from a residential area near the city of El Paso (31°76,690 N and 106°46,741 W, respectively) within the 79901-zip code (Figure 4.1). The EP3 sampling site was close to the Chihuahuan High Desert adjacent to the ASARCO smelter within a residential area.

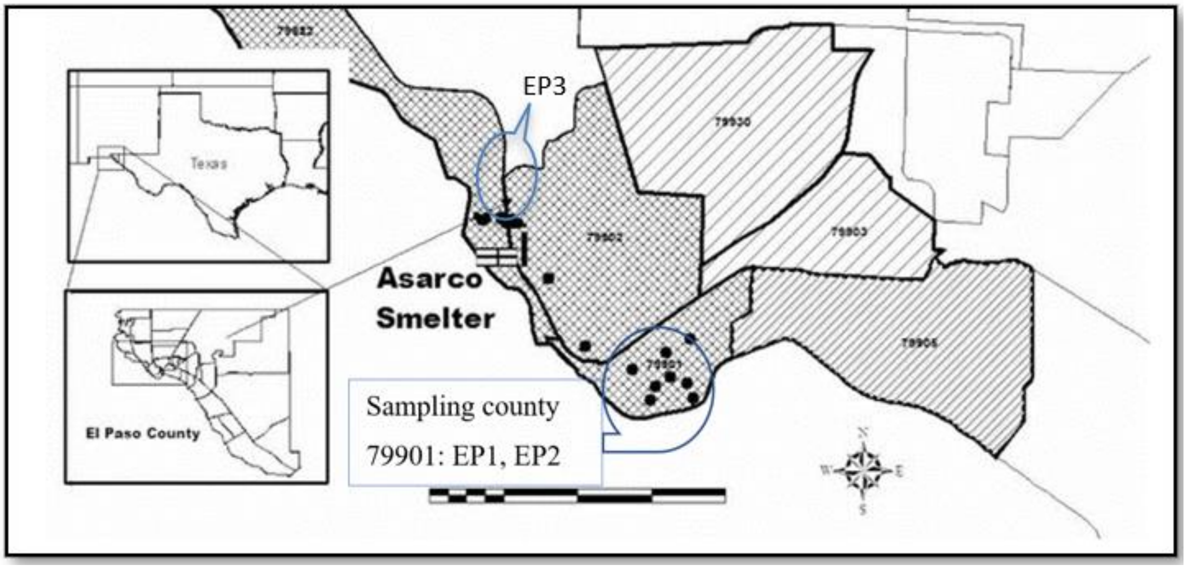

Figure 4.1:Locations of the EP1, EP2 and EP3 of the Asarco smelter northwest of the downtown zip code area (79901) (Source: Cunningham and Tiefenbacher, 2008)

The selection of different locations was based on the initial screening of total Pb concentrations using portable X-ray fluorescence spectroscopy (pXRF) expecting a range of Pb concentrations. The initial screening revealed Pb concentrations of approximately 200 mg kg^{-1} for EP1, 300 ppm for EP2, and 900 ppm for EP3.

Sample Preparation and Experimental Design

All soil samples were air-dried and sieved through a < 2 mm sieve before commencing the experiment. For each treatment and replicate, 100 g of soil sieved through a < 2 mm sieve was measured and placed into 250 g HDPE bottles for the laboratory incubation study. A completely randomized design was used with three replicates. Eight treatments and controls were included, representing two inorganic and organic soil amendments at two different rates for each. Inorganic soil amendments were commercial fertilizers, triple superphosphate (TSP) and monoammonium phosphate (MAP). Their application rates were 1:4 and 1:6 based on Pb: P molar ratio in contaminated soil. Organic soil amendments were the biosolids (BS) and compost (CO) applied as 5 and 10 percent of Mehlich-III extractable P concentration in these materials. Biosolids were obtained from Blue Plains wastewater treatment facility, Washington DC, and characterized as Class A. An additional noteworthy feature is its heightened Fe concentration (10%), surpassing that of conventional biosolids from various wastewater treatment facilities. The incorporation of iron salts such as ferric chloride and ferrous sulfate, utilized for phosphorus removal, contributes to the elevated iron levels found in these biosolids. The compost used in this study was from Agronomy North Farm, Kansas State University, Manhattan, KS, and was produced from food and crop residue materials. Before beginning the experiments selected physiochemical properties such as pH, cation exchange capacity, organic C, soil texture, maximum water holding capacity (MWHC), and total elements (P, Fe, Ca, K, Mn, Na, Pb, Cd, As, Zn, Ni, Cu) in all the soils, biosolids, and compost were measured using the standard methods.

The soil was subsequently prewetted to 40% of its maximum water holding capacity (MWHC) by mass, using Milli-Q water. The TSP and MAP granules were ground into fine particles using a motar and pestle before being added to the soil, ensuring thorough, even treatment mixing. The moisture content of biosolids and compost treatments was considered prior to wetting the soil. After addition of treatments, moisture was balanced to achieve a 40% of MWHC. The treated soil in HDPE bottles was mixed using a ball mill for approximately one hour. After mixing, the bottle openings were sealed with Parafilm to facilitate air exchange but minimize moisture loss. The samples were incubated at a temperature of 25°C for a period of 24 weeks. Moisture adjustments were carried out periodically, guided by weight loss measurements during the incubation period.

Samples of 20 g treated soil were collected from each treatment at times, of 2, 4, 12, and 24 weeks, and subsequently dried at 40°C for approximately 24-48 hours. A subsample was utilized for pH measurement, while the fraction passing through a < 250 µm sieve was employed for bioaccessibility measurements. After 24 weeks a sub-sample of < 2 mm fraction was used for sequential chemical extraction and a sub-sample of < 250 µm was used for Pb speciation detection using synchrotron analysis.

Wet Chemical Analysis

Composite samples from each dried < 2-mm size sieved soil were digested using EPA method 3051A (USEPA, 2007) for total Pb. In addition, a subsample of the <2-mm size fraction was further sieved to <250-µm size fraction and total Pb was determined using the same procedure. The aqua regia digestion method was employed to analyze selected other elements (As, Cd, Fe, Mn, K, Ca, Mg, Al, Si, Se, Ni, Cu, Na, and Zn), followed by subsequent analysis using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Varian 720-ES).

In method 3051A microwave-assisted acid digestion, 10 mL of trace metal-grade concentrated HNO_3 was added to 0.5 g of soil (n=5) in Teflon tubes and the content was digested in a microwave digestion unit (MARS Xpress, CEM Corp. Matthews, NC). The temperature of the soil-acid mixture was initially raised to 165°C within the first 5.5 minutes. It was subsequently increased to 175°C in the second step over the next 4.5 minutes. Finally, a temperature of 175°C was maintained for 5 minutes to complete the digestion process. After digestion, the tubes were allowed to cool in the fume hood until their contents reached room temperature. The solution was filtered using Whatman No. 42 filter paper into screw-capped plastic scintillation vials under a fume hood. The filtered solution was then stored at 4°C until analysis using ICP-OES.

For aqua regia digestion, a mixture of 10 mL trace-metal-grade 1:3 HNO3 to HCl was added to 1 g of soil. The digestion process occurred with a continuous increase in temperature at 30-minute intervals (75, 100, 110, 140°C) using a hot plate block digestion unit. The final step in digestion with 140 ° C is completed when the solution is evaporated yielding about 1 mL solution. The remaining 1 mL solution was diluted with 0.1% HNO_3 and filtered using a Whatman No. 42

filter paper into screw-capped plastic scintillation vials and the solution was then analyzed for total elemental concentration using ICP-OES.

Additionally, selected physiochemical properties such as total organic carbon (TOC), total N, CEC, soil carbonate, and soil texture were assessed using standard procedures at the soil testing laboratory of Kansas State University. Soil pH measurements were conducted using a 1:2 and 1:5 soil-to-water ratio (Orion Star A111, Thermo Scientific), and soil texture was determined using the hydrometer method.

In vitro Soil Pb Bioaccessibility Tests: Physiologically based extraction method (PBET)

In-vitro bioaccessibilty Pb determination methods were developed to mimic the human gastric or gastrointestinal dissolution process. The modified physiologically based extraction method (PBET) (Attanayake et al., 2017) targeted to measure gastric phase dissolution was employed to evaluate Pb bioaccessibility in each soil sample. Originally developed by Ruby et al. (1996), with further modifications introduced by Brown and Chaney (1997) and Medlin (1997), the PBET method was initially designed to assess both gastric and intestinal phase bioaccessibility (Ruby et al., 1996; Brown and Chaney 1997; Medlin ,1997). However, given that many studies reporting low and variable bioaccessible Pb extractability in the intestinal phase due to its neutral pH, the present study focused solely on the gastric phase. The Gastric phase PBET method exhibited a strong linear correlation with in vivo–in vitro correlation tests (Henry et al, 2015). At a simulated stomach pH of 2- 2.5, the correlation coefficient (r^2) was > 0.90 (n=7), based on the gastric phase (Ruby et al., 1996; Hettiarachchi et al., 2003). The PBET analysis used the <250 µm size fraction, representing particles of a size similar to those that could adhere to children's hands (Kelley et al., 2002).

The gastric solution was prepared by dissolving 1.25 g of pepsin, 0.5 g of sodium L-malate, 0.5 g of sodium citrate dihydrate, 420 µL of L(+)-lactic acid, and 500 µL of trace metal-grade acetic acid in 1 L of Milli-Q water. The pH of the solution was then adjusted to ~2.5 by adding of trace-metal grade HCl. The extraction procedure was commenced with the weighing of 1.00±0.05 g of soil (sieved to < 250 µm) into a 250 mL wide mouth HDPE bottle, followed by the addition of 100 mL gastric solution heated to 37°C (soil: solution ratio 1:100). The soil solution mixture was mixed properly and then the pH of the soil solution was measured. Different volumes of trace metal-grade concentrated HCl were added to samples adjusting pH 2.5 ± 0.5 before extraction. The bottles containing the mixture were placed in a Queue incubator orbital shaker (Parkersburg, WV) with a temperature maintained at 37°C. They were shaken for one hour at 150 rpm for extraction. After one hour of extraction, the pH of the soil solution was assessed to confirm the pH is 2.5 ± 0.5. If the pH has deviated from 2.5 ± 0.5 the samples were re-extracted. The supernatant solution was filtered using 0.45-µm syringe filters into plastic scintillation vials and then analyzed for Pb by ICP-OES.

The calculation of the bioaccessible Pb percentage was conducted as follows to assess the effects of treatments (soil amendments) on reduced bioaccessibility and was subsequently used for statistical analysis.

$$\%\, Bioaccessible\ Pb = \frac{Bioaccessible\ (Pb\ mgkg^{-1})}{Total\ Pb\ (mgkg^{-1})} \times 100$$

Sequential Extraction

Tessier et al., (1979) introduced a sequential extraction procedure as an analytical method that involves sequential chemical extractions to separate various trace metals (Cd, Co, Cu, Ni, Pb, Zn, Fe, and Mn) in soils and sediments into five operationally defined fractions. These fractions include exchangeable, bound to carbonates, bound to Fe-oxides, bound to organic matter, and residual fractions (Tessier et al., 1979). The original procedure, later adapted by Zwonitzer et al., (2003) and Scheckel et al., (2003) with some modifications, was employed in this study. It aimed to estimate the quantity of Pb and several other trace metals bound to different soil fractions such as Fe/Al oxides, carbonates, organic matter, etc. The soil used in the sequential extraction was <2 mm sieved fraction from treated soil after the 24-week incubation period.

The method started with, about 2.00±0.05 g of soil carefully weighted and placed into pre-weighted 50 mL centrifuge tubes. The soils then underwent a sequential exposure to a set of extraction solutions designed to isolate five operationally defined fractions: exchangeable, bound to carbonates, bound to Fe and Mn oxides, bound to organic matter, and residual. Following each extraction step, the samples were centrifuged at 1000 rpm for 30 minutes. The resulting supernatants were carefully decanted and filtered through Whatman No. 42 filter paper into screw-capped plastic scintillation vials. These vials were then stored at 4°C in the refrigerator until analysis using ICP-OES and graphite furnace atomic absorption spectrometer (GF AAS) (Varian GTA 120 Graphite Tube Atomizer w/ AA 240Z Zeeman) (Zwonitzer et al., 2003).

In between each extraction step 8 mL of milli-Q water was added, shaken for 10 minutes, and then centrifuged for 1000 rpm in 30 minutes. The supernatant was carefully discarded and the remaining solid residue was used for the next extraction step. The five extraction steps followed in the sequential extraction procedure were as follows:

Step 1: Exchangeable fraction (F1). In a 50 mL centrifuge tube, 2 g of soil was combined with 25 mL of 1 M $MgCl_2$, and the tube was securely sealed. The soil and $MgCl_2$ mixture were shaken for one hour in a reciprocal shaker at room temperature. The mixture was centrifuged at 1000 rpm for 30 minutes, and the supernatant was filtered through Whatman No. 42 filter paper into screwed plastic scintillation vials, and stored at 4°C in the refrigerator.

Step 2: Carbonates-bound fraction (F2). One molar $NaCOOCH_3$ (Sodium acetate) was prepared and adjusted to pH 5.0 with trace-metal grade acetic acid (HOAc). Addition of 25 mL of $NaCOOCH_3$ to each of the residual treatments in step 1, sealed tubes were shaken for five hours at room temperature. After centrifugation at 1000 rpm for 30 minutes, filtration procedures were followed as in step 1.

Step 3: Iron and manganese oxides-bound fraction (F3). The residue from step 2 underwent extraction using 40 mL of 0.04 M NH_2OH-HCl in 25% (v/v) CH_3COOH (acetic acid) solution. The soil solution mixture was heated for six hours at 96°C with intermittent agitation. Then, centrifugation and filtration procedures were executed following the same steps as explained in step 1.

Step 4: Organic matter-bound fraction (F4). First, 6 mL of 0.02 M HNO_3 and 10 mL of 30% H_2O_2 were added to the residue of step 3 and heated at 85°C for two hours followed by adding another 6 mL of 30% H_2O_2 and continued heating for an additional three hours. Secondly, after cooling 10 mL of 3.2 M NH_4COOCH_3 in 20% (v/v) HNO_3 and 10 mL of milli-Q water was added, mixed, and centrifuged and filtration procedures were followed as in step 1.

Step 5: Residual fractions (F5). The remaining residue from step 4 was treated with 20 mL of 4 M HNO_3 and the mixture was heated for two hours at 80°C. Centrifugation and filtration procedures were followed as in step 1.

Quality assurance and quality control for all the wet chemical analysis

All reagents used in this work were analytical or trace metal grade. Ultrapure water with a resistivity of 18.2 MΩ-cm was used to prepare all solutions. All the Glassware was treated with a 5% HNO_3 solution for >12 hours and rinsed three times with distilled water before use. High-density polyethylene (HDPE) plastic containers were used. Certified reference materials NIST 2711a (Montana II) soil was used to evaluate the reproducibility and the accuracy of the tested analytical procedures. Recovery percentages of trace elements in the samples showed reliable analytical data accuracy for the analyses of total contents. In addition, reagent blanks, duplicate samples, samples spiked with known amounts of Pb concentration, and blanks were routinely included in the PBET extraction and analysis.

Synchrotron-based Lead Speciation

Soil sample preparation

A composite soil sample, consisting of three replicates from each treatment after 24 weeks of incubation, was created for the Pb speciation study. Equivalent quantities of the <250 µm fraction of soil from each replicate of the treatments were ground to a fine powder with an agate mortar and pestle to facilitate improved homogenization. For the Pb speciation study of the bulk soil, composite soil samples were packed into Plexiglass sample holders with a thickness of 2 mm, using Kapton® tape for support. The samples were packed in the holder in two steps: firstly, one side of the sample holder slit was sealed with Kapton® tape, filled, and packed with the soil. Secondly, the other side was sealed using Kapton® tape. The labelled sample holders were then securely mounted on the sample stage (45° angle to the beam), enabling the X-ray beam to pass through the soil sample positioned between the two pieces of Kapton in the holder slit.

X-ray absorption measurements

X-ray fluorescence analyses were done at Advanced Photon Source (APS), Argonne National Laboratory in Argonne, IL. Bulk Pb X-ray absorption near-edge spectroscopy (XANES) analysis was performed at the 5 BM-D beam line of DND-CAT. Data were collected at Pb L_{III}-edge (13,035 eV) in fluorescence mode with the Canberra Inc.13-element Ge solid-state detector operated in an energy range between 4.5-25 keV. The energy was calibrated using the Pyromorphite standard. This standard spectrum was applied to distinguish any energy drifts induced by the monochromator drifts during the sample run. The number of spectra collected per sample was 6 to 8 (mostly 6).

Data processing and analysis of XANES and X-ray absorption fine structure (XAFS) spectroscopy were done using Athena-Demeter software version 0.9.26 in IFEFFIT (Ravel and Newville, 2005). In the data analysis process first, the 3-4 scan spectra of a sample were merged to reduce noise. Then the background was corrected using pre-edge subtraction, and the spectrum was normalized using Athena default normalization algorithm. The sample and standard spectra were aligned for correcting any energy drifts using the normalized spectra. For XAFS spectra, the data were converted to k-space, ranging from 2-10 $\acute{\text{Å}}^{-1}$, k^2 weighted to compensate for the dampening of the XAFS amplitude with increasing k. Linear combination fitting (LCF) was employed to achieve the optimal fit by combining standard Pb compounds that were previously collected.

The following 23 standard Pb reference spectra were used for LCF: pyromorphite, chloro-pyromorphite, Pb-montmorillonite, Pb-goethite, Pb-calcite, Pb-kaolinite, $Pb(OH)_2$, hydro-cerussite, cerussite, Pb-citrate, Plumboferrite, Pb-adsorbed hydroxyapatite, hydroxy-pyromorphite, sorbed-Pb, Pb bound to humic acid, Pb bound to fulvic acid, anglesite, galena, plumboferrite, plumbogummite, litharge, plattenerite and plumbocarnite.

Statistical Analysis

Collected data for pH and bioaccessible Pb were entered into an Excel database, and checked twice for accuracy. Bioaccessible Pb percentage was used to evaluate treatment (soil amendments) effects on reduced bioaccessibilty. All analyses were conducted using SAS 9.4 software (2016 by SAS Institute Inc., Cary, NC, USA). Changes in bioaccessible Pb percentages were separately evaluated for each soil as a function of time, pH and treatment. Comparisons by treatment and time were tested with 9 x 4 ANOVAs ($p < 0.05$) with post hoc comparison of means for percent bioaccessible Pb and pH. For main effects, post hoc comparisons of LSM differences were calculated using the Tukey–Kramer adjustment for multiple comparisons.

The sequential Pb fractionation data were first converted into percentages of each fraction relative to the total Pb. Subsequently, the percentage of Pb fractions at each step was analyzed using the PROC GLM procedure in SAS, employing least square means to compare all treatments at a significance level of 0.05.

Results and Discussion

Initial soil characterization

Selected total elemental concentrations of the EP1, EP2, and EP3 soil are presented in Table 4.1. Both EP1 and EP2 soils are classified as Gila series (Taxonomic class: Coarse-loamy, mixed, superactive, calcareous, thermic Typic Torrifluvents) according to the NRCS soil taxonomic classification. The location of EP3 soil was collected is close the boundary of two series of soil and thus found to be more closely related to the Delnorte-Canutio Association. The Delnorte soil series belongs to the soil taxonomic class: Loamy-skeletal, mixed, superactive, thermic, shallow Calcic Petrocalcids and Canutio soil series classified in taxonomic class: Loamy-skeletal, mixed, superactive, calcareous, thermic Typic Torriorthents (NRCS, 2024).

Both EP1 and EP2 soil textures are identified as loamy sand, with a composition of 78-80% sand, 10-12% silt, and 10-12% clay, while EP3 soil is described as sandy loam with 68% sand, 20% silt, and 12% clay. The soil in El Paso is a blend of colluvial sediments sourced from the neighboring mountains and fluvial sediments originating from the Rio Grande. Typical fluvial sediments in the region are categorized as ranging from fine to coarse grains, including gravel and sand, along with a mixture of silt and clay (Texas Commission on Environmental Quality in 2023). Therefore, all three soils exhibited the characteristic soil texture commonly found in the

El Paso area (Elkekli, 2013; Paz et al.,2017). The soil pH varied among the samples, with EP1 showing a slightly alkaline pH of 7.76, EP2 displaying an alkaline pH of 8.09, and EP3 exhibiting a highly alkaline pH of 8.91. These measurements were conducted using a 1:2 soil: water ratio. The EP3 soil recorded the lowest values for electrical conductivity (EC) and CEC (60 µS cm^{-1} and 4.72 $cmol_c$ kg^{-1}). In contrast, EP1 and EP2 soils exhibited somewhat similar values for EC and CEC (EP1 was 270 µS cm^{-1} and 8.57 $cmol_c$ kg^{-1}, and EP2 was 244 µS cm^{-1} and 7.77 $cmol_c$ kg^{-1}). The soil, EP1 exhibited the highest percentages of MWHC and TOC (45% and 5.34%, respectively), in comparison, EP2 soil showed MWHC and TOC percentages of 31% and 3.1%, while EP3 soil had the lowest MWHC and TOC values (29% for MWHC and 0.48% for TOC). The arid to semi-arid desert climate in El Paso is marked by abundant sunlight year-round, low humidity, extremely low annual precipitation ranging between 8 to 10 inches, and an exceptionally high evaporation rate of approximately 150 inches per year. Lack of well-established natural vegetation and the dry and loose soil is typical to this EL Paso area (El-Hage & Moulton, 1998). Therefore, all three soils exhibited an alkaline pH, lower MWHC, and very low TOC.

The total Pb concentration in EP1, EP2, and EP3 soils was 157, 327, and 727 mg kg^{-1}, respectively, in the <2 mm soil fraction, indicating a range from mildly to moderately high Pb contamination. However, the concentrations in the <250 µm soil fraction were considerably higher, 329, 543, and 1091 mg kg^{-1} for EP1, EP2, and EP3, respectively, pushing EP3 toward the high Pb contamination category. Notably, EP3 soil also exhibited considerably higher concentrations of As and Cd (103 and 29 mg kg^{-1}, respectively). This elevated As and Cd in EP3 soil is indicative of its proximity to the ASARCO smelter. The bioaccessible Pb concentrations in EP1, EP2, and EP3 soils were 68±2.4, 158±20, and 514±21 mg kg^{-1}, constituting 43%, 48%, and 71% of the total Pb content in each respective soil. Additionally, the bioaccessible As and PBET extractable Cd percentages in EP3 soil were 49 and > 90%, respectively. US EPA (2024), reported that concentrations of trace elements determined in this study (Pb, As and Cd) were compared with the Regional Screening Levels (RSLs) for residential sites (Pb- 200 mg kg^{-1}(US EPA 2024). Many previous studies have consistently reported higher bioavailability for As and Cd compared to Pb in mining and smelter-impacted soil (Navarro et al., 2006; Pelfrêne et al., 2011; Li et al., 2019; Zhu et al., 2019). However, in the current study, we observed > 90% of PBET extractable Cd in EP3 soil.

Class A biosolids exhibited a slightly alkaline pH (7.47±0.02), whereas compost exhibited a slightly acidic pH (6.62±0.06). Biosolids contained a higher moisture content (62%) than the compost (26%). The concentrations of both Pb and As in Class A biosolids and compost were found to be < 20 mg kg^{-1} in Pb and 7 mg kg^{-1} in As or very low concentrations. Additionally, Cd was not detected in either the biosolids or the compost. Approximately 10% of the total Fe and 3.5% of the total P were identified in Class A biosolids, with 744 mg kg^{-1} of Mehlich-III P being detected. Interestingly, despite the compost having a total phosphorus concentration of 1467 mg kg^{-1}, the Mehlich-III P content in the compost was notably higher (669 mg kg^{-1}) than the biosolids. Class A biosolids are regulated for potentially toxic elements and disinfected to a level that effectively inactivates pathogens, and they are subject to fewer site-specific controls. They are also referred to as EQ (exceptional quality) biosolids due to their sufficiently low concentrations of heavy metals (WEF, 2024).

Table 4.1: Selected total elemental concentrations of the EP1, EP2 and EP3 soils, Class A biosolids, and composts

Element	EP1	EP2	EP3	Class A biosolids*	Compost*
Pb mg/kg	157±55	327±85	727±43	20.7	4.14
As mg/kg	6.37±1.1	12±0.9	103±14	7.8	1.3
Cd mg/kg	1.66±0.4	3.04±0.2	29±1.8	< DL	< DL
Ni mg/kg	42±14	40±7	16±5	17.4	5.8
Mo mg/kg	5.97±0.8	8.46±1.8	78±8	-	-
Se mg/kg	< DL	1.10±0.5	1.31±0.9	-	-
Mn mg/kg	194±27	273±97	393±9	434	432
P mg/kg	407±41	452±12	643±37	34603	1467
K mg/kg	2138±81	2244±71	3030±62	1365	2803
Zn mg/kg	749±126	736±166	1883±107	700	93
Cu mg/kg	2375±303	2291±254	2634±168	369	9
Na mg/kg	393±26	552±45	429±29	481	756
Al mg/kg	5342	5418	11118	-	-
Ca mg/kg	5162	42700	23245	28377	16247
Fe mg/Kg	14799	19590	34144	97519	11705

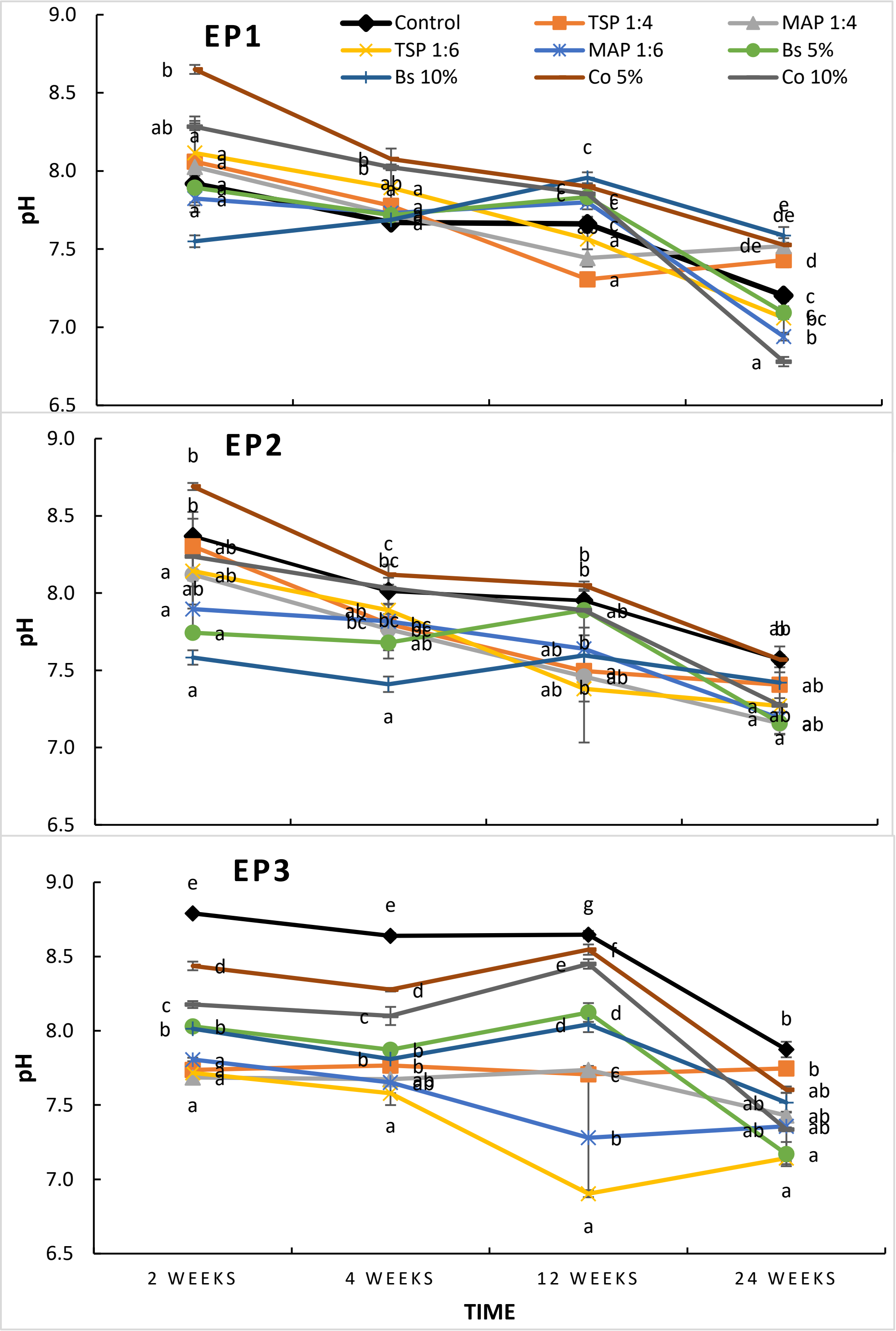

Figure 4.2: Changes in pH with time in three soils (EP1, EP2, EP3). TSP: triple superphosphate; MAP: monoammonium phosphate; Bs: biosolids; Co: compost. (Means within each time with the same letter are not significantly different at P = 0.05).

Changes in pH with time

A gradual reduction in pH was observed in each soil and treatment over time, including the control (Figure 4.2; EP1, EP2, EP3). After 24 weeks of incubation, EP1 soil exhibited significant differences in pH of most treatments, except for MAP1:4 and 5% biosolids compared to the control (Figure 4.2; EP1). Significantly higher pH values were observed in the 10% biosolids, 5% compost, and both TSP treatments compared to the control. Conversely, significantly lower pH was found in the 10% compost and MAP1:6 treatments compared to the control in EP1 soil. Surprisingly, biosolids 10% treatment stabilized its pH (7.6) after 2 and 24 weeks of incubation with small changes during other sampling times. While both compost treatment rates initially displayed significantly higher pH at the 2-week mark, the 10% compost treatment experienced a drastic reduction in pH (from 8.28 to 6.78) compared to all other treatments after 24 weeks. Additionally, the 5% compost treatment showed a pH reduction of approximately 1 unit (from 8.65 to 7.53). The overall pH reduction in EP1 soil does not exhibit a clear separation between organic and inorganic treatments.

In EP2 soil, no significant differences among treatments were observed at 24 weeks (Figure 4.2; EP2). However, compared to the 2-week, all treatments exhibited a significant reduction in pH. Both biosolid treatments showed the lowest pH at 2 weeks, and their reduction was also the lowest among other treatments after 24 weeks. The highest pH was recorded in the compost 5%, consistently at all times, followed by the control.

Similar to the other two soils, EP3 soil exhibited a reduction in pH in all treatments except TSP 1:4 after 24 weeks (Figure 4.2; EP3). When compared to the control, biosolid and compost 10%, both MAP and TSP 1:6 treatments displayed significantly lower pH after 24 weeks. For the initial 12-week period, organic treatments showed significantly higher pH compared to inorganic treatments. Overall, after 24 weeks, EP3 soil control, compost, and biosolids 5% showed a significantly greater reduction compared to the pH at 2 weeks (approximately 1 unit).

Overall, noteworthy declines in pH were observed across all three soils after four weeks of applying inorganic P treatments. Both control and compost treatments demonstrated clear pH reductions by the 24th week across all soil types. Conversely, soils treated with biosolids initially saw a decrease in pH by the 4th week but displayed an increase by the 12th week. Notably, applying 10% biosolids resulted in pH values similar to earlier time points by the 24th week, while the 5% treatment showed a significant decline in pH across all three soils during the same interval. Notable differences were observed in the amounts of TSP and MAP applied to each soil, with EP3 receiving the highest amount followed by EP2, based on the Pb:P molar ratio. Consequently, in EP3 soil, both MAP and TSP significantly contributed to pH reduction compared to EP2 and EP1, despite EP3 soil having the highest initial pH (8.9). Conversely, EP1 soil experienced the least reduction in pH, receiving the lowest amount of both fertilizers.

Both TSP and MAP introduce phosphorus into soil in the form of the $H_2PO_4^-$ (orthophosphate ion dihydrogenphosphate) ion, and in alkaline soil solutions $H_2PO_4^-$ release of H^+ ions leading to soil acidification. Direct acidification by TSP ($Ca(H_2PO_4)_2$) occurs via phosphoric acid (H_3PO_4). Upon dissolution in soil moisture, TSP dissociates, releasing H^+ ions into the soil solution, thereby lowering soil pH. On the other hand, MAP contains ammonium (NH_4^+) ions, which can undergo nitrification to form nitrate (NO_3^-) ions. During nitrification, H^+ ions are released, contributing to soil acidification (Zeng et al., 2017). Organic soil amendments typically increase pH in acidic soil by releasing base cations, thus further enhancing soil alkalinity (Chen et al., 2022). Codling et al. (2020) reported increased pH with biosolid treatments in slightly acidic soil in their respective studies (Codling et al., 2021). These studies have reported a reduction in bioaccessibility of Pb with increasing pH, attributed to Pb immobilization through a precipitation process.

In our current study, we observed significant reductions in pH across all three soils compared to their original (untreated) states in all treatments, including the control, over time. Numerous soil reactions can contribute to this decline in soil pH when soil moisture is present. These reactions encompass microbial decomposition and the humification of organic matter in the soil, resulting in the release of organic acids. Additionally, the dissolution of CO_2 in soil water leads to the production of carbonic acid. Furthermore, soil conditions may feature high-density carboxyl and phenolic groups that dissociate, releasing H^+. Moreover, the increased adsorption and precipitation of basic cations (such as (Ca, Mg, K, and Na) can leave H^+ in the soil solution. Finally, nitrification, is another well-known process that contributes to soil acidification (Neina, 2019). Consequently, we posit that one or more of these reactions may have facilitated the observed reduction in pH in the treatments, aside from the direct effects of soil amendments.

Changes in bioaccessible Pb with time

The effects of treatment, time, and their interaction were found to be significant in all soils, except for EP1 soil, where the interaction between treatment and time did not reach significance ($p<0.05$). Over time, a consistent reduction in bioaccessible Pb was observed across all soils and treatments, including the control (Figure 4.3; EP1, EP2, EP3). Despite the inherent differences among the three soils across various factors, it is noteworthy that the 10% biosolids treatment consistently and significantly decreased bioaccessible Pb compared to all other treatments in all three soils after 24 weeks of incubation.

In EP1 and EP2 soil, at 24 weeks, only biosolids treatments (5% and 10%) exhibited a significant reduction in bioaccessible Pb compared to the control. The reduction in biosolids 5% was 20 and 25% while in biosolids 10% was 30 and 47% when compared to the control in EP1 and EP2 soil, respectively. When compared to the original (untreated) soil bioaccessibility, the percentage reduction was 67% for EP1 and 79% for EP2. Additionally, the control treatment also demonstrated a 54 and 60 % reduction compared to the original soil by the 24th week period in EP1 and EP2 soil, respectively. The EP3 soil exhibited similar behavior, with the highest reduction observed in the biosolids 10% treatment, along with biosolids 5% and TSP1:6 treatments compared to the control after the 24 weeks. The reduction in EP3 soil from the biosolids 10% treatment after 24 weeks was 26% compared to the control and 56% compared to the original (untreated) soil. In all three soils, compost treatments did not exhibit a significant difference in bioaccessible Pb reduction compared to the control. Furthermore, both EP2 and EP3 soils showed a significant reduction in bioaccessible Pb with higher rates of TSP and MAP (1:6).

It is widely recognized that the activity of free Pb^{2+} increases as pH decreases, as most Pb minerals exhibit lower solubility at higher pH. Sauve et al, (1998), reported that at near-neutral pH, the activity of Pb^{2+} displayed no distinct correlation with pH but showed a minor yet noteworthy increase attributable to variations in organic matter content. This trend suggests that labile Pb-organic matter complexes might introduce a positive bias in estimating free Pb^{2+}. This observation implies that labile Pb-organic matter complexes might introduce a positive bias in estimating free Pb^{2+} levels. Consequently, within the near-neutral pH range, elevated soil organic matter content enhances levels of dissolved organic matter (DOM), thereby promoting the formation of organo-Pb complexes and increasing Pb solubility (Sauvé et al., 1998). However, in our present study, we observed a decrease in pH within the range of 6.8 to 7.6 across all three soils with the addition of amendments, along with a significant decrease in bioaccessible Pb when biosolids were applied. Hence, we anticipate that there is indeed a relationship between the formation of organo-Pb complexes and the enhancement of Pb solubility with the treatments rather than pH effect.

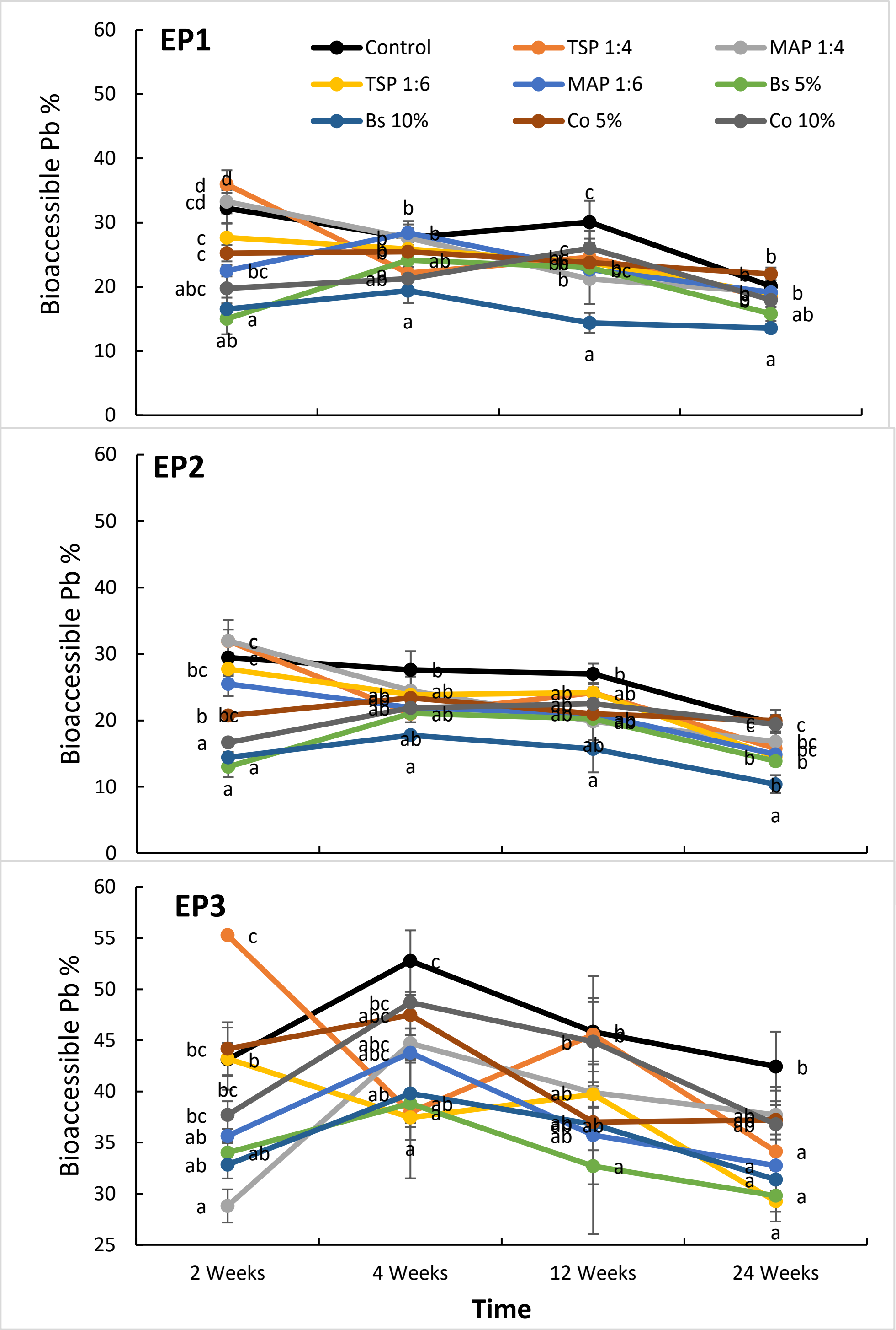

Figure 4.3: Percentage bioaccessible Pb (PBET) changes with time during the incubation study period in three soils-EP1, EP2, and EP3. TSP: triple superphosphate; MAP: monoammonium phosphate; Bs: biosolids; Co: compost. (Means within each time containing the same letter are not significantly different at P = 0.05).

Relationship between the percentage of bioaccessible Pb and various fractions obtained through sequential extraction.

Five operationally-defined Pb fractions in soils were extracted and measured by following the sequential extraction procedure (Tessier et al., 1979). The concentrations of Pb in the treated soil fractions vary with the different treatments. In the untreated EP1 original soil, Pb concentrations in various soil fractions followed this order: carbonate-bound > Fe-Mn oxide-bound > residual > organic-bound > exchangeable (78.8 >59.8 >5.0 >58.8 >0.29 mg kg^{-1}, respectively). In all three soils, the exchangeable fraction had the lowest lead concentration. Specifically, the exchangeable fraction in EP1 and EP2 was less than 0.5%, while in EP3, it was less than 4%. EP3 exhibited a significantly lower ($p < 0.0001$) exchangeable Pb fraction in biosolids treatments (1.5-2.3 mg kg^{-1}) compared to the control (9.5 mg kg^{-1}) and other treatments (7.3-16.3 mg kg^{-1}) (Figure 4.4).

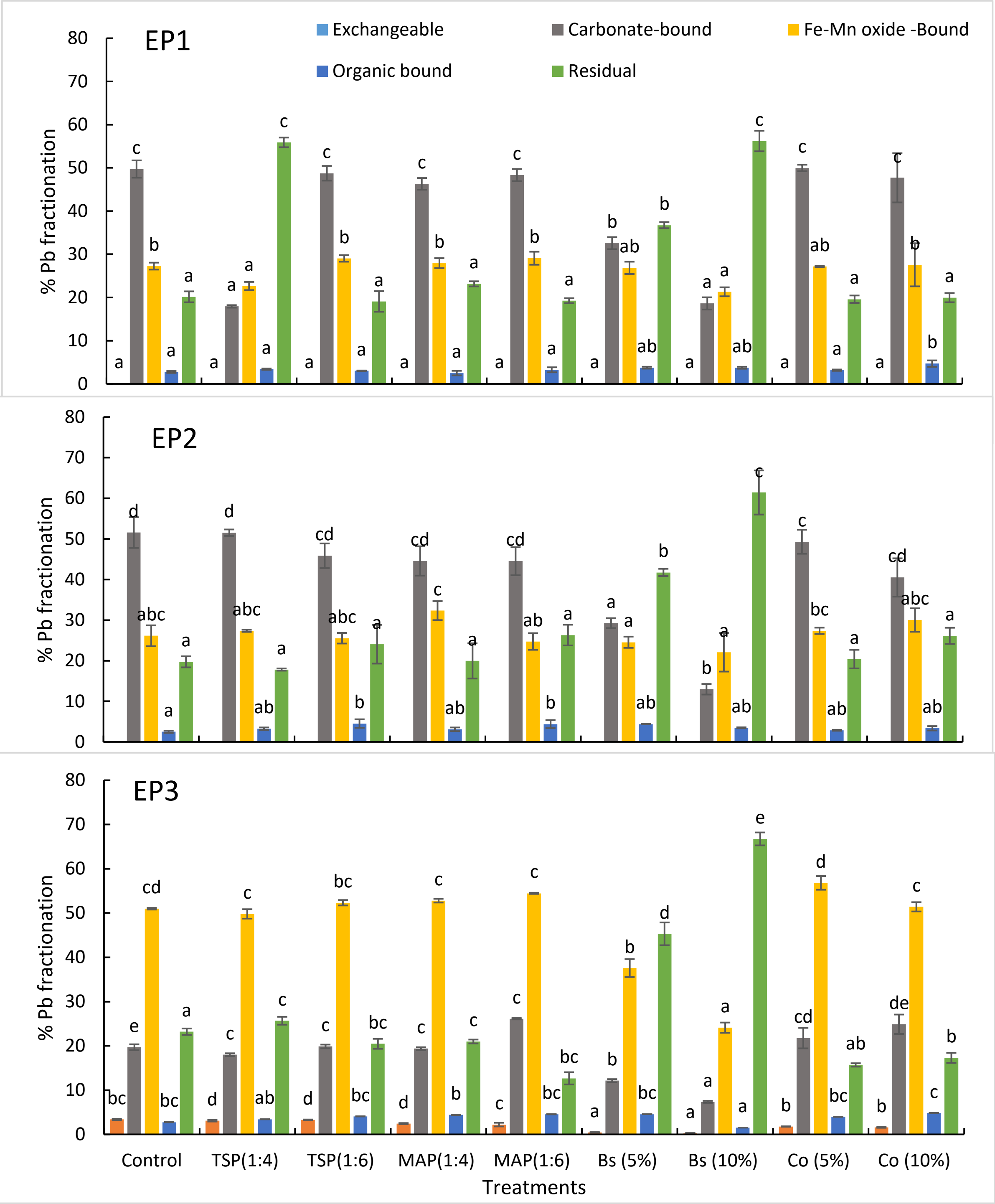

Figure 4.4: Percentage of total Pb measured in each fraction of sequential extraction, performed on 24 weeks of incubation in three soils EP1, EP2, and EP3. TSP, triple superphosphate; MAP, monoammonium phosphate; Bs, biosolids; Co, compost. (Means within the same fraction containing the same letter are not significantly different at P = 0.05).

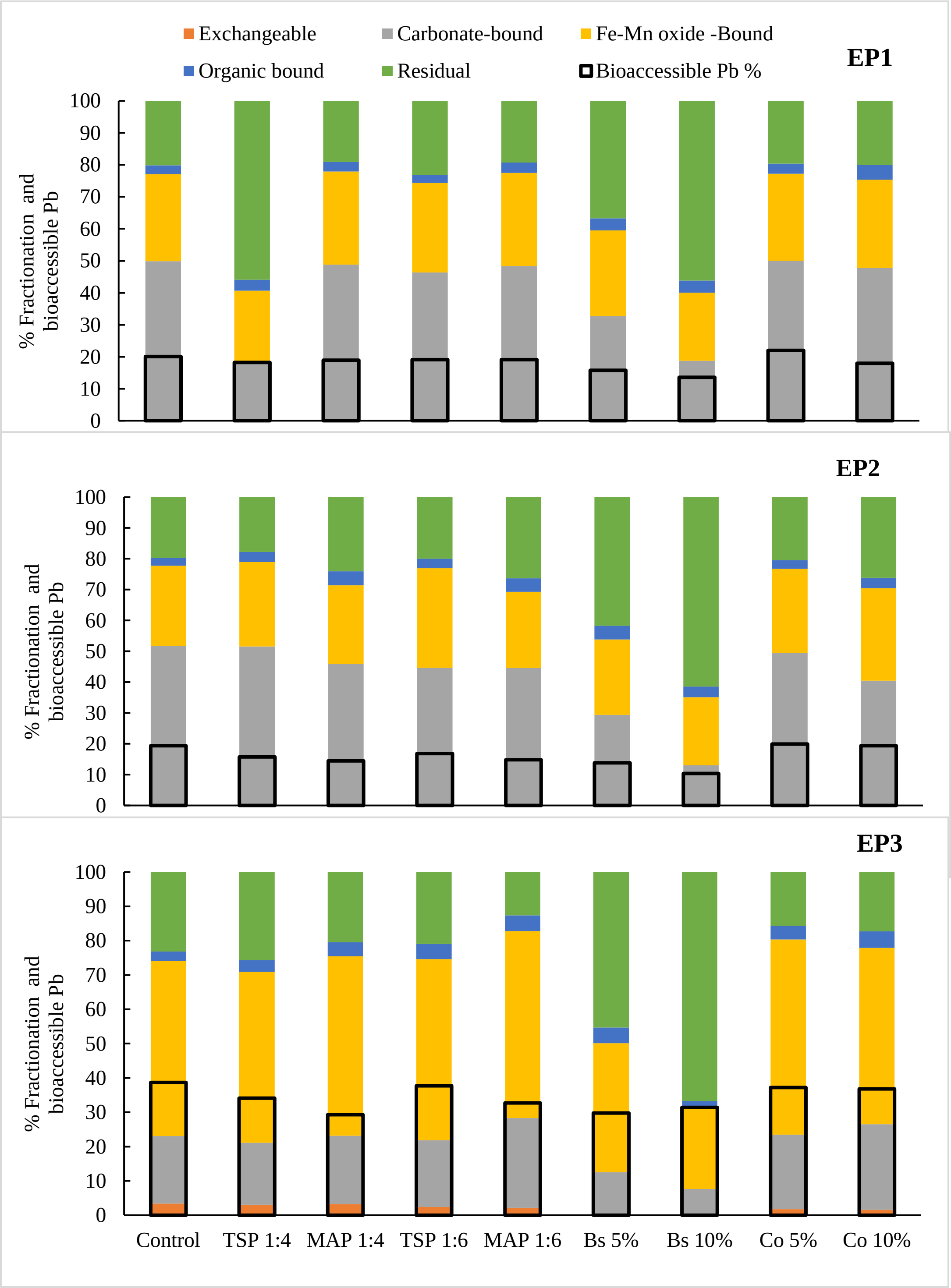

Figure 4.5: Percentage of bioaccessible Pb overlaid upon the % total Pb measured in each fraction of sequential extraction, performed on 24 weeks of incubation in three soils EP1, EP2, and EP3. TSP, triple superphosphate; MAP, monoammonium phosphate; Bs, biosolid; Co, compost.

In EP1 and EP2, changes in the exchangeable Pb fraction were not obvious due to its lower Pb concentrations (< 0.3 mg kg^{-1}). Both biosolids treatments (5% and 10%) showed significantly higher residual fractions (> 50% from total soil) in all three soils (EP1, 2, 3) compared to other P-containing organic and inorganic treatments (Figure 4.4; EP1, EP2, EP3). There was a negative correlation (R^2=0.30 - 0.39) between increased residual fraction and reduced bioaccessibility, making lead less bioavailable to humans and plants. Alasmary, (2020) found similar results in their study, evaluating the effect of soil amendments and biofuel crop growth on Pb uptake and bioaccessibility in a skeet range soil based on a greenhouse study (Alasmary, 2020). The transformation of exchangeable, carbonate, and Fe-Mn-bound Pb fractions into the residual fraction was evident with the biosolids treatments in his study.

While EP1 and EP2 showed the highest Pb concentration in the carbonate-bound fraction, EP3 displayed the highest lead concentration in the Fe-Mn oxide-bound fraction. This could be attributed to the EP3 soil, collected in close proximity to the smelter, which likely experienced greater release of iron (Fe) during the smelting process compared to the other two sites. Additionally, this soil inherently contains lower levels of organic carbon (TOC; 0.48%), which may further contribute to the observed differences. The concentration of carbonate-bound Pb varied among the three soils and treatments. In EP1 soil, the lowest carbonate-bound Pb was observed in both TSP1:4 and biosolid 10% treatments (41.3 and 42.5 mg kg^{-1}), comprising up to 18 and 19% of total Pb in the soil. The highest carbonate-bound Pb in EP1 soil was found in compost treatments (compost 5%- 113.2 mg kg^{-1} and compost 10%-142.1 mg kg^{-1}) constituting 48-50% of the total Pb in the soil. Although carbonate-bound Pb in EP1 soil did not exhibit a strong positive correlation (R^2 =0.26) with bioaccessible Pb, some treatments showed increased

Pb bioaccessibility with higher carbonate-bound Pb, such as compost and control (Figures 4.4 and 4.5, EP1). In EP2 soil, biosolids treatments exhibited the lowest carbonate-bound Pb (5% - 82.3 and 10% - 41.0 mg kg-1), while control, compost, and TSP1:4 showed the highest carbonate-bound Pb ranging from 142.5 to 151.9 mg kg^{-1}. As a result, the biosolids 10% treatment comprised only 13%, while control, TSP1:4, and compost showed 49-52% carbonate-bound Pb of the total soil. Similar to EP1, EP2 also didn't exhibit a strong positive correlation ($R^2 = 0.26$) between carbonate-bound Pb and bioaccessible Pb. However, the biosolids treatment showed a relationship, indicating reduced Pb bioaccessibility was associated with decreased carbonate-bound Pb. Third soil, EP3, also followed a similar trend for carbonate-bound Pb, with biosolids treatments showing the lowest carbonate-bound Pb (biosolids 5%-61.5 mg kg^{-1} and biosolids 10%- 41.4 mg kg^{-1}) while control and compost displayed the highest (102.3, 112.0 mg kg^{-1}) carbonate-bound Pb. In EP3 soil, the percentage of carbonate-bound Pb ranged from 7 to 26% of the total soil representing biosolids 10% (lowest) and control (highest) treatments, respectively. The lowest Fe-Mn bound Pb was observed in the biosolid 10% treatment in all three soils. This suggests that Fe-bound Pb in biosolids 10% treatment likely appeared in the residual fraction. Both control and compost treatments exhibited higher Fe-Mn oxide-bound Pb in all three soils, although not significantly different from some other treatments. In EP3 soil, the percentage of organic-bound Pb ranged from 1.5 to 5.0%, with concentrations ranging from 8.7 to 23.3 mg kg^{-1}. Biosolids 10% treatment in EP3 soil exhibited the significantly lowest organic-bound Pb (1.5%).

The organic-bound Pb fraction ranked as the second lowest in these soils, primarily attributed to lower soil organic matter contents across all three soils. This might be due to suboptimal vegetation growth, and accelerated organic matter decomposition due to the elevated regional temperature (Błońska and Lasota, 2017). In a study by Domanska et al. (2013) regarding the use of treated municipal sewage, showed the highest Pb concentrations in the Fe-Mn oxides and organic matter-bound fractions and followed by carbonate-bound and residual fractions. They further suggested that the significantly higher binding of Pb (21 to 30%) compared to Cd (9 to 11%) to organic bound fraction is attributed to Pb having a strong affinity for organic matter. This is evident in the formation of complex bonds between Pb and organic compounds present in the soil (Domanska et al., 2013).

Effect of Treatment on Pb Speciation

At the end of the incubation study, for 24 weeks sample, X-ray absorption spectroscopy studies were performed. Based on the results of the linear combination fitting of XANES and XAFS, XAFS analysis gave a more meaningful and highly correlative Pb speciation explaining the treatments effect. The XAFS spectroscopy, with its sensitivity to local atomic structure and versatility in soil sample analysis, is a valuable tool for investigating the properties and behavior of elements in various scientific contexts. The analysis and modelling of XAFS spectra play a crucial role in extracting meaningful information about the atomic environment surrounding the selected element such as oxidation state and coordination chemistry of the selected element. Additionally, XAFS can often be done on low-concentration elements (few ppm), and so has applications in a trace element such as Pb analysis (Newville, 2004).

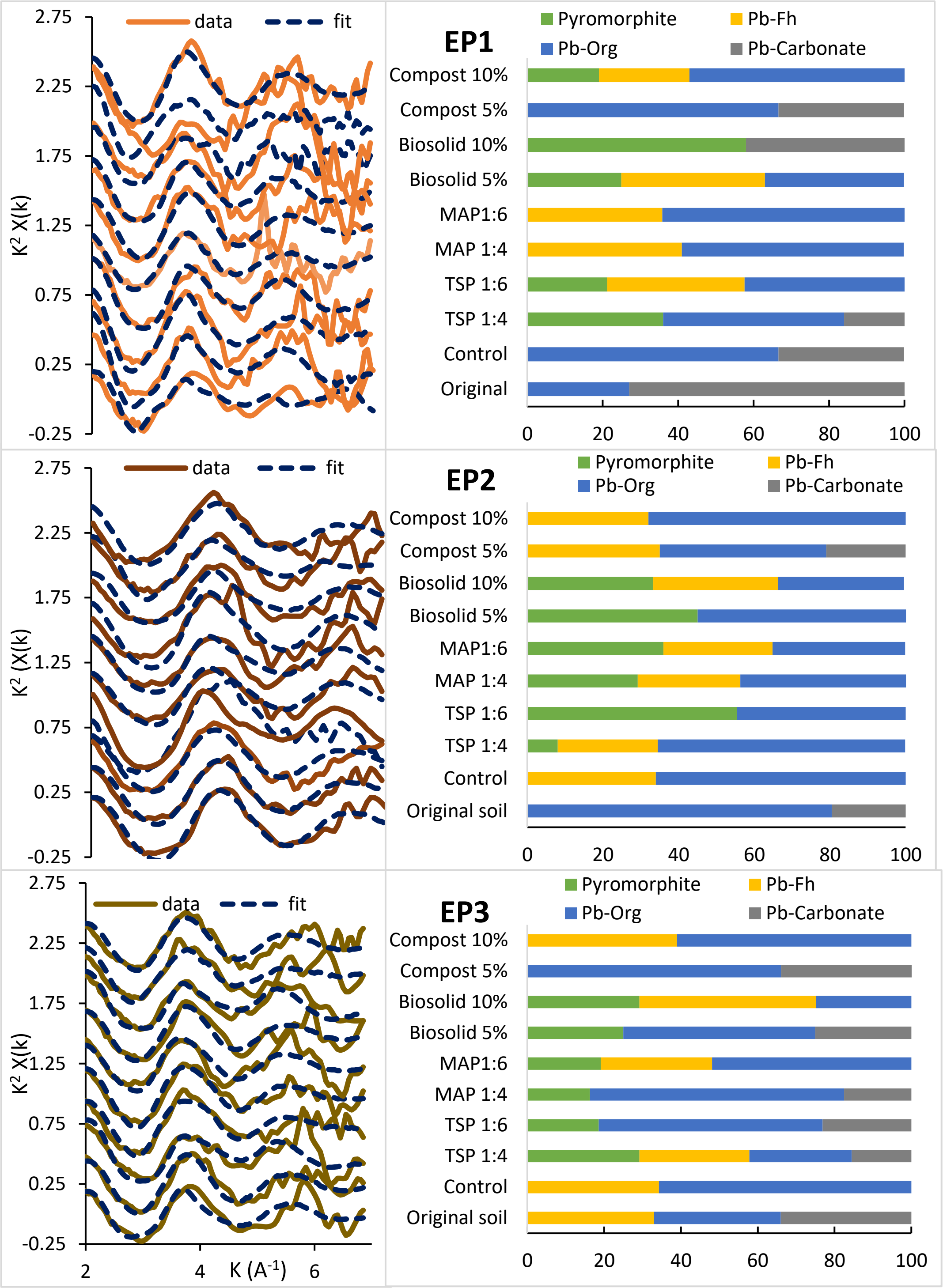

Figure 4.6: Normalized Pb K-edge X-ray absorption fine structure spectra of three soils (EP1, EP2, EP3) with results of linear combination fitting as percent distribution of Pb species. TSP, triple superphosphate; MAP, monoammonium phosphate; Bs, biosolids; Co, compost; Pb-Fh, Fe-ferrihydrite sorbed Pb and Pb-Org, Pb bound to organic compounds.

The EP1 soil exhibited the lowest total Pb concentration (157 mg kg^{-1}) and thus displayed elevated signal to noise in XAFS spectra compared to the other two soil samples (Figure 4.6). Attempts to mitigate this noise through slight spectral smoothing proved ineffective in accurately discerning Pb species. Consequently, normalized, XAFS spectra were employed for species identification for all three soils.

In the EP1 soil, original soil and control and compost 5% treatments, Pb was primarily found to be associated with carbonate-bound mineral compounds, including cerussite, hydrocerussite, and plumbonacrite. Additionally, organic-bound Pb complexes identified as humic acid, fulvic acid, and citrate were identified in those soil samples. Conversely, both inorganic treatments TSP and MAP, biosolid 5% and compost 10% treatments led to the depletion of carbonate-bound Pb species, replaced by Pb sorbed to Fe-ferrihydrite compounds (35-41%), pyromorphite (chloropyromorphite, hydroxy pyromorphite, Pb-adsorbed-hydroxyapatite) (21- 36%), and organic Pb complexes (42-57%). The biosolid 10% treatment was characterized by an abundance of pyromorphite (58%) than other soluble Pb species.

In the EP2 soil, the original sample displayed a Pb species distribution similar to EP1, with a predominant association of Pb with humic acid (81%) followed by hydrocerussite (19%) (Figure 4.6). Control and compost 10% treatments exhibited Pb predominantly bound to humic acid or citrate (66-68%) which are proxy for organic bound Pb and Fe-ferrihydrite minerals (32-34%) (Fe-bound Pb), whereas the compost 5% treatment showed hydrocerussite (21%). Notably, Pb carbonate was absent in inorganic P (TSP and MAP) and biosolids treatments, with a concomitant depletion of the organic-bound Pb fraction and the emergence of pyromorphite and

Fe-bound Pb species.

In EP3 soil, the distribution of Pb species in the original untreated soil, as well as in control and compost treatments, exhibited similarities (Figure 4.6, EP3). More than 60% of Pb was bound to organic species, with carbonate-bound Pb comprising 34%, and Pb bound to Fe ranging from 33% to 39%, with the no detectable pyromorphite. Inorganic P (MAP and TSP) and biosolids treatments shared a comparable Pb species distribution, clearly showing pyromorphite, thereby compensating for the reduction or absence of organic-bound Pb and carbonate-bound Pb species.

The observed reduction in bioaccessibility in both inorganic P (TSP and MAP) and biosolid treatments aligns well with the presence of pyromorphite. Among various Pb minerals, pyromorphite stands out as the most stable form of Pb phosphates in soils, as documented by Lindsay (1979) and Ryan et al. (2001). Iavazzo et al., (2012) and Li et al., (2021) reported that most of the alkaline pH conditions, Pb are predominantly associated with soil carbonates. Under neutral to slightly acidic soil conditions, soluble Pb carbonate minerals such as cerussite ($PbCO_3$) and hydrocerussite [$Pb(CO_3)_2(OH)_2$] have the potential to transform more stable Pb phosphate-like minerals over time. This transformation is facilitated by the presence of adequate amounts of soluble phosphorus under alkaline conditions, as elucidated by Sever (1993) and Hashimoto et al. (2009).

Our study provides further evidence for this phenomenon, as the addition of TSP and MAP-like P fertilizers, and class A P-enriched biosolids has promoted pyromorphite formation. The disappearance of Pb carbonate minerals with these treatments and the concurrent formation of pyromorphite is corroborated by sequential chemical extraction techniques and synchrotron analysis employed in our study (Figures 4.4, 4.5 and 4.6). Ryan et al. (2001) confirmed pyromorphite formation using XAFS spectroscopy after a 240-day incubation period of hydroxyapatite treatment. This finding was further supported by sequential chemical extraction experiments, which demonstrated that hydroxyapatite treatment led to a reduction in the first four fractions of extractable Pb and a corresponding increase in the recalcitrant extraction residue fraction, accounting for 35% of the total Pb at the beginning of incubation and 45% after 240 days (Ryan et al., 2001).

In all three soils subjected to high amounts of biosolids (10%), TSP, and MAP (1:6) treatments, a notable transformation of soluble Pb species such as cerussite and hydrocerussite, representing the Pb carbonate fraction, into pyromorphite (residual fraction) was observed. This transformation was evident in both XAFS analysis and sequential extraction, particularly following a 24-week incubation period. Despite notable pyromorphite formation, it did not exceed 50% in the majority of these treatments. Furthermore, all treatments exhibited a decrease in bioaccessibility over time compared to the original untreated soil, indicating limitations in forming stable Pb compounds like pyromorphite in these soils following phosphorus treatments.

The transformation of Pb into pyromorphite is heavily influenced by soil pH, with optimal conditions typically found in environments characterized by acidity (pH < 5) to weak acidity (pH 6 to 7) (Zhang and Ryan, 1999; Apple and Ma, 2002; Guanxing et al., 2011). However, the restricted formation of pyromorphite after phosphorus amendments may also be attributed to the relatively high presence of organic bound Pb, notably Pb bound to humic acid, across all three soils specifically in compost treatments. Studies have highlighted the prevalence of organically complexed Pb in soils with elevated organic carbon content, as revealed by XAFS analysis (Lang and Kaupenjohann, 2003; Scheckel and Ryan, 2004; Andrunik et al., 2020). Moreover, the introduction of composted biosolids at 10% concentration combined with TSP at 1% has been linked to an increase in dissolved organic carbon (DOC)-complexed Pb, leading to a decrease in the proportion of pyromorphite formation (Scheckel and Ryan, 2004). Andrunik et al. (2020) also suggested that the reduced formation of pyromorphite involves the strong adsorption of Pb as stable Pb-organic surface complexes, supported by a substantial increase (up to 50%) in organic Pb fractions (F4) observed in the sequential extraction data.

Furthermore, the conversion of soluble Pb into Fe-bound Pb or Fe-Mn oxide may contribute to the limited formation of pyromorphite in these soils. Iron and Mn oxyhydroxides are recognized for their strong sorption affinity for anions, including phosphorus. Phosphorus fixation may occur through diverse pathways, including the precipitation of insoluble Fe-P directly onto the surface of Fe-oxides, adsorption and retention of P within the structure of Fe-oxides, and specific adsorption of P onto Fe-oxides through ligand exchange processes (Bigham et al., 2002). Soil acidification has been identified as a crucial factor for facilitating the release of Pb to from Pb-minerals in alkaline soil pH conditions (Hashimoto et al., 2009).

The reduction in pH observed after the application of all treatments, including the control, may be attributed to the dissolution of certain Pb minerals in these soils, thereby enhancing the formation of Fe-bound Pb and the strong adsorption of Pb as stable Pb-organic surface complexes. Additionally, loosely bound or surface-bound Pb within organic compounds and carbonate-bound Pb may have been dissolved and subsequently transformed into more stable complexes during the incubation period. These hypotheses were supported by the observed reduction in organic-bound Pb (Figure 4.6) and carbonate-bound Pb in the original soil, along with the increased formation of either organic-bound or Fe-bound Pb in the control treatment across all soils. Consequently, a reduction in bioaccessible Pb was noted.

These soils originated from dry and arid climatic environments with limited rainfall/moisture, exhibiting sandy loam to loamy sand textures with less than 45% maximum water-holding capacity (MWHC). Therefore, soil moisture/water availability likely serves as a limiting factor, restricting the transfer of solid-phase Pb into the solution phase via desorption and dissolution of the soil solid fraction and subsequent sorption into more stable Pb forms. Throughout the incubation period, maintaining stable MWHC in the control treatment facilitated soil chemical reactions, resulting in reduction in bioaccessible Pb. Furthermore, the study underscores the crucial role of soil moisture/water in facilitating the transformation of soluble Pb compounds into stable Pb compounds, particularly in regions characterized by dry and arid climatic conditions where the original Pb species are preserved. Given that these Pb species are highly soluble in acidic environments and pose significant health hazards upon exposure through ingestion or inhalation.

Further, the addition of soil amendments alleviated some of the other limiting factors, such as the availability of soluble P. This effect was less pronounced in EP1 soil due to its low total Pb concentration, resulting in a minimal soluble Pb fraction. Hence, only the biosolids at 10% concentration exhibited a significant reduction in bioaccessible Pb, accompanied by a more pyromorphite formation (58%). The presence of lead carbonate observed after 24 weeks of incubation in biosolids 10% treatment may be attributed to minimal variation in soil pH (7.55 and 7.59 at 2 weeks and 24 weeks, respectively). Similarly, the presence of Pb carbonate in EP1 soil following TSP1:4 and compost 5% treatments after 24 weeks may also be attributed to limited pH changes (Figure 4.2; EP1).

Addition of compost (crop residue and food waste), especially the 5% treatment, led to nearly identical alterations in pH, reduction of bioaccessible Pb, sequential extraction Pb fractionation, and XAFS Pb species distribution compared to the control treatment across all three soils. Although the compost at 10% treatment exhibited some changes, they were not as significant as those observed with other treatments. Attanayake et al, (2017) in their field studies and Palansooriya et al, (2020) highlighted that the effects of compost and plant residues on the immobilization of trace elements vary depending on material maturity levels, composition, and soil properties. Similarly, Rizwan et al. (2021) found that the incorporation of finely ground rice straw had minimal impact on pH and slight changes in sequentially extracted Pb fractions compared to the control. Furthermore, Popescu et al. (2023) observed no changes in plant tissue or soil-available Pb with the application of yard compost, with or without a low rate of diammonium phosphate (DAP) application, in lead arsenate-contaminated soil while growing carrots.

The compost utilized in our study exhibited a notably high concentration of Mehlich-III P (669 mg kg^{-1}). There is a higher likelihood of Mehlich-III P precipitation as calcium-phosphate minerals when pH rises above 6.5 (Penn et al., 2018). Consequently, the availability of phosphorus decreases. Without a significant pH change following compost addition, Pb solubility is also limited. As a result, the outcome could be a poor reduction of bioaccessible Pb with compost treatments, as observed in our study.

Conclusions

The results obtained from both sequential chemical extraction and synchrotron analysis revealed significant impacts of biosolids, TSP, and MAP on the transformation of more soluble Pb fractions, such as carbonate and organic-associated Pb, into stable or residual Pb fractions, including Fe-bound Pb and pyromorphite, across all three soils. Furthermore, the maintenance of soil moisture in these soils was found to reduce the bioaccessible Pb levels effectively, likely due to natural attenuation processes occurring in soils as observed in the control and compost-amended soils, which exhibited similar percentages of bioaccessible Pb levels and Pb speciation profiles.

Overall, the findings suggest that the lower percentage of bioaccessible Pb observed in soils treated with biosolids may be attributed to the formation of stable Pb compounds. These could likely represented by Pb-P, Pb-OC-Fe species due to the specialty of biosolids used in the current study. Additionally, inorganic soil amendments like TSP and MAP demonstrated the ability to form stable Pb-pyromorphite compounds in alkaline Pb-contaminated soil. The formation of pyromorphite, Fe-bound, and organic-bound Pb species is associated with reduced bioaccessible Pb levels.

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Chapter 5 - Estimating the Proportion of Bioaccessible Lead (BaPb) in Household Dust Wipe Samples: A Comparison of IVBA and PBET Methods

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Abstract

Established methods for using standardized dust wipes to collect and measure total lead in household dust are readily available but the use of dust wipes to measure bioaccessible lead (BaPb) is less clear. This study compared two in vitro methods for estimating the proportion of BaPb in dust collected into dust wipes including the US-EPA's *in-vitro* bioaccessible assay (IVBA) method at two pH (1.5 and 2.5) values; and the physiologically-based extraction test (PBET 2.5 pH). Two types of simulated household dust samples (Pb-soil contaminated and Pb-paint contaminated) each with three Pb concentrations were created. Equal amounts of simulated dust were applied to a smooth surface and collected following the standard EPA dust wipe protocol and were analyzed for BaPb and total Pb (ASTM-E1644-17, ICP-OES). Estimated BaPb levels differed significantly by the method of extraction. Mean percent BaPb were IVBA pH 1.5, > 90% (Pb-paint) and 59 to 63% (Pb-soil); IVBA pH 2.5 78 to 86% (Pb-paint) and 45 to 50% (Pb-soil); PBET pH 2.5 56 to 61% (Pb-paint) and 41 to 50% Pb-soil). Particularly for lead-

paint contaminated dust, PBET showed significantly greater discrimination as suggested by the broader range of BaPb values and closer approximation to total lead concentrations in simulated household dust samples.

Introduction

With growing acceptance that no level of Pb is “safe” for children and developing fetuses (CDC, 2002; Thomason et al., 2019) accurate identification of home Pb hazard sources is critical for reducing blood lead levels (BLLs) in children and infants. Ingested and inhaled household dust is recognized as one of the major sources of Pb exposure for all children (Han et al., 2012) and exposure due to dust ingestion is particularly prevalent among children age 5 and below CDC, 2002). Calabrese et al, (1991) reported that 11% of toddlers may exhibit pica behavior (eating nonfood items), and may consume up to 10 to13 g of soil per day. Younger children are likely to ingest significant quantities of interior household dust when they spend significant amounts of time indoors, playing on the floor, and putting their hands and other objects into their mouths. According to the United States environmental protection agency (USEPA), it is estimated that children under the age of 6 ingest or breathe twice as much dust as older children, and up to ten times more than adults (US EPA, 2002).

Lead in household dust most commonly results from deteriorating lead-based paint, lead-contaminated soil that is tracked indoors, and somewhat less commonly, lead dust carried into the home on work clothes and skin from adults’ occupational exposures (Yu et al., 2006; Pelley, 2017; US EPA, 2022). Inhaled Pb is uniquely toxic because it is not believed to be directly influenced by biochemical processes that alter its absorption. In the gut however, ingested lead encounters stomach acids that usually alter its absorption. Thus, the toxicity of ingested lead is associated with its bioaccessibility, defined as the fraction of total lead available for absorption into the blood stream via the gut (Kelley et al., 2002; Hettiarachchi & Pierzynski, 2004; Turner and Ip, 2007). Dissolved Pb in the form of free ion (Pb^{2+}) is the form that can enter the body and mimic and antagonize the action of calcium (Ca^{2+}) and zinc (Zn^{2+}) ions (Sanders et al., 2009; Gorkhali et al., 2016). Ingested lead undergoes a dissolution process in the gastrointestinal tract and the dissolution process varies according to the chemical form of the Pb (Ruby et al., 1993; Pelfrêne & Douay, 2017). Materials containing $PbCO_3$ (lead carbonate) or Pb-manganese (Pb-Mn) oxides as the dominant Pb species showed high bioaccessibility (ranging from 60 to 100%) in many in vivo and in vitro studies (Schoof et al., 1995; Casteel et al., 2006; Denys et al., 2007; Drexler & Brattin, 2007; Rasmussen et al., 2011; Li et al., 2012; Sowers et al., 2021; Haque et al., 2022). In addition, many studies reported the low solubility of Pb-phosphate and anglesite, galena, and some Fe-Pb, Pb-O and Pb sulphate species (Hettiarachchi & Pierzynski, 2004; Bosso et al., 2008; Scheckel et al., 2013; Attanayake et al., 2017; Karna et al., 2021). Lead in soil can exist as Pb containing minerals or Pb-bound to other soil minerals. However, the overall solubility and bioaccessibility of soil Pb depend on the relative amounts and reactivity of the different chemical forms (such as crystallinity) and physical forms of Pb and Pb-bound minerals (e.g., accessibility). Most synthetic Pb-associated products contain highly bioaccessible Pb compounds, i.e., leaded paint: $PbCrO_4$ (lead(II) chromate), Pb_3O_4 (lead(II, IV) oxide), and $PbCO_3$ (lead(II) carbonate), leaded gasoline: $Pb(C_2H_5)_4$ (tetraethyl and/or tetramethyl lead), and lead arsenate pesticide ($PbHAsO_4$).

Interest in characterizing household dust samples for the purpose of identifying child Pb hazards in the home grew during the 1990's, however these studies tended to focus only on the relationship between total Pb in household dust and child BLLs (Lanphear et al., 1996), despite the fact that the dissolution process of ingested Pb by stomach acids differs for different Pb species (Mushak, 1991; Ooman et al., 2003; Sowers et al., 2021). Studies have shown a strong relationship between child BLLs and the proportion of bioaccessibile lead (BaPb) present in ingested soil and dust, using either *in vivo* or *in vitro* methods (Lanphear et al., 1996; Zia et al., 2011; Bradham et al., 2017). To our knowledge, there are no studies that determined BaPb in dust wipe samples, whereas bulk vacuum samples have been used in determining BaPb in house dust samples (Yu et al., 2006; Rasmussen et al., 2011; Boros et al., 2017; Sowers et al., 2021).

The methods for determining the proportion of BaPb fall into two broader categories, in vivo and in vitro methods. Although it could be superior to in vitro methods, in vivo methods cannot be practiced as a routine test (Zia et al., 2011; Deshommes et al., 2012; Henry et al., 2015). In vitro approaches BaPb have some disadvantages, such as over or under estimation, effect of solution matrix, pH, and sample properties (Zia et al., 2011; Deshommes et al., 2012). In all in vitro bioaccessibility methods HCl acid regulates the extraction solution pH. Among the published in vitro bioaccessibility methods including IVBA [(USEPA method 1340)/ RBALP (relative bioavailability leaching procedure)/ SBRC (Solubility/Bioavailability Research Consortium)] at gastric phase (uses 30.03 g l^{-1} glycine adjusted to pH 1.5 with concentrated HCl), PBET and the Unified Bioaccessibility Method (UBM) (uses combination of inorganic solution mixture, organic solution mixture and specific enzymes), PBET is the one with the least pH buffering.[38] PBET extraction solution is comprised of pepsin, organic acids (acetate and citrate, lactate, and malate), and HCl, which mimics a complex mixture of gastric solution than simple glycine extraction (Ruby et al., 1993).

Comparison studies of the different potential in vitro bioaccessible methods are needed to identify an optimal approach for determining in vitro BaPb in dust wipe samples. The goal of this study was to compare the two main approaches currently suggested in the literature for determining BaPb in household dust wipe samples including the USEPA's *in vitro* bioaccessible assay (IVBA) Method 1340 at two pH values (IVBA 1.5 and IVBA 2.5 pH); and the physiologically-based extraction test (PBET 2.5 pH).

Materials and methods

Collection and initial testing of household dust

Interior household dust was collected from the vacuum cleaners of 6 homes in El Paso, Texas and combined to get the dust mass adequate for the study. The collected dust was sieved from < 250 µm sieve (manually removed large pet hair as much as possible), tested for Pb, and was determined to have similar in background amounts of lead (Pb concentration < 30 mg kg^{-1}).

Collection, initial testing and preparation of Pb contaminated soil

The soil-lead contamination source was created using different proportions of lead-contaminated and uncontaminated soil of similar geological composition. Lead contaminated soil was collected from El Paso, Texas close to the ASARCO smelting site. Both soil sources were sieved (250 µm) and tested for lead. The uncontaminated soil was confirmed less than the background soil lead (Pb < 30 mg kg^{-1}) and Pb-contaminated soil determined to have a Pb concentration of 4023 mg

kg^{-1} (±188.2) by USEPA method SW846–3051A with concentrated HNO_3 (nitric acid) (US EPA 2007), followed by the inductively coupled plasma - optical emission spectrometer (ICP-OES) (Varian 720-ES, USA) analytical procedure. Both soil and dust total Pb were determined by USEPA method SW846–3051A (Table 1). Briefly, 10 mL of trace metal–grade concentrated HNO_3 was added to 0.5 g of soil and the content was digested in a microwave digestion unit (MARS Xpress, CEM Corp.). The solution was filtered using a Whatman No. 42 filter paper and was analyzed for Pb using an ICP- OES. Expected Pb based on the p-XRF and mixing ratio of Pb sources and dust has shown in Table 1. A standard reference soil (NIST 2711a-Montana II) was digested as a quality assurance/quality control (QA/QC) sample to evaluate the validity of digestion and analytical procedures for initial soil, dust and dust mixture preparation.

Simulated house dust

Two types of simulated lead-contaminated household dust were prepared using household dust collected by a vacuum cleaner from 6 different homes and two Pb sources, including lead-contaminated soil (collected from the closed Asarco smelter site) and Pb in powdered paint (2% Pb, lead in powdered paint, Sigma-Aldrich, St. Louis). The collected dust was sieved (250 µm) and hair strands and other large debris were removed manually. The sieved dust was tested by using both portable X-Ray fluorescence spectroscopy (pXRF) and laboratory analytical procedure (US EPA, 2008), and the Pb concentration was determined to be below the detectable limit (< 30 mg kg^{-1}). For each lead source, three Pb concentrations were created (“low,” “medium,” and “high”) by combining different proportions household dust and the Pb source material. The USEPA’s clearance levels are, for floors, 10 micrograms (µg) of lead in dust per square foot (ft^2); and for window sills, 100 µg of lead in dust per ft^{-2} (US EPA, 2020). We estimated 0.2 g per ft^2 and measured dust lead loading based on the lead concentrations in each created dust mixture. Using these estimates, “low” represented lead in 80 -100 µg ft^{-2}; "medium" represented lead in 130 -150 µg ft^{-2}; "high" represented lead in 180 - 200 µg ft^{-2}, In this way, "low” (400 mg kg^{-1}), "medium” (800-700 mg kg^{-1}), and "high" (1000-1200 mg kg^{-1}) Pb concentrations were based on lead loading values of 100 µg ft^{-2} (lower limit); 150 µg ft^{-2} (medium); and 200 µg ft^{-2} (higher limit).

Details regarding the mixtures follow. Although the target Pb concentrations were different in each simulated dust mixture, the ratio of lead-contaminated soil and house dust was held constant and the percent soil fraction in contaminated soil and dust mixture was 30%. The simulated Pb paint dust 1000, 700, and 400 Pb fractions were 50%, 33%, and 20%.

Simulated Pb soil-contaminated household dust preparation.

Table 1 shows the Pb levels expected for the “high,” “medium,” and “low” concentration lead-contaminated soil sources. The mixture ratios for the levels were 3:0.3; 3:2; and 3:7 respectively with Pb contaminated and uncontaminated soil. Table 1 shows the lead concentrations achieved with these ratios and recovery percentages. The three levels of lead-contaminated soil mixtures were then mixed using a ball mill (US Stoneware, Ohio) at about 20 rpm together with the prepared household dust using a 1:2 ratio. Table 1 also shows the final Pb concentrations (initial levels measured by p-XRF) determined by ICP-OES in the simulated lead-contaminated dust (Pb soil dust) samples and their approximation to the calculated expected Pb concentrations.

Simulated Pb paint-contaminated dust preparation.

Lead in powdered paint certified material (lead concentration ~2000 mg kg^{-1} as lead dichloride) was mixed with sieved (250 µm) house dust in the ratio of 1:1, 1:3, and 1:4 with expected final lead concentration of 1000, 700, and 400 mg kg^{-1}, respectively. Table 1 shows the measured Pb in the simulated lead paint-contaminated dust as determined by ICP-OES.

As shown in Table 1, the mean total lead recovery was 95% for Pb soil contaminated dust and 88% for Pb paint contaminated dust, indicating that the components of the simulated dust types had high affinity and good adherence without clear separation of dust and soil or paint.

Table 5.1: Total lead concentration in initial soil, dust, and dust mixtures

Sample	Measured Pb# mg kg^{-1} ± STDV	Expected Pb* Con. mg kg^{-1}	Recovery %
House dust	< 6	None	(-)
Uncontaminated soil	< 6	None	(-)
Contaminated soil	4023±188.2	4000	101
Soil mixture 1	3702±120.3	3800	97
Soil mixture 2	2363±342.0	2600	91
Soil mixture 3	1188±268.1	1200	99
Simulated soil dust 1200	1157±17.8	1200	96
Simulated soil dust 800	759±15.5	800	95
Simulated soil dust 400	381±0.8	400	95
Simulated paint dust 1000	856±8.34	1000	86
Simulated paint dust 700	594±17.43	700	85
Simulated paint dust 400	376±14.11	400	94

USEPA method SW846–3051A analyzed by ICP-OES

[*] Based on the mixing ratio and the Pb concentrations used in certified powdered paint material

Table 5.2: Expected Total Pb, Measured Total Pb, Percent Bioaccessible Pb in the original Dust Mixtures (n=3 per Type and Pb Concentration)

Sample	Expected Pb# mg kg^{-1} ± STDV	Measured Pb# mg kg^{-1} ± STDV	Recovery %*	Bioaccessible Pb %± STDV IVBA 1.5pH	IVBA 2.5pH	PBET 2.5pH
Pb Soil dust 1200	1157±18	1254±47	108	66±1.0	58±13	62±14
Pb Soil dust 800	759±16	781±32	103	66±1.6	50±1.1	50±0.3
Pb Soil dust 400	381±0.8	418±27	110	65±2.5	47±0.4	55±6.5
Pb Paint dust 1000	856±9	934±84	109	100±0	97±1.0	66±2.2
Pb Paint dust 700	594±17	695±53	117	100±0	97±0.3	65±2.0
Pb Paint dust 400	376±14	444±26	118	100±0	95±2.4	66±4.4

* Recovery % acceptable within 100 ± 20 due to method differences.

USEPA method SW846–3051A dust only and ASTM E1644-17 method with dust wipe analyzed by ICP-OES

Dust wipe sample collection

The recommended USEPA protocol for collecting household dust samples was followed in this study. The ASTM premoistened towelettes (ASTM E1792) were used to collect a sample within a recommended sampling area using a specific number of passes (5-6) within a standard 1’ x 1’ template. This wipe method measures lead loading as $\mu g\ ft^{-2}$ or $\mu g\ cm^{-2}$ (Sowers et al., 2021)

A box chamber with two separate compartments were constructed (44 cm width x 72 cm length x 48 cm height) using plexiglass sides to ensure undisturbed dust collection. The front of the box was fixed with a sliding door to facilitate easy access to the sampling area. The sampling areas were marked using colored tape in both compartments with the aid of a 30 cm x 30 cm template. Blended 0.2 g of simulated dust-mix was spread as evenly as possible inside the marked area (30 cm x 30 cm).

ASTM E1792 dust wipes were used for all dust collection. Each compartment of the box floor (30 cm x 30 cm area) was wiped following the standard dust wipe procedure given by ASTM E 1728 (ASTM, 2020). For each dust mixture, dust collection was repeated 10 times.

Dust wipes were placed in the enclosed chamber to completely dry for 24 to 48 hours. Ten random blank wipes were dried fully in a desiccator to obtain the weight of the empty wipe to calculate the collected dust amount required in the BaPb calculation. Dust weight (D) was calculated using the difference between the blank, pre-determined dry weight of the wipe and the dried, dust-containing, dust wipe (equation 1).

Pb bioaccessibility (BaPb) using IVBA 1.5 pH, IVBA 2.5 pH, and PBET in vitro assays

The IVBA (US EPA, 2019) and PBET (Ruby et al., 1996) methods were used to assess Pb bioaccessibility in dust samples. In-vitro BaPb determination methods were developed to mimic the human gastric or gastro-intestinal dissolution process. Based on the applied in vitro BaPb determination method BaPb amount can vary because of different pH and extraction solution conditions (Attanayake et al., 2014; Obrycki et al., 2016; Boros et al., 2017). The gastric phase was used in both methods.

IVBA. The USEPA method 1340 *in vitro* bioaccessibility assay was employed using 0.4 mol L^{-1} glycine ($C_2H_5NO_2$) solution adjusted to two different pH as recommended pH of 1.5 and 2.5 using trace metal grade HCl (hydrochloric acid) (US EPA, 2019).

PBET. The physiologically based extraction test originally developed by Ruby et al, (1996), modified by Brown and Chaney (1997) used in this study. The gastric solution was prepared by dissolving 1.25 g of pepsin, 0.5 g of sodium malate, 0.5 g of sodium citrate, 420 µL of lactic acid, and 500 µL of acetic acid in 1 L of Milli-Q water whose pH was subsequently adjusted to 2.5 by drop-wise addition of HCl (Ruby et al., 1996; Attanayake et al., 2017).

In vitro bioaccessible Pb extraction procedure

Extraction solution (S1) was added at a 1:100 ratio (dust: solution) based on the amount of dust collected (D). Extraction solution preparation and pH adjustment was done following the standard procedures and maintaining the temperature at 37±2°C. After adding the extraction solution, the folded dust wipe was carefully opened to facilitate the contact between collected dust with extraction solution properly inside the 125 mL screw-capped, wide-necked high-density polyethylene (HDPE) bottle, while ensuring that there was no loss of dust. Empty bottles (B1) weight was measured and recorded before the addition of solution and wipes. Initial pH was measured and adjusted 2.5±2 only for PBET, while IVBA pH was not adjusted. According to the IVBA, the extraction and filtration process should complete within 90 minutes. Although it is not recommended to adjust pH before extraction, if the fluid pH is not within ±0.5 pH units of the starting pH or is unable to complete within the elapsed total time, the test must be discarded and the sample needs to be re-extracted.

The contents were then placed on the specific extraction device where an electric motor-driven flywheel, drives a rotating block situated inside a temperature-controlled water bath. This facilitated end-over-end mixing of the content, and the electric motor adjusted to 30±2 rotations per minute (rpm). The temperature in the water bath was maintained at 37±2°C using an immersion circulator heater. After a one-hour extraction period, pH was recorded, and the supernatant of extraction solution was poured into a pre-weighed 50 mL polypropylene centrifuge tube (C1). The contents were centrifuged at about 1200 rpm for 5 minutes. The supernatant was filtered through a 0.45 µm nylon filter. The remaining contents of the HDPE bottle (B2) and centrifuged tube (C2) were weighted. The filtered solution used for the bioaccessible lead (R1) determination using ICP-OES.

To confirm the validity of the simulated dust samples and for the purposes of extraction method comparison, simulated dust alone was tested for Total Pb and BaPb. Table 2 shows the expected Pb based on the USEPA method SW846–3051A total digestion of simulated dust and the BaPb percentages obtained with each of the extraction methods for dust alone. The measured Pb for each type and Pb level was obtained from 3 samples per type and Pb level was measured by 0.2 g of each simulated dust mixture added into the dust wipe and digested following the recommended procedure (ASTM E1644-17 method) (ASTM, 2017). Recovery exceeding 100% simply indicated the variability associated with weighing, the two different digestion methods used, and analytical variability in the measure of total Pb in the soil and paint sources.

In addition, 0.2 g of each simulated dust mixture in triplicate directly (without dust wipe) was digested following the ASTM E1644-17 method. The total Pb obtained following this procedure was used in the percent BaPb calculation (equation 6). The resulted total Pb was 1196±50, 758±24, 395±28 mg kg^{-1} for simulated Pb soil dust mixtures 1200, 800, and 400 mg kg^{-1} respectively showing that 95 to 100% recovery based on expected total Pb. Simulated Pb paint dust total Pb was 856±8, 594±17, 376±14 mg kg^{-1} in Pb paint dust mixtures 1000, 700, and 400 mg kg^{-1} respectively showing comparatively low recovery (86, 85, 94% respectively) percentage. This may be due to the higher variability recorded in Pb paint material (acceptance window 1580 to 2350 ± 128 mg kg^{-1}) rather than its certified value of 1960±26 mg kg^{-1} indicated in the product.

During total Pb concentration in dust mixtures and BaPb extraction all the QA/QC checks mentioned (reagent blank, method blank, laboratory control sample, matrix spike sample and control soil-NIST 2011a-Montana II) in the USEPA method 1340 was included (US EPA, 2019).

Total Pb determination in dust wipes

The ASTM E1644-17 hot plate digestion of dust wipe samples for determination of total lead was used with some modifications. All the remaining dust and wipe samples from the 125 mL

HDPE bottle and centrifuged tube were quantitatively transferred into a 250 mL beaker using 12.5 mL Mili-Q water. Trace metal grade con. HNO_3 12.5 mL was added to the beaker to get 25 mL of 1:1 water and HNO_3. Digestion followed the procedure recommended in the ASTM E 1644-17 method (Mushak, 1991). Briefly, the digestion has proceeded beaker covered with watch glass gently heat the content to 85-100°C on the hot plate and reflux for 10- 15 minutes. Contents were allowed to cool near room temperature, and add 10 mL of concentrated nitric acid, replacing the watch glass, and reflux for 30 minutes without boiling. The watch glass was removed and allowed the solution to evaporate to approximately 10 mL without boiling. The evaporated sample was allowed to cool near room temperature and add 5 mL of water and 5 mL of 30% hydrogen peroxide. The beaker was covered with the watch-glass and returned covered beaker to the hot plate for warming and to start the peroxide reaction. When the peroxide reaction was completed, the watch glass was removed and continued heating until the volume has been reduced to approximately 10 mL of digestion mixture. The digestion mixture was quantitatively transferred through a funnel equipped with a Whatman No. 42 filter paper into a 100 mL volumetric flask. A filtered, diluted extraction solution was used for remaining total Pb determination using ICP-OES (ICP-OES reading mg L^{-1}: R2).

Bioaccessible lead was calculated as follows:

$$\text{Dust weight(D)} = \text{Dried dust containing wipe} - \text{ dry weight of empty (blank) wipe} \quad (1)$$

$$\text{Bioaccessible Pb (BaPb)} = \text{R1} \times \left(\frac{S1}{D}\right) \text{mg } kg^{-1} \quad (2)$$

Where,

R1: ICP-OES reading (mg L^{-1}) for bioaccessible lead extraction; S1: Volume of extraction solution; and D: Weight of collected dust (g)

If we assume weight of the remaining extracting solution from bioaccessible lead extraction = X mL

$$\mathrm{X\ mL} = \{(\mathrm{B2} - \mathrm{B1}) + (\mathrm{C2} - \mathrm{C1})\}\ \mathrm{g} \qquad (3)$$

Where,
B1: weight of the empty HDPE bottle
B2: weight of the remaining contents and the HDPE bottle
C1; weight of the empty 50 mL centrifuged tube
C2: weight of the remaining contents and the 50 mL centrifuged tube
(Note g=mL as density of aqueous solution is 1 g mL^{-1})

$$\text{Remaining total Pb in dust wipes after removing bioaccessible Pb (ToPb)} =$$

$$\{\mathrm{R2\ mg}\ L^{-1} - [\mathrm{R1\ mg}\ L^{-1} \times (X\ ml\ per\ 100\ ml]\} \times (\frac{100}{D})\ (4)$$

Where,
R2: ICP-OES mg L^{-1} reading for remaining total lead extraction

$$\text{Recovered total Pb} = \text{ToPb} + \text{BaPb} \qquad (5)$$

$$\%\ \text{Bioaccessible Pb} = (\frac{BaPb}{Total\ Pb}) \times 100 \qquad (6)$$

Where,
Total Pb: from 0.2 g directly digested following the ASTM E1644-17 method

Bioaccessible lead in dust mixtures.

The BaPb in dust mixtures without wipes (n=3) was also measured by adding extraction solution and dust mixtures at a 1:100 ratio into HDPE bottle. Extraction solution preparation, pH adjustment, and maintaining the temperature were done following the standard procedures. After a one-hour extraction period under 30 rpm, pH was recorded, and the supernatant was filtered through a 0.45 µm nylon filter. The filtered solution used for the BaPb determination using an ICP-OES. The percent BaPb was calculated as % of total lead concentration in dust samples.

Simulated Pb soil dust percent BaPb range from 65 to 66, 47 to 58, and 50 to 62% in IVBA pH 1.5, IVBA pH 2.5, and PBET respectively. Simulated Pb paint dust had a far higher percent BaPb and ranged from 100, 95 to 97, and 65 to 66% in IVBA pH 1.5, IVBA pH 2.5, and PBET respectively. It was noted that the BaPb differences across Pb concentrations were substantially smaller than the BaPb differences across the extraction methods.

Statistical analysis

Data were collected, entered into an Excel database and double-checked for accuracy. All analyses were conducted using SAS 9.4 software (2016 by SAS Institute Inc., Cary, NC, USA). Comparisons by method (3), matrix (2), and lead levels (3) were tested with 3 × 2 × 3 ANOVAs ($p < 0.05$) with post-hoc comparison of means for BaPb percentage and total Pb. When the fixed effect F-value was statistically significant, relevant post-hoc tests of least square means (LSM) (for categorical effects) were evaluated. For main effects, post-hoc comparisons of LSM differences were calculated using the Tukey–Kramer adjustment for multiple comparisons.

We analyzed BaPb percentage as a major variability factor. In addition, recovered total Pb was also analyzed to investigate whether there is any effect on measuring remaining total Pb after BaPb extraction and adding them together to obtain recovered total concentration.

Results and discussion

Comparison of BaPb extraction methods for lead soil contaminated dust in wipe samples and lead paint contaminated dust in wipe samples

For the comparison of BaPb extraction methods, 10 wipes per method and level of Pb contamination were tested. Table 3 shows the average values (n=10) for total Pb, BaPb, and BaPb percentage of dust wipes. The average total lead recovery (Table 3) after extraction of BaPb was determined by summation of total Pb concentration in residue soil after BaPb extraction and BaPb calculation (Methods, Equation 5), and expressed as % of total lead concentration expected in dust samples. Percent recoveries ranged from 74 to 96% of all dust mixtures regardless of the bioaccessible lead extraction methods. The highest total lead recovery was found for the Pb paint dust mixtures using IVBA 1.5 pH (89 to 96%), followed by the Pb paint dust mixtures IVBA pH 2.5 (87 to 89%), with the lowest estimates from the paint dust mixtures PBET (74 to 76%). Lead soil dust mixtures gave the highest total Pb recovery after applying PBET (84 to 88%) followed by IVBA pH 2.5 (77 to 85%) and IVBA pH 1.5 (76 to 77%).

Lead paint dust was found 100% BaPb (Table 3) when the percent BaPb was calculated using recovered total Pb in each dust mixture (Pb paint 1000, 700, 400) for IVBA pH 1.5. The recovery percentage varied from 96, 92, and 89% in Pb paint dust mixture 1000, 700, and 400, respectively (Table 3). Percent BaPb in soil dust were 80, 83, 82% in IVBA pH 1.5 although recovered total recovery % varied as 77, 76, 77% in Pb soil dust mixtures 1200, 800, 400 respectively (Table 3). We calculated total Pb (expected based on the used amount) in each dust mixture as well as determined the recovered total Pb by the summation of BaPb and the total Pb in in vitro test residues for each dust sample. The recovered total Pb had variability among the dust mixtures (Table 3) although they were within the acceptable range (100 ± 25%). Due to this variability in recovered total Pb, the current study used total Pb determined directly by digesting 0.2 g without dust wipe following ASTM E1644-17 method for BaPb percentage calculation and further data analysis (Methods, Equation 6).

Significant type III fixed effects and parameter estimates

Tables 4 and 5 summarize the Type III fixed effects and tests of parameter estimates for the main effects of the method (IVBA 1.5pH, IVBA 2.5pH, PBET), matrix (soil and dust), lead level (high, medium, low), and the interactions of method × matrix, method × level, and matrix × level. Significant effects of all these parameters were found for both BaPb % and recovered total Pb. These results (significant effects of both BaPb percentage and recovered total Pb) suggested that the method followed in the current study was applicable for measuring both BaPb and total Pb in dust wipe samples.

Table 5.3: Comparison of IVBA and PBET Methods for Extracted BaPb percentage from Dust Wipe Samples with Lead Soil Contaminated Dust and Lead Paint Contaminated Dust (n=10 per Type and Concentration)

Method	Sample Name	Pb Soil dust			Pb Paint dust		
		1200	800	400	1000	700	400
IVBA 1.5pH	Recovered total Pb mg kg^{-1}	890±30	580±21	292±16	820±33	552±30	339±17
	Total Pb recovery %*	77	76	77	96	92	89
	BaPb mg kg^{-1}	713±29	480±19	237±8	820±33	552±30	339±17
	BaPb %	80±3	83±3	82±3	100±0	100±0	100±0
IVBA 2.5pH	Recovered total Pb mg kg^{-1}	908±100	581±27	324±35	764±35	524±28	328±21
	Total Pb recovery %*	78	77	85	89	88	87
	BaPb mg kg^{-1}	600±17	374±14	177±6	743±31	492±22	297±12
	BaPb %	67±7	65±1	55±6	97±2	94±3	91±4
PBET 2.5pH	Recovered total Pb mg kg^{-1}	971±86	668±107	337±53	651±53	448±23	279±19
	Total Pb recovery %*	84	88	88	76	75	74
	BaPb mg kg^{-1}	558±60	380±36	292±9	534±36	360±21	216±16
	BaPb %	58±6	55±8	49±6	82±5	81±5	78±8

*Total Pb Recovery % was calculated as a percentage of total Pb concentration (USEPA method SW846–3051A) in dust samples.

Table 5.4: Significant Type III fixed effects estimates showing associations of bioaccessible Pb and recovered total Pb

Significant Type III fixed effect		
	F	p
Bioaccessible Pb %		
Method	709.38	<.0001
Matrix	322.99	<.0001
Level	31.55	<.0001
Method*Matrix	50.18	<.0001
Method*Level	5.15	0.0006
Matrix*Level	3.11	0.0471
Recovered total Pb		
Method	12	<.0001
Matrix	943	<.0001
Level	4582	<.0001
Method*Matrix	57	<.0001
Method*Level	10	<.0001
Matrix*Level	1318	<.0001

Table 5.5: Tests of parameter estimates for significant Type III fixed effects

Solutions for fixed effects	Bioaccessible Pb %					Recovered total Pb				
	Est	SE	DE	t value	p	Est	SE	DE	t value	p
Intercept	82.7022	1.22277	13	67.64	<.0001	556.542	19.0369		29.23	<.0001
Method PBET	-24.895	1.60098	2	-15.55	<.0001	126.152	24.9251		5.06	<.0001
Method_IVBA pH_2.5	-17.945	1.60098	2	-11.21	<.0001	33.2723	24.9251		1.33	0.1837
Method_IVBA pH_1.5	0	.	2	.	.	0	.		.	.
Matrix Paint	8.75741	1.46148	1	5.99	<.0001	12.871	22.7534		0.57	0.5724
Matrix Soil	0	.	1	.	.	0	.		.	.
Level High	-0.9306	1.60098	2	-0.58	0.5618	392.229	24.9251		15.74	<.0001
Level Low	-2.7371	1.60098	2	-1.71	0.0892	-299.86	24.9251		-12.03	<.0001
Level Medium	0	.	2	.	.	0	.		.	.
Method*Matrix										
PBET Paint	-6.9321	1.60098		-4.33	<.0001	-263.82	24.9251		-10.58	<.0001
PBET Soil	0	.		.	.	0	.		.	.
IVBA pH_2.5 Paint	9.05989	1.60098		5.66	<.0001	-95.259	24.9251		-3.82	0.0002
IVBA pH_2.5 Soil	0	.		.	.	0	.		.	.
IVBA pH_1.5 Paint	0	.		.	.	0	.		.	.
IVBA pH_1.5 Soil	0	.		.	.	0	.		.	.
Method*Level										
PBET High	0.066	1.96079		0.03	0.9732	-164.3	30.5269		-5.38	<.0001
PBET Low	-5.4334	1.96079		-2.77	0.0062	0.51197	30.5269		0.02	0.9866
PBET Medium	0	.		.	.	0	.		.	.
IVBA pH_2.5 High	1.93367	1.96079		0.99	0.3255	-72.23	30.5269		-2.37	0.0191

IVBA pH_2.5 Low	-6.1722	1.96079	-3.15	0.002	22.7998	30.5269	0.75	0.4562
IVBA pH_2.5 Medium	0	.	.	.	0	.	.	.
Matrix*Level								
Paint High	3.11674	1.60098	1.95	0.0532	1155.82	24.9251	46.37	<.0001
Paint Low	3.72221	1.60098	2.32	0.0213	101.907	24.9251	4.09	<.0001
Paint Medium	0	.	.	.	0	.	.	.
Soil High	0	.	.	.	0	.	.	.
Soil Low	0	.	.	.	0	.	.	.
Soil Medium	0	.	.	.	0	.	.	.

Table 5.6: Mean pH in each method (n=10, mean ± SD) after 1-hour extraction

Sample	**IVBA 1.5pH**	**IVBA 2.5pH**	**PBET 2.5pH**
Pb Soil dust 1200	1.65±0.02	2.81±0.03	2.74±0.17
Pb Soil dust 800	1.88±0.01	2.86±0.03	2.55±0.12
Pb Soil dust 400	1.95±0.02	2.87±0.01	2.58±0.06
Pb Paint dust 1000	1.90±0.05	2.65±0.02	2.33±0.22
Pb Paint dust 700	1.90±0.08	2.70±0.03	2.38±0.17
Pb Paint dust 400	1.98±0.06	2.75±0.04	2.56±0.33

Lead soil dust BaPb percentage ranged from 41 to 63% (Figure 1) compared to the Pb paint dust which had a higher BaPb i.e., 56 to 95% (Figure 2). Post-hoc tests of LSM differences were significant for all comparisons: IVBA 1.5 pH and PBET (diff estimate = 30.15, SE =1.60, t = 37.66, p < .0001, 95% CI 28.25 to 32.04) and between IVBA pH 2.5 and PBET (diff estimate = 15.32, SE =1.60, t = 19.14, p < .0001, 95% CI 13.42 to 17.21). The LSM difference between IVBA pH 1.5 and IVBA pH 2.5 (diff estimate = 14.83, SE =1.60, t = 18.52, p < .0001, 95% CI 12.94 to 16.72) was also significant. The IVBA 1.5 pH had a substantially higher percent BaPb (87%) compared to PBET (57%) and IVBA pH 2.5 (72%), confirming IVBA 1.5 pH extract significantly more Pb due to a strong pH effect on BaPb extraction.

While percent BaPb differed significantly in Pb soil and Pb paint dust mixtures as shown in Table 4, tests of the parameter estimates (Table 5) showed how IVBA 1.5 and 2.5 did not differ significantly in their discrimination of percent BaPb, as suggested by their uniformly high estimates.

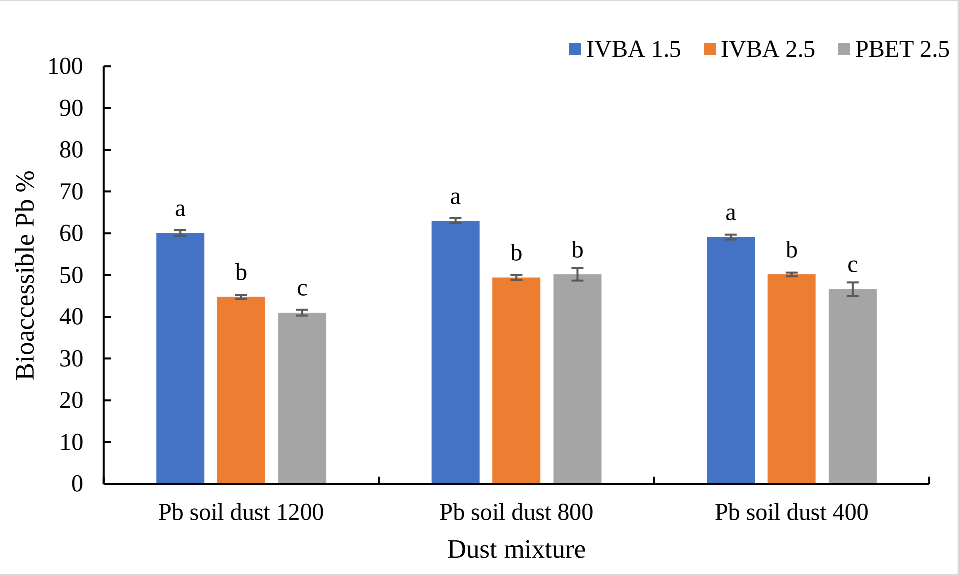

Figure 5.1: Bioaccessible lead percentage of simulated Pb contaminated soil dust in wipe based on expected total Pb (total Pb from digesting 0.2 g without dust wipe following ASTM E1644-17 method). Means with the same letter within a dust mixture not significantly different at $p < 0.05$.

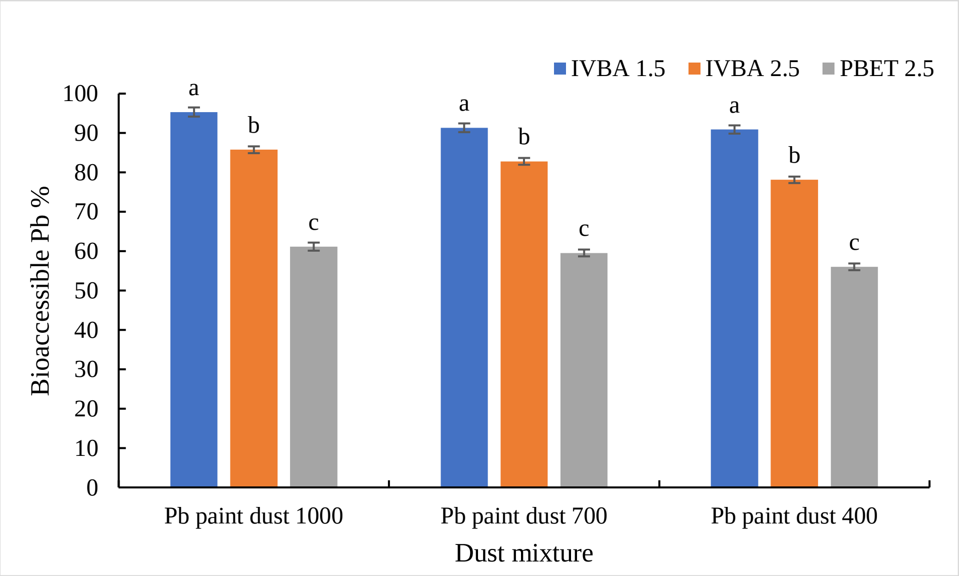

Figure 5.2: Bioaccessible lead percentage of simulated Pb paint contaimanted dust in wipe based on expected total Pb (total Pb from digesting 0.2 g without dust wipe following ASTM E1644-17 method). Means with the same letter within a dust mixture not significantly different at $p < 0.05$.

Effect of dust matrix (soil vs paint) and in vitro bioaccessible method (PBET vs IVBA)

All the Pb soil dust mixture percent BaPb estimates also differed significantly ($p < 0.05$) between IVBA pH 1.5 and IVBA pH 2.5; and IVBA pH 1.5 and PBET (Figure 1). Lead soil dust 400 and 1200 differed between IVBA pH 2.5 and PBET, while Pb soil dust 800 showed that there was no significant difference between IVBA pH 2.5 and PBET in BaPb percentage (Figure 1). Although significant, the difference between IVBA pH 2.5 and PBET for Pb soil dust 400 and 1200 was 4% and 3% compared to the larger difference (12 to 19%) observed between IVBA pH 1.5 and PBET. Lead paint dust mixtures differed significantly among IVBA pH 1.5, IVBA pH 2.5, and PBET in BaPb percentage (Figure 2). Compared to Pb soil dust mixtures, Pb paint dust mixtures had a greater difference (32 to 35%) between IVBA pH 1.5 and PBET. The difference between IVBA pH 1.5 and IVBA pH 2.5 in Pb paint dust mixtures was also larger (9 to 13%) compared to Pb soil dust mixtures. Again, the significantly greater percent BaPb in Pb soil and Pb paint dust mixtures measured with IVBA pH 1.5 indicated significantly more Pb extraction by this method.

Large differences were also observed for percent BaPb in soil versus paint materials. This was not surprising. As compared to a complex Pb chemistry and highly heterogenous soil matrix, paint materials are composed of simpler matrix and fewer (one or two) lead compounds, which could be readily available for dissolution in acidic solutions (US EPA, 2007). For this reason, Pb in paint dust has greater bioaccessibility than Pb in soil dust. A recent in vivo mouse study that fed Pb (hydroxy)carbonate in house paint delivered in either dust or soil mixtures reported up to 100% lead relative bioavailability (RBA) showing that higher solubility of this added paint even after mixing with soil or dust (Sowers et al., 2021). Showers et al, (2024) found that based on X-ray absorption spectroscopic-resolved Pb speciation and in vivo mouse Pb RBA, Pb speciation was well-related to variations in RBA results that use house dust and residential soils. Highly bioavailable Pb (hydroxy)carbonate was the major Pb species present in house dusts while Pb RBA was up to 100% and is likely driven by paint Pb. In vivo swine study using NIST paint (lead carbonate) mixed soil as feeding material found that 86, 73, 55 and 74% RBA in blood, liver, kidney and bone respectively (Casteel et al., 2006). Interestingly, in another study, settled house dust from urban Canadian homes evaluated for BaPb concentrations, higher proportions of BaPb compounds were found in pigments from older paints (Rasmussen et al., 2011).

In the current study, we found a similarity between percent BaPb irrespectively to the total Pb concentration in both dust mixtures. However, there were differences in bioaccesassible Pb as expected for the mixtures with different Pb levels. The matrix, solubility of Pb, and the in vitro extracting solution characteristics (pH, composition) determine the soluble Pb in the extraction (Zia et al., 2011). Further soil and dust characteristics, such as pH, organic matter and particle size, and concentrations of other interacting elements were significantly correlated with the Pb bioaccessibility (Mushak, 1991; Deshommes et al., 2012).

Effect of pH and composition in extracting solutions

The IVBA pH 1.5 had the lowest final pH (Table 6) in all the simulated dust mixtures ranging from 1.65 to 1.98 (± 0.02 -0.08) while IVBA 2.5 showed the highest pH ranging from 2.65 to 2.87 (± 0.01-0.04). IVBA 1.5 and 2.5 however did not deviate from their initial pH±0.5. Although the average PBET pH lies between 2.33 to 2.74 it showed the lowest percent BaPb values, and BaPb percentage were lower compared to IVBA 2.5 which had a higher pH. The main reason for this difference is that glycine buffer in IVBA pH 2.5 uses a comparatively higher amount of HCl to reach pH 2.5; in contrast, the PBET solution has no strong pH buffering capacity. Therefore, other than differences in the solution matrix, amount of HCl which controls the pH in extracting solutions is likely to have a direct effect on the solubility of the lead compound.

The broad range in percentage BaPb for all dust mixtures illustrated the strong effect of extraction pH and extraction solution composition on the *in vitro* methods indicating that the in vitro method to be used for a given practical application must be selected carefully (Scheckel et al., 2005; Scheckel et al., 2013; Kastury et al., 2019). Greater proportion of lead were extracted using methods with lower extraction solution pH, such as pH 1.5, compared with methods with higher pH, such as pH 2.5 (Brown et al., 2004; US EPA, 2019; Scheckel et al., 2013; Attanayake et al., 2014) because lower as compared to higher pH enables greater lead solubility (Hettiarachchi & Pierzynski, 2004; Li et al., 2014).

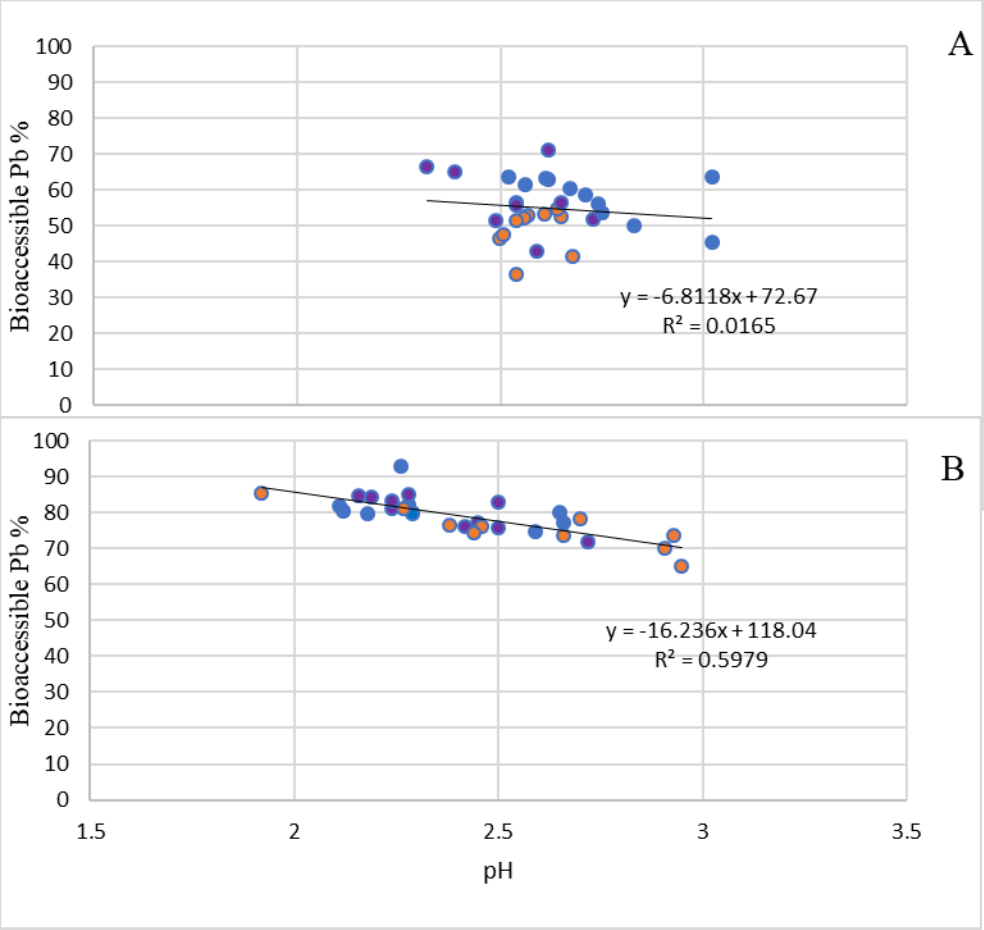

Figure 5.3: Correlations between solution pH at the end of PBET extraction and the percent BaPb A: Pb soil dust and B: Pb paint dust. Blue color circles Pb soil dust 1200 and Pb paint dust 1000, purple color circles soil dust 800 and Pb paint dust 700, orange color circles soil dust 400 and Pb paint dust 400

Overall PBET method provided significantly higher percent BaPb discrimination (lower pH and differences in solution matrix) as compared to IVBA however the PBET procedure has a few challenges. For example, monitoring and maintaining pH while performing the PBET extraction is required. The lack of pH buffering in the PBET extraction solution causes pH values to rise as soon as the dust mixture is added. The target recommended pH was 2.5 ± 0.2, but this value had to be lowered initially and then monitored carefully and adjusted as needed until the end of extraction using concentrated and /or diluted HCl. As expected, the amounts to be added varied with the dust mixture. As shown in Figure 3, the final pH range for PBET was 2.5±0.5 rather than 2.5±0.2. However, given the natural buffering capacity of the soil mixtures, pH variation was maintained at 2.5 ± 0.2 in most of the samples for PBET. Deviation from this pH range resulted in significant changes in lead bioaccessibility observed in the current study. In addition, pH changed significantly depending on the matrix and lead concentrations in the simulated dust mixtures. Controlling the pH values in the PBET method were a more challenging in the current study since unfolded dust wipe and less amount (< 20 mL) of solution mixture.

The IVBA USEPA Method 1340 uses 0.4 mol L^{-1} glycine solution adjusted to pH 1.5 using HCl. In the current study, we used this method for testing two values of pH (1.5 and 2.5) as comparison methods for PBET pH 2.5. Although the pH of 1.5 is used in the recommendations, stomach fluid pH varies from 1 to 4 depending on several factors including but not limited to inter-individual variability, health status, and feeding status of the individual (Armstrong, 2004). However, Tutuian et al, (2002) reported that day time mean pH for healthy persons in the US was 2.80±0.17 and night time was 1.38±0.07 with a 24-hour mean of 2.05±0.13. Huang et al. (2002) reported that a mean pH of 2.26 to 2.52 was a baseline for adult males used in their study in Canada. In addition, the mean (+/- SD) gastric fluid pH was 1.80 +/- 0.79 in 53% of children studied in fasting state (Crawford et al., 1990) although pediatric gastric pH is quite variable among individuals, and depends strongly on nutritional status. Both adults and pediatric gastric pH values rise to more than 4 and subsequently return to basal values within 2 hours as food ingestion and following emptied from the stomach (2 to 2.5). The pH value of 2.5 was selected for testing in the PBET to provide an intermediate pH which represents a status of intermediate between fasting and fed states (Ruby et al., 1996).

To simulate gastric digestion in BaPb extraction methods, glycine is added before the acidification of the samples to obtain a pH value which resembles the human digestive tract. Although the fasting state human gastric pH values range from 1 to 4, a pH of 1.5 was selected in USEPA method 1340 because the highest amounts of lead and arsenic are extracted at a pH of 1.5 (US EPA 2019). However, similar to our results, past studies have concluded that IVBA pH 1.5 extracts nearly 100% of total lead as “bioaccessible” due to its low pH. Obrycki et al, (2016) found that phosphate treatments where promoted Pb phosphate formation were ineffective in reducing BaPb in treated soils as measured by IVBA pH 1.5, whereas IVBA pH 2.5 showing greater treatment effects in reducing BaPb. As shown in this study, pH 1.5 IVBA appears to dissolve some hardly soluble lead-containing compounds in real gastric conditions like Pb-phosphate.

Glycine is an important constituent of metabolic activities (Razak et al., 2017) and is also used as a buffering agent. Glycine at low concentrations prevents pH decrease in solutions (Pikal-Cleland et al., 2002). When it uses a significantly lower pH value it has a higher opportunity to regulate that pH. Therefore, when pH 1.5 is used with glycine buffer IVBA rarely exceeds the initial solution pH±0.5 after one hour of extraction time as restricted by the method 9US EPA 2019). However, pH 1.5 and 2.5 with glycine were buffered the pH within this limit (±0.5) in the current study. Ruby et al, (1993) adopted PBET from Miller et al, (1981) which was originally developed for evaluating an in vitro method for estimation of iron availability from meals. Pepsin is the major constituent in PBET, the powerful enzyme in gastric juice that digests proteins within the normal gastric juice pH 1.5–2.5 when the presence of HCl. Acidification of the samples to pH 2 or 4 is important because pepsin begins to denature itself and thus will lose its activity at pH $\geq$ 5 (Britannica, 2020). In several animal feeding studies, PBET 2.5 best represented bioaccessible lead concentration as compared to PBET pH 1.5 and 2.0 (Ruby et al., 1996).

In vitro methods are continued to be validated as a tool for rapid and feasible assessment of Pb oral bioavailability. It is important to determine the most suitable method and solution pH which resembles the human gastric condition. The bioaccessible lead fraction is highly related to the total lead and its matrix (Yang & Cattle, 2015). Lead contaminated soil for this study was collected from El Paso, Texas close to the ASARCO smelting site. Therefore, the high bioaccessibility of > 55% in PBET and IVBA pH 2.5 while > 80% in IVBA pH 1.5 (Figures 1 and 2) is not surprising for soil dust mixtures. A study conducted at San Luis Potosi, Mexico with soils impacted by smelter activities found PBET measured percent BaPb range from 30 to 50 (Romero et al., 2008) and another study used a 0.011 M HCl (pH 2) solution to estimate BaPb also recorded 30 to 52% BaPb (Yang et al., 2002). Detailed analysis of dust mixtures including Pb mineralogy would also give a reasonable explanation for the observed Pb solubility. Although the detailed analysis of individual dust mixtures was not the focus of the current study, Pb soil containing dust always gave a relatively low percent BaPb compared to the Pb paint material containing dust emphasizing the importance of matrix and the forms of Pb on BaPb.

Conclusion

Measuring the bioavailability of lead in household dust samples is critical for understanding the possible risk of child lead exposure, even when the absolute Pb concentration of lead in household dust may appear to be low. This study demonstrated that the two most commonly used methods, IVBA and PBET, differ broadly in their estimation of BaPb in home dust wipe samples. Moreover, the results showed how, particularly for lead-paint contaminated household dust, the IVBA methods significantly extract more Pb as the bioavailable lead in dust wipe samples raising questions regarding their suitability for in vitro BaPb measurement. The comparability of BaPb estimates for analyses that did and did not use dust wipes suggested that using ASTM-grade dust wipes is an acceptable approach. More studies evaluating the comparability of methods are needed.

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Chapter 6 - Side by Side Comparison of Micro Vacuum Dust Collection vs Dust Wipe for Pb Determination in Household Dust Samples

Abstract

Standardized dust wipes have several limitations on application, and getting important measurements such as bioaccessible Pb in household dust samples. Therefore, the objective of this study was to develop a modified micro vacuum sampling method that is simpler, more precise and more reliable than the currently recommended dust wipe method, for household dust collection. Two different simulated dust mixtures i.e. simulated paint and soil dust with three different lead concentrations were used. Standard dust wipe method and micro vacuum method with modification (20 Lpm flow rate, inlet of the filter cassette fixed 2 cm length Nalgene Tygon® tube, 2 minutes per 30×30 cm area). Undisturbed dust collection was repeated randomly side-by-side, for both methods and each dust mixture using a two-compartment box chamber. Collected dust in micro vacuum-assisted filter cassettes and dust wipes were digested following the standard procedure. Lead concentrations 1200 and 1000 mg kg^{-1} for soil and paint dust, respectively, showed no significant difference between methods. However, at low lead concentrations, soil 800, paint 700, soil and paint 400 mg kg^{-1} dust mixtures, significantly higher lead loading (20-13%) were found in the dust wipe method compared to the micro vacuum method. This may be due to weaknesses in the dust transferring procedure followed in the micro vacuum method. The Bland and Altman plots indicate that there was a good agreement between

the dust wipe and micro vacuum method. Accordingly, there is great potential for developing a standardized micro vacuum method that is low-cost, efficient, and collects dust for detailed and critical analysis, such as bioaccessibility of Pb.

Introduction

Indoor dust is comprised of small solid particles, together with organic and inorganic inert materials, which are usually characterized by fine size fractions (< 100 μm) that settled under the influence of gravity (Turner and Ip, 2007; WHO, 1999). Research investigating human exposures to priority pollutants has suggested that settled house dust may be a significant source of indoor exposure (Maertens et al., 2004). Trace metal contaminants in indoor dust pose a significant potential exposure risk to people because of the time spent indoors and the readily ingested and inhaled fine-grained composition of indoor dust ((Doyi et al., 2020). Young children (< 5 years old) have the potential for exposure to toxic substances through non-dietary ingestion pathways other than soil ingestion. Frequent mouthing of objects or their fingers in their environment, as an exploring behavior or a habit, enable exposure to potentially toxic substances that may exceed that of other routes of direct ingestion and dermal exposure. Concern about the potential loading of toxicants, nondietary ingestion may result in higher ingestion rates of contaminated material (USEPA 2002). The health risks associated with contaminants in the indoor environment are of significant concern, and therefore, the potential hazards of indoor pollutants have continued to be widely investigated.

Despite the regulatory and practical measures implemented in all most all countries, including the United States, to eliminate lead exposure over the last 4 decades, one in two children living in the US has detectable levels of lead in their blood (Hauptman et al., 2021). One in three children globally is reported to have blood lead levels at or above 5 micrograms per deciliter (µg dL^{-1}) (Rees and Fuller 2020). Traces of legacy leaded paints, leaded gasoline, and active or inactive lead-based industrial sites located near residential sites were found to be responsible for the high accumulation of house dust contaminated with lead.

Dust collection is extremely important in tracing the source and application of possible mitigation measures. Dust sampling methods, protocols, and analyses should be capable of collecting and measuring the lead concentration on various surfaces with proven accuracy and precision. The standardized wet wipe methods typically utilize a premoistened towelette or paper wipe to collect a sample within a sampling area using a specific wipe pattern or a number of passes. In addition, a variety of vacuum devices are available that range from commercial vacuums to hand-held personal air sampling pumps with controlled flow rates and sampling areas of interest for dust collection (US EPA, 2000).

However, wipe methods for sampling lead in house dust do not collect lead concentration or dust loading data. Dust loading is the amount of dust per unit area expressed as micrograms per square meter (µg m^{-2}) or micrograms per square foot (µg ft^{-2}) whereas lead concentration is the mass concentration of lead per mass of dust, typically reported as micrograms per gram (µg g^{-1} or ppm). The integrated exposure uptake biokinetic (IEUBK) model, an exposure (dose)-response model that uses children's environmental lead exposures to estimate risk of elevated blood lead, uses lead concentration as the metric to represent the extent and magnitude of lead in residential dust at a site. Dust wipe methods are routinely used by the United States Department of Housing and Urban Development (HUD) and collect data that are informative for HUD, especially lead-based paint risk assessment and clearance programs but, are not recommended for Superfund lead risk assessments that utilize the IEUBK model (USEPA, 2008). The IEUBK model identified high-volume cyclonic vacuums with some modifications. High-volume cyclonic vacuums are able to produce Pb concentration data best fit with the model application.

Dust wipes have several limitations when considering the amount of dust collected, the area sampled and the measurement is restricted to lead loading. In addition, a bioavailable fraction of collected dust samples cannot be measured with the dust wipe samples. However, dust wipe is important in identifying the specific lead-contaminated places/ areas in the household environment, which is important in the application of mitigation measures.

Vacuum methods allow for determining dust lead concentration, lead loading, and dust loading. In addition, vacuum methods are typically capable of collecting masses adequate to use for prescribed analytical methods and are generally useful for a wider variety of surfaces (smooth/ hard or rough/ porous: carpets, concrete, apparel, tile, steel, wood, vinyl, etc.) than the wipe method. The accuracy and efficiency of the different vacuum samplers were studied by various researchers (Lanphear et al., 1995, Sterling et al., 1999, US EPA, 1995a). Based on these studies comparing collection efficiency and precision, the high-volume surface sampler (HVS) and the high volume cyclone vacuum methods had a higher collection efficiency and precision than the low flow vacuum methods (such as dust vacuum method: DVM; micro vacuum method: MVM). High-volume cyclonic vacuums are recommended in the IEUBK model because these studies indicated that they meet the several recommended criteria desirable for lead risk assessment specified in the model. However, the typical DVM sampler used in previous studies mentioned consists of a common personal air-monitoring pump, with a 2.0 to 3.0 liters per minute (Lpm) flow rate, and is connected to a three-piece air-monitoring cassette containing a 0.8 µm polycellulose acetate filter. They are easier to use than high-volume samplers.

Objectives

Due to observed poor performance in early studies, the micro vacuum sampling method is rarely used. An improved micro vacuum sampling method would be an alternative method that can be used efficiently and precisely while getting valuable information that the wipe method could not offer. Therefore, the objective of this study was to develop a modified method for household dust collection that is simpler, more precise and more reliable than the currently recommended dust wipe sample method. This modified method will allow for collecting dust lead concentration and dust loading in addition to lead loading. It will also facilitate collecting adequate quantities of dust for other critical measurements, such as bioaccessible lead.

Materials and Methods

Simulated house dust preparation

A two compartment plexiglass chamber was used in this study to prevent dust loss. Simulated house dust was prepared using two lead sources, mixing with real interior house dust sample which was collected from El Paso, Texas. Simulated soil dust was created by mixing uncontaminated house dust with contaminated surface soil (4000 mg kg^{-1}, collected from El Paso, Texas close to the ASARCO smelting site) and uncontaminated surface soil (collected from the same area), to get dust mixtures with lead concentrations of 1200, 800 and 400 mg kg^{-1} while maintaining the dust to soil ratio at 1:2. Simulated paint dust was created by mixing uncontaminated house dust with certified paint materials (Powdered paint certified material ~2100 mg kg^{-1}, Sigma-Aldrich, St. Louis, MO, USA) in the ratio of 1:2, 1:3 and 1.4 paint and dust resulting dust lead concentration 1000, 700 and 400 mg kg^{-1}. Detail description of dust mixture creation has mentioned in Wekumbura *et.al,* (2022).

Dust collection

The two methods used for dust collection side-by-side were:

Wipe method: The procedure given by ASTM E 1728: "Standard Practice for Field Collection of Settled Dust Samples Using Wipe Sampling Methods for Subsequent Lead Determination" (*http://www.astm.org/Standards/E1728.htm*) was followed. Briefly, first wipe the marked sampling area side-to-side in an S-like motion, with firm pressure. Then fold the wipe in half with the contaminated side facing inward. Following the same procedure make the second wipe pass from top to bottom and fold the wipe again in half with the contaminated side facing inward. Make the third wipe pass around the perimeter of the sampled area then fold it with the contaminated side facing inward.

Micro-vacuum method: Rotaryvane sampling vacuum pump (2 to 40 liters per minute (Lpm)) (Jarless)/ P/N's 9801 & 9801-88) was adjusted to the flow rate of 20 Lpm with the aid of a rotameter. The filter cassette containing a 0.8 µm polycellulose acetate filter outlet was connected via Nalgene Tygon® tubes to the pump. The Inlet of the filter cassette was connected to the Tygon® tubing (2 cm length) cut at a 45-degree angle in the open end. After a few trials, the time needed to complete the 30 x 30 cm^2 for dust collection was confirmed to 2 minutes. The 45-degree cut end of the Nalgene Tygon® tube was placed very close to the floor at one corner of the area to be sampled and the tube was moved side to side and up and down (side-to-side and top to bottom with as many “S”-like motions as are necessary to completely cover the entire sample area) within 2 minutes period. Filter cassette weight was recorded before and after dust collection.

A box chamber (44 cm width x 72 cm length x 48 cm height) with two separate compartments was used for undisturbed dust collection. About 0.2 g of dust samples were spread evenly in both compartments on 30 cm^2 areas of the plexiglass chamber floor. Randomly, on one of the sides, dust collection using wipe method, was performed while on the other side the micro vacuum method was used. Dust collection was repeated 25 times, side-by-side, randomly for both methods and each dust mixture.

Total lead determination

Collected dust in filter cassette and dust wipe were digested following the ASTM E1644-17. The whole dust wipe was placed in a 250 mL beaker and 25 mL of 1:1 trace metal grade concentrated nitric acid (HNO_3) acid and Milli-Q H_2O were added. To extract all the dust on the filter cassettes, cassettes were first washed with 1:3 HNO_3: water into a 250 mL beaker and additional conc. HNO_3 was added to get 25 mL volume 1:1 HNO_3 and water. The beakers were covered with a watch glass and were placed on the hot plate to start digestion about 15 minutes under 85 to 100°C temperature. This was followed by addition of 10 mL of concentrated HNO_3, and reflux for 30 min without boiling. Approximately 10 mL of the evaporated sample was added with 5 mL of water and 5 mL of 30% hydrogen peroxide (H_2O_2). Further digestion was allowed with evaporation to reach 10 mL. Digested samples were filtered using Whatman No. 42 filter paper and analyzed for lead using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Varian 720-ES, Santa Clara, CA, USA).

A standard reference soil (NIST 2711a-Montana II) was digested along with every batch of dust samples and blank wipes, blank filter cassette and reagent blanks were included as a quality assurance/quality control (QA/QC) sample to evaluate digestion and analytical procedures. Triplicate analysis, spiked sample recoveries, and check values were included during the analysis of Pb concentration in dust mixture digests.

To investigate whether improvements to the dust recovery from the filter cassettes can be made, a complementary study was conducted using three samples from each dust mixtures (800, 700 and 400 mg kg^{-1}). For that, after cassettes were first washed with 1:3 HNO_3: water into a 250 mL beaker, they were then placed in the same beaker, for an additional step, sonification. The sonication was performed by an ultrasonic cleaner (FS60, Pittsburgh, PA, USA) for the above mentioned dust mixtures (n=3) for 10 minutes period without applying any heat. Then followed the same digestion and analysis procedure explained in section 2.3 for total lead determination.

Lead (Pb) loading was calculated as follows.

$$Pb\ loading\ \frac{\mu g}{30cm^2} = (ICP - OES\ reading\ \frac{mg}{L} \times 1000\ \frac{\mu g}{mg} \times 0.1\ L\ (solution\ volume)$$

$$Pb\ loading\ \frac{\mu g}{ft^2} = Pb\ loading \frac{\mu g}{30cm^2} \times 1.032256\ \frac{30cm^2}{ft^2}$$

Statistical analysis

All analyses were conducted using SAS 9.4 software (2016 by SAS Institute Inc., Cary, NC, USA). Descriptive analyses were conducted to determine the means and distributions of the dust wipe and micro vacuum variables in different dust mixtures. Comparisons by method (3), matrix (2), and lead concentrations (3) were tested with 3 × 2 × 3 ANOVAs ($p < 0.05$) with post-hoc comparison of means for total Pb loading. When the fixed effect F-value was statistically significant, relevant post-hoc tests of least square means (LSM) (for categorical effects) were evaluated. For main effects, post-hoc comparisons of LSM differences were calculated using the Tukey–Kramer adjustment for multiple comparisons.

Bland–Altman analysis was designed to assess the comparability between methods rather than their differences or linear relationship. A Bland Altman plot showing the difference between the two paired measurements (y-axis) was plotted against the mean of the two measurements (x-axis) to determine the degree of agreement between the two methods. Mean comparisons were done using the independent sample t-test ($p < 0.05$) to test the statistically significant differences between the dust wipe and micro vacuum method with sonification.

Results and Discussion

The descriptive statistics for Pb loading in dust wipe and micro vacuum method in each dust mixture are shown in Table 6.1. The skewness metric showed that the data from each method were negatively skewed in dust mixtures with soil dust 1200 and paint dust 1000 mg kg^{-1} lead concentrations whereas positively skewed in dust mixtures with soil dust 800, paint 700, soil and paint dust 400 mg kg^{-1} lead concentration dust mixtures. The skewed distributions were less than one in most of the samples which showed that medians were very close to the means for both dust wipe and micro vacuum measurement. Also, there was less variability in lead loading within a method.

Type III fixed effects and tests of parameter estimates for the main effects of the method (wipes and micro-vacuum), matrix (soil and dust), lead concentrations (high:1000-1200, medium: 700-800, low:400 mg kg^{-1}), and the interactions of method × matrix, method × level, and matrix × level. Significant effects of all these parameters were found for Pb loading except interactions of method × matrix. Both soil and paint dust mixtures with lead concentrations 1200 and 1000 mg kg^{-1} for soil and paint dust respectively, showed that there is no significant difference (least squares means for effect method × concentration) in lead loading when compared to dust wipes and micro vacuum (Figures 6.1 and 6.2). However, with lead concentrations of soil 800, paint 700, soil and paint 400 mg kg^{-1} dust mixtures significantly higher lead loading were found in the dust wipe method compared to the micro vacuum method. In addition, these soil and paint (800, 700 and 400 mg kg^{-1}) dust mixtures gave higher dust wipe Pb loading in mean, median, minimum, maximum, and percentile compared to the micro vacuum (Table 6.1).

Bland and Altman quantified the difference between measurements using a graphical method. The scatterplot (Figure 6.3) in which the X-axis represented the average [(dust wipe + micro vacuum)/2], and the Y-axis represented the difference (dust wipe - micro vacuum) of two measurements. The data points were limited in upper and lower limits using a 95% confidence interval (CI: mean ± 1.96 standard deviations) of distributed data. The scatterplot can be evaluated according to the scatter dispersion. In a good agreement, the scattering of points is diminished, and points lie relatively close to the line which represents mean bias. The lead loading measured by dust wipe were 51, 7.5, 8.4 units below or 48, 50, 27 above micro vacuum in soil dust 1200, 800, 400 respectively (Figure 6.3 a, b, c) while 26, 11, 23 units below or 37, 35, 3 above in paint dust 1000, 700, 400 respectively (Figure 6.3 d, e, f). The Bland and Altman plots in the current study indicate that there was a good agreement between dust wipe and micro vacuum method. However, the scatter plot does not say if the agreement is sufficient or suitable to use one method or the other indifferently.

Table 6.1: Descriptive statistics for lead loading (µg/ ft2) in dust wipe samples and micro vacuum, N=25

		Soil dust 1200		**Soil dust 800**		**Soil dust 400**		**Paint dust 1000**		**Paint dust 700**		**Paint dust 400**	
		Dust wipe	Micro Vacuum	Dust wipe	Micro Vacuum	Dust wipe	Micro Vacuum	Dust wipe	Micro Vacuum	Dust wipe	Micro Vacuum	Dust wipe	Micro Vacuum
Mean		197	199	141	113	67	58	167	162	127	115	82	72
Std. Error of Mean		3.6	2.59	1.46	1.75	1.70	0.90	3.60	1.24	1.82	2.09	0.80	0.97
Median		195	199	141	111	67	56	164	162	127	113	82	71
Std. Deviation		18.1	12.9	7.2	8.6	8.5	4.5	16.9	5.8	9.3	10.7	4.0	4.9
Variance		327	167	51	73	72	20	286	34	86	114	16	24
Skewness		-0.90	-0.08	0.69	0.70	0.08	1.52	-0.25	-0.13	0.40	3.64	0.17	0.16
Range		86	58	25	33	26	17	86	23	44	55	17	20
Minimum		141	170	132	100	55	52	121	151	109	106	74	61
Maximum		210	211	149	119	76	59	184	167	134	117	85	76
Percentiles	25	188	193	136	107	75	56	158	159	122	110	80	68
	50	195	199	146	111	67	56	164	162	127	113	82	71

	75	208	209	146	118	59	58	177	167	133	116	85	74

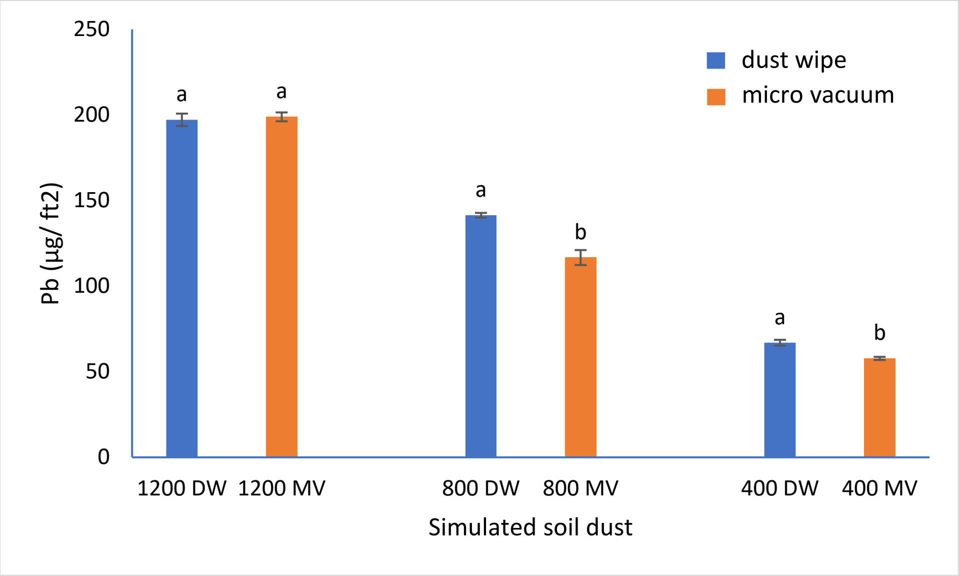

Figure 6.1: Lead loading (µg ft^{-2}) of simulated soil dust concentration 1200, 800 and 400 mg kg^{-1} (DW: Dust wipe; MV: Micro vacuum). Different letters indicate significant difference between methods within the same lead concentration category at 0.05 probability level.

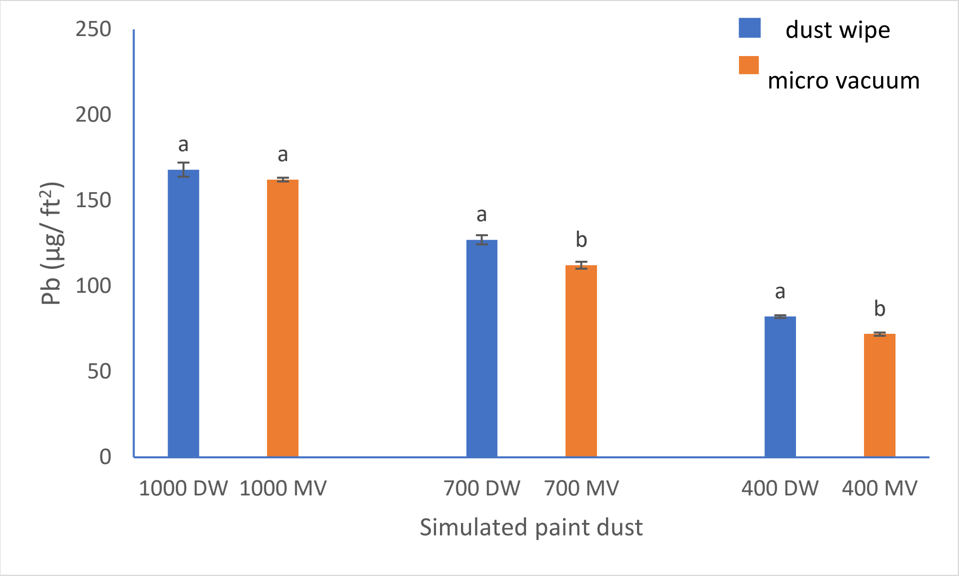

Figure 6.2: Lead loading (µg/ ft2) of simulated soil dust concentration 2000, 1000, 700, and 400 mg kg-1 (DW: Dust wipe; MV: Micro vacuum). Different letters indicate significant difference between methods within the same lead concentration category at 0.05.

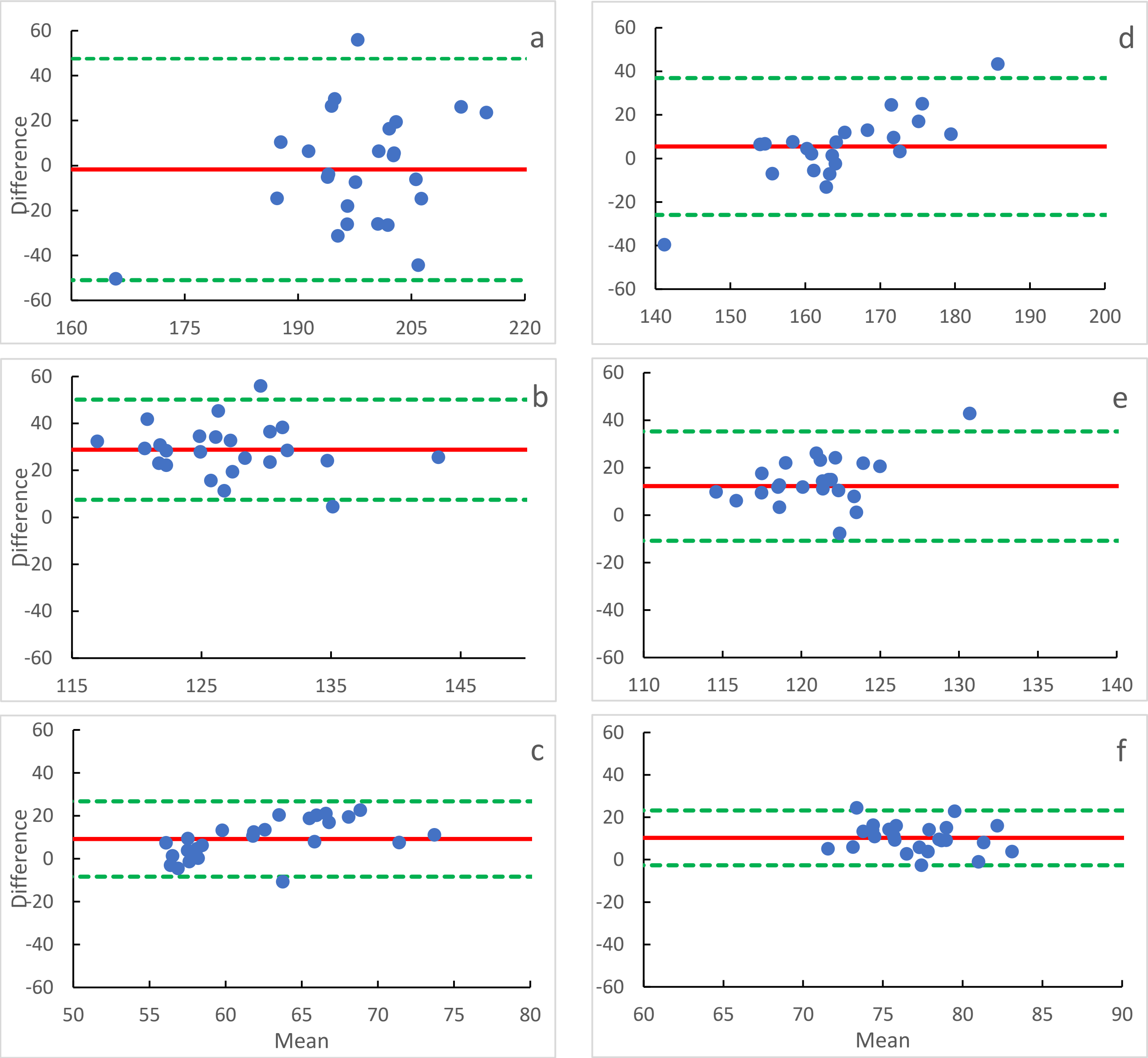

Figure 6.3: Bland–Altman plot a, b, c: soil dust 1200, 800, 400; d, e, f: paint dust 1000, 700, 400 mg kg-1 showing the agreement between dust wipe and micro vacuum for analyzing dust samples for lead loading (Difference: dust wipe – micro vacuum; Mean (dust wipe – micro vacuum)/2). The straight horizontal line represented the average difference in measurements between the two methods (mean bias). The dotted horizontal lines represented upper and lower limit of the 95% confidence interval for the average difference.

The collection efficiency of micro vacuum was 99 to 100% in all dust mixtures based on weight differences of empty and dust-collected filter cassettes, while dust wipes collection efficiency ranged from 90 to 99%. Therefore, both methods showed good dust collection efficiencies. Compared to the current study, studies that compare side by side dust wipes and micro vacuum found that dust wipes are a significantly more efficient method of sampling than micro vacuum techniques (Farfel et al 1993, Lopez 2016). However, micro vacuum sampling they used 2-2.5 Lpm flow rate in the vacuum pump (i.e., lower than the current study).

Two different dust matrices (soil and paint) with three different Pb concentrations were evaluated in the current study to identify an alternative method for collection and analysis of household dust as a better predictor of source/ point of Pb contamination with other critical information, such as total Pb concentration, bioaccessible Pb fraction and dust mass. Although the dust collection efficiency of micro vacuum showed a high efficiency (99 to 100%), except for the 1000 and 1200 mg kg^{-1} Pb concentration dust mixtures other dust mixtures (800, 700, 400 mg kg^{-1}) showed significantly lower Pb loading from the micro vacuum method compared to the dust wipe method. In the current study, we used ASTM E1644-17 method to digest dust in both filter cassette, dust wipe, and control samples. The whole dust wipe was placed in the 250 ml beaker and added the 1:1 HNO_3 and water and then followed the rest of the procedure. Therefore, we could expect no or minimal loss of dust as a consequence of nearly 100% recovery.

However, transferring the dust collected into a filter cassette through a micro vacuum for analysis was not direct. First, visible dust was carefully transferred into the beaker, and then 20 ml of 1:3 HNO_3 and water mixture was used to wash out dust that was still retained/adhered to the filter cassette in to the beaker. Significantly reduced Pb recovery in the low Pb concentration

(800, 700, 400 mg kg^{-1}) dust mixtures can be due to greater effect of losing a small fraction of dust compared to the samples with high Pb concentration (1000 and 1200 mg kg^{-1}). The poor dust transfer efficiency for digestion might be the reason for low Pb loading resulting in micro vacuum-assisted medium and low Pb concentrated samples observed in the current study. ASTM E1644-17 method was originally recommended for the digestion of lead in dust wipe samples collected during various lead hazard activities. We tried to follow the same procedure with the digestion of dust collected into the filter cassette and further modifications to this method will be needed to ensure complete recovery of the collected dust in filter cassettes.

Effect of ultrasonic cleaning for dust recovery: Due to observed significant differences between the two methods at all low Pb concentrations (800, 700, 400 mg kg^{-1}), a complementary study with a sonification step for cleaning the cassettes was conducted to improve dust recovery. Both soil 800 and 400 mg kg^{-1} dust mixtures showed greater recovery after ultrasonic cleaning and values were not significantly different ($p < 0.05$) from the dust wipe method. However, both 700 and 400 mg kg^{-1} paint dust mixtures still showed significantly lower Pb recoveries compared to the dust wipe method. These results suggest that paint materials might need a more aggressive extraction procedure to recover all dust (paint dust) from collection cassettes, as dust surface dust collection proved to be effective (99 to 100% collection efficiency). Inclusion of ultrasonic cleaning as a sample extraction technique could enhance the dust recovery from the internal cassette wall for some household dust as shown for the soil dust mixtures. The national institute for occupational safety and health (NIOSH) discussed the inclusion of materials adhering to internal cassette surfaces during sampling and analysis of aerosol samples. The specified procedures facilitate the inclusion of all particles in the sampler as part of the sample whether they deposit on the filter or on the inside surfaces of the sampler (Asley et. al., 2001; Ashley and

Harper, 2014). Similarly, it is important to extract all dust materials from the filter cassettes.

There were only a few studies that compared dust wipes and micro vacuums for the collection of house dust in the literature and they reported that micro vacuums were inferior in dust collection and subsequent analysis of lead and other heavy metals (Farfel et. al., 1994; Lanphear et. al., 1995; Lopez, 2016; Reynolds et. al., 1997; USEPA, 1995a; USEPA, 1995b). Farfel et al. (1994) conducted a field study comparing side by side wipe and micro vacuum method. They found that wipe lead loading was greater than 3.9 and 5.7 times for uncarpeted floors and window sills, respectively, compared to the micro vacuum method. Two USEPA studies (USEPA, 1995a and USEPA, 1995b) compared side by side wipe and vacuum dust samples including a micro-vacuum. One of the studies was conducted in the laboratory and the other in a real household environment. Interestingly, both studies and a study conducted by Lanphear et al. (1995) found that wipe lead loadings were 5 to 10 times higher than the micro vacuum method depending on the sampling surface, i.e. floor vs window stool. USEPA, 1995b reported that the average dust recovery was 30% (14 to 47%) while dust recovery declined as the dust particle size increased when the micro vacuum method was used. The average Pb recovery was 26% for the micro vacuum method compared to 63% for the wipe method in that study. Reynolds et. al., 1997 reported that on the carpet surfaces, the vacuum method had a higher recovery, but the recoveries were < 50% while the wipe sampling methods yielded significantly higher recoveries (100%) on smooth, hard surfaces and produced more accurate results.

Lopez (2016) performed micro-vacuum and surface wet wipe sampling techniques side-by-side and found that the micro-vacuum and wet wipe techniques were statistically different and wet wipe technique picked up more metals from the surface than the micro-vacuum technique. They further explained that surface dust characteristics such as layers of grime (thickness) or oil that the micro-vacuum technique had difficulty penetrating could be one of the reasons for the wet wipe technique pick up more metals in their study. This study emphasized the importance of evaluation of surface characteristics (porosity and texture) of the sampling area and the surface dust characteristics (thickness, oiliness) before selecting a proper surface sampling technique. Ashley et al. (2007) evaluated the standardized micro-vacuum method (ASTM D7144 procedure) for collecting surface dust found significantly less Pb recoveries ranging from a low (20%) for industrial carpet and apparent high (85%) for concrete floor. In this study, they found that part of the dust (about 40%) was retained on the collection nozzle. This might be due to low flow rate used in the study which is not capable of getting the dust into the filter cassettes. They have suggested that use of high-volume sampling pumps affording sampling flow rates of >10 Lpm could improve micro-vacuum sampling efficiencies. In the current study, we did not observe retention of dust on the collection nozzle.

Previous studies mentioned above were conducted using micro vacuum flow rates of 2 to 2.5 Lpm, which has been standardized as the ASTM D7144 procedure (ASTM, 2011). The ASTM D7144 procedure uses a standard 37 mm three-piece filter cassette with a filter and backup pad. The cassette inlet is connected to a flexible tube extension, with the tube cut at a 45° angle. A sampling pump calibrated to 2.5 Lpm is used as the suction source and the sample collection time is 1 min per 100 cm^2 area.

Creek et al. (2006) discussed various vacuum sampling techniques and their potential uses, the importance of harmonization as well as modification of sampling methods appropriately to meet the requirements and study objectives and further recommendations. In the current study, we modified the sampling time, area, and more importantly suction flow rate into a 20 Lpm that was 10 times higher than the ASTM D7144 recommended value. Our results showed that with the current method, we could achieve more than 90% dust recovery on smooth hard surfaces using the micro vacuum method. Statistically significant differences were only observed in 800, 700 and 400 mg kg^{-1} lead concentration samples in the wipe and micro vacuum method in this study. We believe that difference is more related to the poor dust transfer procedure used in the micro vacuum method. Compared to the wipe method Pb loading was being, 20 and 13% less for soil dust 800 and 400 mg kg^{-1} and 10 and 13% less for paint dust at 700 and 400 mg kg^{-1} for the micro vacuum method. This might be due to inability to recover small fraction of the collected dust that greatly affects the low and medium lead concentration (800,700, 400 mg kg^{-1}) dust compared to high lead concentration (1200, 100 mg kg^{-1}) dust, i.e., little loss of dust that was not affected by Pb loading values and produces statistically nonsignificant results. We found that micro vacuum is an acceptable alternative method for dust collection with the modified higher flow rate and time. However, the procedure we used for extracting dust from filter cassettes needs to be improved.

Conclusions

There are numerous dust sampling methods and vacuum methods for surface dust sampling. The wet wipe has been used in many HUD studies. Although, they are more effective on smooth surfaces, some other surfaces such a porous and uneven rough surfaces and occasions such as require details on lead concentration, dust mass and bioaccessibility limit their use. There is a great potential for developing a standardized micro vacuum method that is low-cost, and easy-to-practice. It offers additional benefits such as being used for smaller scale and or specific areas, and collected dust also can be used in the different analyses (particle size, bioaccessible Pb) and measure lead concentration rather than Pb loading. The current study we used a smooth hard surface for evaluating modified micro vacuum efficiency. Therefore, we are suggesting further studies to confirm the flow rate and time needed for different floor or other surface types.

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Chapter 7 - Overall Summary and Conclusions

The increasing exposure of children to lead (Pb) contaminated soil and dust continues to be a public health issue. The health hazard posed by Pb highlights the need to explore low-cost and feasible soil remediation methods to address widespread soil Pb contamination. These methods must demonstrate effectiveness across various soil and climatic conditions, a wide range of soil Pb concentrations and sources, and in the presence of co-contaminants, while ensuring long-term stability. Soil amendments have been identified as viable and cost-effective techniques for on-site modification of the soil Pb bioavailability. This modification aims to alter the chemical form of Pb, thereby reducing the bioaccessible Pb fraction in the soil.

This dissertation consists of seven chapters. Chapter 1 provides an overview of the research topics, while Chapter 2 presents a comprehensive review of the literature. Subsequent chapters dedicated into the experimental work, results, and conclusions. The final chapter offers a summary of the main findings and proposes avenues for future research.

The initial two studies evaluated the efficacy of soil amendments, either containing P alone or a combination of P and Fe, in addition to organic or inorganic compounds, aimed at reducing Pb bioaccessibility in soil. However, assessing the effectiveness of these amendments is complex due to the spatial and temporal variability of soil properties and biogeochemical reactions. The aim of the first study was to understand the changes in Pb speciation within slightly alkaline soil located in close proximity to a smelter. This soil exhibited considerably high total Pb concentration (> 3000 mg kg^{-1}) and was accompanied by co-contaminants such as As, Cd, Zn, and Cu. The study investigated the effects of applying both inorganic and organic soil amendments on altering the speciation of Pb in this environment. The second study was designed to investigate changes in Pb speciation in urban residential soil, which is categorized as alkaline and exhibits mild to moderate Pb contamination. This soil type typically contains Pb from various sources associated with urban residential activities.

Chapter 3 describes the use of class A biosolids, compost, TSP and MAP for reducing in vitro bioaccessibilty. Sequential chemical extraction, synchrotron-based X-ray analysis to explore the correlation between the formation of Pb species and their impact on Pb bioaccessibility in alkaline smelter-impacted soil co-contaminated with As, Cd and other potentially toxic elements. Biosolids, TSP and MAP resulted in a significant (~40%) reduction in bioaccessible Pb together with bioaccessibility of As and Cd. This reduction was strongly correlated (negatively) with an increase in the residual Pb fraction, particularly evident in the biosolids treatment. Moreover, a greater proportion of exchangeable, carbonate and Fe-Mn bound Pb transformed into a residual or stable fraction of Pb in inorganic P (TSP, MAP) and biosolids treatments. Synchrotron X-ray based analysis revealed formation of pyromorphite, Fe-ferrihydrite sorbed, and organic-bound Pb species was associated with reduced bioaccessible Pb in TSP, MAP and biosolids treatments.

Chapter 4 explored the reduction of bioaccessibility of Pb in mildly to moderately Pb-contaminated residential soils (~150-750 mg kg-1) utilizing the same amendments. The findings of this study revealed that biosolids, TSP, and MAP exhibited reductions in bioaccessible Pb and facilitated the formation of stable Pb compounds. This transformation involved converting more soluble Pb fractions, such as carbonate and organic-associated Pb, into stable or residual Pb fractions, including Fe-bound Pb and pyromorphite, across three different mildly to moderately Pb-contaminated residential soils. In addition, over time, all treatments, including the control, showed gradual decreases in pH and the amount of bioaccessible Pb in all soils. Maintaining soil moisture effectively reduced bioaccessible Pb levels in the control soil, likely facilitating the natural attenuation processes to occur in soil. Control and compost-amended soils exhibited similar levels of bioaccessible Pb and speciation profiles. This study also highlighs the significance of soil moisture in transforming soluble Pb into stable compounds, particularly in dry and arid climates where original forms of Pb are preserved. Future research should be conducted to verify these results under field conditions, with different soil, environmental and climatic conditions.

Bioaccessibility of Pb-contaminated household dust is equally important as soil and yet few studies have evaluated the bioaccessible Pb fraction of dusts. Standard dust collection procedures, such as dust wipes, are limited in their ability to assess bioaccessible Pb, thus prompting the need for alternative methods. Therefore, one of our subsequent studies compared two in vitro methods for estimating the proportion of bioaccessible Pb in dust collected using dust wipes.

Chapter 5 describes the two types of simulated household dust samples (Pb-soil contaminated and Pb-paint contaminated), each with three different Pb concentrations, underwent evaluation using the US-EPA’s in-vitro bioaccessible assay (IVBA) method at two pH values (1.5 and 2.5), as well as the physiologically based extraction test (PBET) at pH 2.5. Dust samples were collected following the standard EPA dust wipe protocol and analyzed for bioaccessible Pb and total Pb. The estimated bioaccessible Pb levels varied significantly depending on the extraction method and the source of Pb. Specifically, the mean percent bioaccessible Pb was highest with the IVBA method at pH 1.5, followed by the IVBA method at pH 2.5, with the PBET method at pH 2.5 showing the lowest values. Furthermore, lead-paint-contaminated dust exhibited higher bioaccessible Pb values compared to Pb-soil dust. Notably, the PBET method demonstrated greater discrimination, as indicated by a wider range of bioaccessible Pb values and closer alignment with total lead concentrations in simulated household dust samples, particularly for lead-paint-contaminated dust.

Chapter 6 details in parallel, as we aimed to develop a modified micro vacuum sampling method for household dust collection that is simpler, more precise, and reliable, particularly for collecting dust in specific areas of interest and generating detailed measurements, including bioaccessibility. Both micro vacuum sampling and dust wipe methods demonstrated 90-100% collection efficiency, with no significant differences observed at high lead concentrations (> 1000 mg kg-1) for simulated paint and soil dust used in total Pb measurements. However, at lower lead concentrations (800-400 mg kg-1) in dust mixtures, significantly higher lead loading (20-13%) was found in the dust wipe method compared to the micro vacuum method, potentially due to limitations in the dust-transferring procedure in the micro vacuum method. The application of sonication for dust extraction from filter cassettes in the micro vacuum method improved recovery simulated soil dust. The micro vacuum method represents an acceptable alternative for dust collection, provided modifications such as increased flow rate (20 Lpm) and duration (2 minutes per 30×30 cm area) are implemented. However, improvements in the dust extraction procedure from filter cassettes are warranted. Future research should be conducted to verify these results with real dust samples collected, with different surfaces and floor types.