

Geochemical mechanisms of lead stabilization in phosphate-amended urban soils
and prediction of physicochemical properties using proximal soil sensors

by

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Abstract

Soil lead (Pb) is one of the most common environmental contaminants in urban areas and remains a persistent global public health concern. Although Pb occurs naturally in soils at average concentrations of approximately 10 mg kg^{-1} , levels in contaminated urban and residential areas frequently exceed 200 mg kg^{-1} , which is the current residential soil limit set by the U.S. Environmental Protection Agency (EPA) to protect human health. However, total soil Pb concentration alone does not adequately represent contamination risk, as soils with similar total Pb concentrations may differ in chemical species that control Pb bioaccessibility. Phosphorus (P) based amendments are widely used to immobilize soil Pb by promoting the formation of relatively stable Pb-phosphate minerals. This process reduces Pb bioaccessibility. Biochar has also gained attention as a complementary remediation material due to its high porosity, large surface area, and ability to modify soil physicochemical properties, which supports this role. However, uncertainties remain regarding the consistency of its stabilization mechanisms and long-term effectiveness under field conditions.

The first phase of this doctoral research aimed to elucidate the mechanisms by which organic and inorganic P amendments and biochar reduce Pb bioaccessibility in urban soils. Nine urban soils from Kansas City, Missouri, representing a wide range of Pb contamination levels, were evaluated through 12-month laboratory incubation studies. Four of these studies were followed up by field validation of selected treatments in 1-year-long field experiments. The assessment integrated conventional wet-chemistry analyses with advanced spectroscopic techniques. Bulk X-ray absorption near-edge structure (XANES) was employed for multiple sites for overall changes in soil Pb speciation. In one study, μ -X-ray fluorescence (μ -XRF) mapping was used to perform spatially resolved Pb speciation using μ -XANES to better understand Pb

transformation pathways. The results from laboratory incubation and field experiments indicated that bioaccessible Pb was primarily influenced by soil pH and extractable P. Additionally, the P amendments significantly reduced Pb bioaccessibility, from 16% to 36%, and promoted redistribution of Pb into more stable soil fractions. Biochar also reduced desorbed Pb up to 1.67-fold relative to the unamended control. Comparative evaluation showed contrasting immobilization mechanisms among amendments. Mechanisms involving inorganic P treatments were dominated by the formation of pyromorphite-like species, up to 34% according to bulk XANES, while μ -XANES identified localized spots with high predominance (> 80%). In contrast, biochar, biochar modified with inorganic P treatment, and organic P treatments primarily immobilized Pb through complexation to organic matter and sorption to Fe-associated phases. These findings demonstrate that inorganic phosphates remain the most effective strategy for promoting the formation of relatively stable Pb minerals with low bioaccessibility. Organic P treatments increased acid phosphatase activity during the early sampling period (1-6 months), whereas inorganic sources generally did not differ from the control. In contrast, β -glucosidase and arylsulfatase had limited sensitivity to the amendments. Enzyme activities appeared to be strongly governed by soil physicochemical properties (pH, TOC, and resin-P) rather than total Pb concentration. These findings indicate that Pb stabilization with P amendments does not necessarily result in uniform recovery of soil biological activity.

The second phase of this doctoral research evaluated proximal soil sensing technologies as rapid tools for soil characterization. Although traditional wet-chemistry methods provide accurate measurements, they are limited by low sampling density and high spatial variability, which are typical of agricultural fields. Precision agriculture increasingly relies on proximal soil sensors to generate high-resolution datasets on soil chemical and physical properties that support

site-specific management. This study evaluated relationships between multiple soil sensor outputs and selected physicochemical soil properties at two depths and assessed how these influence sensor signal intensity and predictive performance across two contrasting agricultural fields in the U.S. Midwest using the Veris P4000 soil sensor. In this study several soil properties can be estimated with high accuracy when appropriate preprocessing and modeling approaches are applied. For instance, the NIR and VIS sensors had the strongest predictive performance for soil organic carbon and exchangeable phosphorus ($R^2 > 0.7$), whereas EC_a sensor was mainly associated with soil pH, exchangeable cations, and clay content ($R^2 > 0.50$). Sensor performance was strongly site-dependent, underscoring the need for site-specific calibration and prior knowledge of local soil conditions to ensure robust interpretation.

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Chapter 1 - Introduction

Part I. Lead contamination and stabilization mechanisms in urban soils

Lead (Pb) is a naturally occurring element in soils, with an average concentration of approximately 10 mg kg^{-1} (Lindsay, 1979). However, Pb is also one of the most toxic and persistent contaminants in the environment. In urban areas, soil Pb contamination has increased significantly due to anthropogenic activities, including the historical use of lead gasoline, deterioration of lead-based paints in older buildings, industrial emissions, and the deposition of mining-derived materials used in urban landfills or construction backfills (Ahmad et al., 2021; Zimdahl et al., 1973). As a result, Pb concentrations in urban soils frequently exceed the current residential soil screening level established by the U.S. Environmental Protection Agency (EPA), 2024, approximately $260 \text{ mg Pb kg}^{-1}$. For example, in surface soil samples (0-2.5 cm) systematically collected across residential areas of metropolitan New Orleans, localized Pb hotspots exceeding $1000 \text{ mg Pb kg}^{-1}$ have been reported, particularly within 1 m of contamination sources such as home foundations and roadways, where proximity strongly influences Pb distribution (Zahran et al., 2013). Soil Pb contamination represents a major public health concern because contaminated soil and dust are primary exposure pathways for humans, particularly through ingestion and inhalation (Hettiarachchi & Pierzynski, 2002). Children are especially vulnerable due to their developing nervous systems and their greater likelihood of soil ingestion during activities. Elevated blood lead levels (BLLs) greater than $5 \text{ } \mu\text{g Pb dL}^{-1}$ have been reported in approximately 20-60% of children living near contaminated environments in the United States, increasing the risk of neurological and developmental impairments such as reduced IQ, behavioral disorders, and long-term cognitive deficits (Centers for Disease Control and Prevention (CDC), 2012; Laidlaw et al., 2017). Since no safe BLLs threshold has been

identified for children, soil Pb contamination remains an urgent global environmental and public health issue.

In addition to human health impacts, Pb contamination can also negatively affect soil quality and ecosystem functioning. Lead toxicity interferes with key biological processes in soil by disrupting microbial activity and enzyme biochemical reactions (Doelman & Haanstra, 1979; Khan et al., 2007; Tejada et al., 2007). Soil enzymes such as phosphatase, β -glucosidase, urease, and arylsulfatase are essential for nutrient cycling and soil fertility, and their activity is highly sensitive to heavy metal contamination (Nannipieri et al., 2011). Several studies have shown that increasing Pb concentrations can significantly reduce microbial respiration and enzyme activity, indicating degradation of soil biological health (Alasmery et al., 2021; Tejada et al., 2007). Therefore, understanding how Pb contamination affects both soil chemical processes and biological functioning is critical for developing effective remediation strategies. Among the available remediation approaches, in situ chemical stabilization has been widely recognized as one of the most cost-effective methods for mitigating Pb contamination in soils (Hettiarachchi & Pierzynski, 2004; Mayer et al., 2022; Scheckel et al., 2013). This strategy aims to reduce Pb mobility and bioavailability by promoting the formation of more stable mineral phases or by enhancing sorption reactions within the soil matrix (Kumpiene et al., 2008). Phosphorus (P) amendments have been extensively studied due to their ability to transform reactive Pb species into highly stable Pb-phosphate minerals, primarily pyromorphite-like phases, which exhibit low solubility across a wide range of environmental conditions (Baker et al., 2014; Hettiarachchi & Pierzynski, 2002; Lindsay, 1979).

Previous studies using advanced spectroscopic techniques have confirmed the formation of these stable mineral phases following P amendment applications. For example, Weber et al.

(2015) demonstrated using X-ray absorption near edge structure (XANES) that soils amended with triple superphosphate (TSP) showed chloropyromorphite-like formation ranging from 29% to 35%. Similarly, Scheckel and Ryan (2004) reported pyromorphite species formation up to 41% following inorganic P application, confirming the effectiveness of phosphate-induced Pb stabilization mechanisms. In addition to inorganic phosphate sources, organic amendments such as biosolids and biochar have also been investigated for their potential to immobilize Pb through sorption, complexation with organic functional groups, and changes in soil physicochemical properties (Beesley et al., 2011; Cao and Harris, 2010). However, despite extensive research supporting P-based remediation strategies, several uncertainties remain. For example, one limitation is that total soil Pb concentration alone does not adequately reflect environmental risk. Instead, the fraction of Pb that becomes bioaccessible after soil ingestion is more directly related to human exposure risk. Bioaccessible Pb refers to the portion of soil Pb that can dissolve under simulated gastrointestinal conditions and therefore become available for absorption by the human body (Hettiarachchi et al., 2001; Sharp & Brabander, 2017). Consequently, evaluating the effectiveness of remediation treatments requires not only measuring total Pb but also assessing changes in Pb bioaccessibility and chemical speciation. Additionally, the effectiveness of different P treatments varies depending on soil properties, amendment type, and environmental conditions. In addition, limited information is available regarding the long-term stability of these immobilization mechanisms and their potential interactions with soil biological processes.

For this dissertation research, organic amendments in the form of Class A biosolids were applied at a rate of 10% (w/w) from three sources: Lawrence, KS (BS-LW), Saint Joseph, MO (BS-SJ), and Washington, D.C. (BS-WDC). In addition, inorganic P-amendments were applied as commercially available fertilizers at a Pb:P molar ratio of 1:4, including monoammonium

phosphate (MAP), diammonium phosphate (DAP), triple superphosphate (TSP), and ammonium polyphosphate (APP). A native mixed-wood biochar was also evaluated at an application rate of 1% (w/w), applied either alone or in combination with inorganic P-sources at the same Pb:P molar ratio. A control treatment with no P or biochar addition was included for comparison. Nine urban soils collected from Kansas City, Missouri, representing a wide range of Pb contamination levels, were evaluated through 12-month laboratory incubation experiments. Subsequently, selected treatments were further validated under field conditions through one-year field experiments conducted in four of these soils. The assessment integrated conventional wet-chemistry analyses with advanced spectroscopic techniques. Bulk X-ray absorption near edge structure was employed for multiple sites for overall changes in soil Pb speciation. In one study, μ -X-ray fluorescence (μ -XRF) mapping was used to perform spatially resolved Pb speciation, with μ -XANES, guided by elemental associations, to better understand Pb transformation pathways. The results from laboratory incubation and field experiments indicated that bioaccessible Pb was primarily influenced by soil pH and extractable P. Additionally, the P amendments significantly reduced Pb bioaccessibility, from 16% to 36%, and promoted redistribution of Pb into more stable soil fractions. Biochar also reduced the amount of desorbed Pb up to 1.67-fold relative to the unamended control. Comparative evaluation revealed distinct immobilization mechanisms among the amendments. Mechanisms involving inorganic P treatments were dominated by the formation of pyromorphite-like species, up to 34% according to bulk XANES, while μ -XANES showed spots with high predominance (> 80%). In contrast, biochar, biochar modified with inorganic P treatment, and organic P treatments primarily immobilized Pb through complexation to organic matter and sorption to Fe-associated phases.

These findings demonstrate that arguably inorganic-P treatments remain the most effective strategy for promoting the formation of relatively stable Pb minerals (pyromorphite group) with low bioaccessibility. Extracellular enzyme activity measurements showed that the effect of organic P treatments increased acid phosphatase activity during the early sampling period (1-6 months), whereas inorganic sources generally did not differ from the control. In contrast, β -glucosidase and arylsulfatase showed limited sensitivity to the amendments. Enzyme activities appeared to be strongly governed by soil physicochemical properties (pH, TOC, and resin-P) rather than total Pb concentration. These findings indicate that Pb stabilization with P amendments does not necessarily result in uniform recovery of soil biological activity.

Overall, this dissertation includes three studies designed to investigate the effectiveness of different soil amendments in reducing Pb bioaccessibility in contaminated urban soils and to understand the mechanisms controlling Pb stabilization. These studies evaluated how organic and inorganic P-amendments, as well as biochar, influence Pb chemical speciation, bioaccessibility, and associated soil physicochemical and biological responses. In addition, this research integrates conventional wet-chemical analyses with advanced synchrotron-based techniques to better understand the processes governing Pb immobilization in contaminated soils.

Objectives

Chapter 2

The objective of this chapter was to evaluate the effectiveness of organic and inorganic phosphorus amendments for reducing Pb bioaccessibility in contaminated urban soils. Specifically, this chapter aimed to: (i) assess the capacity of different P sources to reduce bioaccessible Pb, (ii) determine how these amendments influence Pb chemical fractionation and speciation, and (iii) examine the relationships between soil physicochemical properties and Pb

bioaccessibility. This chapter provides a mechanistic understanding of how phosphorus amendments stabilize Pb through precipitation reactions and interactions with soil components.

Chapter 3

This study aims to bridge this knowledge gap by examining the effectiveness of in situ mitigation strategies (P treatments) for reducing bioaccessible soil Pb in mildly to moderately contaminated urban soils in the field. Additionally, to investigate the long-term effect of organic and inorganic P amendments on soil enzyme activities in Pb contaminated urban soils. Further, to identify the major soil chemical properties that control the bioaccessible soil Pb and the influence on soil enzyme activity, and to determine whether changes in soil Pb speciation induced by the application of P treatments could affect soil enzymatic activity.

Chapter 4

This study investigated the mechanisms by which phosphorus and biochar-based amendments reduce Pb bioaccessibility and compare their relative effectiveness under both controlled laboratory and field conditions. The assessment combined conventional wet-chemistry analyses with spectroscopic techniques, including bulk X-ray absorption near-edge structure and microscale approaches such as micro-X-ray fluorescence mapping and micro-X-ray absorption near-edge structure, to better understand the processes controlling Pb immobilization and transformation in treated soils.

Part II. Proximal soil sensors for soil characterization

Accurate soil characterization is essential for supporting informed agronomic decision-making and improving the efficiency of agricultural management practices. Traditionally, soil physicochemical properties have been determined through laboratory-based wet chemical analyses of soil samples collected in the field. However, due to the high spatial variability of soil physicochemical properties within agricultural fields, conventional soil sampling often requires many samples to adequately capture this variability (Cambardella et al., 1994; Kuang et al., 2012). In practice, systematic sampling strategies such as grid sampling-where multiple soil cores (typically 8-10) are collected at GPS-referenced points and combined into composite samples-are commonly used to generate representative datasets and produce interpolated spatial maps of soil properties across agricultural fields (Marcaida et al., 2025; Virk et al., 2024). These approaches are often labor-intensive and limited in their ability to fully capture soil spatial heterogeneity when the number of samples is small relative to the total field area (Kuang et al., 2012). In contrast, precision agriculture technologies have enabled the development of proximal soil sensing tools capable of rapidly collecting high-resolution spatial data directly in the field. Sensors such as visible-near infrared (VIS-NIR) spectroscopy, apparent electrical conductivity (EC_a), electromagnetic induction systems, and gamma-ray spectroscopy have demonstrated strong potential for predicting key soil properties including soil organic carbon, exchangeable nutrients, pH, soil texture fractions, cation exchange capacity (CEC), and soil moisture content (Ji et al., 2019; Pan et al., 2014). For example, VIS-NIR spectroscopy has shown strong predictive capability for soil organic matter and exchangeable cations such as calcium (Ca), magnesium (Mg), and aluminum (Al) ($R^2 > 0.85$), while gamma-ray spectroscopy and electromagnetic sensors such as DUALEM have also demonstrated good performance for

estimating SOM and exchangeable nutrients (Ji et al., 2019). Similarly, laboratory VIS-NIR spectral measurements have successfully predicted clay content and soil organic carbon in tropical soils with coefficients of determination greater than 0.70 (Pinheiro et al., 2017). However, other studies have shown that EC_a does not directly predict nutrient concentrations with high accuracy unless these nutrients are strongly associated with soil properties influencing conductivity, such as texture, CEC or, soil moisture (Heiniger et al., 2003). Consequently, approaches that subdivide fields into homogeneous soil zones using combinations of EC_a measurements, topography, and soil sampling have been shown to significantly improve model performance and the reliability of spatial interpolation (Corwin & Lesch, 2005). Nevertheless, the performance of proximal soil sensors remains strongly influenced by several sources of variability, including soil physicochemical properties and environmental conditions. In this context, soil texture, pH, cation exchange capacity, salinity, organic matter content, and soil moisture not only influence sensor signals, but also introduce variability that can affect the accuracy of soil property predictions (Corwin & Lesch, 2005). In addition, spectral responses may be affected by mineral composition, organic matter content, and soil depth, as the visible region is sensitive to iron oxides and humic substances, whereas the near-infrared region responds to functional groups associated with organic compounds (Hutengs et al., 2019; Viscarra Rossel & Hicks, 2015; Zhang et al., 2024). To address these limitations, several methodological approaches have been developed to improve predictive model performance. Spectral preprocessing techniques such as multiplicative scatter correction (MSC), derivative transformations, baseline correction, and standard normal variation (SNV) normalization are widely used to reduce noise and enhance chemically relevant spectral features (Savitzky & Golay, 1964; Stenberg et al., 2010). In addition, robust model validation strategies, including

cross-validation and Monte Carlo resampling, are essential for evaluating model predictive performance and reducing the risk of overfitting in spectral datasets characterized by large numbers of correlated variables (Kohavi, 1995; Shi et al., 2023; Wolpert, 1992).

These findings highlight that the performance of proximal soil sensors is strongly influenced by soil physicochemical properties, environmental conditions, and spatial heterogeneity within agricultural systems. Consequently, appropriate spectral preprocessing, feature selection, and rigorous model validation are essential to ensure reliable and transferable predictions of soil properties across different soil types and management conditions. The study was conducted in two NSF EPSCoR research fields located in Ashland Bottoms and Colby, Kansas, both historically managed under wheat-soybean rotation in the Midwestern United States. Soil measurements were obtained using the Veris P4000 soil sensing system (Veris Technologies, Salina, KS, USA), equipped with four sensors: apparent electrical conductivity (EC_a), red visible spectrum (VIS: 557-1269 nm), near-infrared reflectance (NIR: 1323-2652 nm), and a force sensor. Simultaneously, soil cores were collected to a depth of 1 m using a soil core sampler.

Field measurements obtained from the Veris P4000 sensors (EC_a , VIS, NIR, and Force) were averaged by sampling point and soil depth (0-20 cm and 20-60 cm) to generate representative values for each location, allowing direct comparisons with soil samples collected at the same positions and depths. To improve signal quality and reduce spectral noise, sensor data were preprocessed using a Savitzky-Golay smoothing transformation (Savitzky & Golay, 1964). To compare the performance of the soil sensors, several conventional wet-chemical laboratory analyses were conducted to determine key soil physicochemical properties. Soil pH was measured in Milli-Q water using a 1:5 soil-to-water ratio with a pH/conductivity. Cation

exchange capacity (CEC, $\text{cmol}_{(+)}\text{kg}^{-1}$) was determined using the displacement method. Soil organic carbon (SOC%) and total nitrogen (TN%) were measured by dry combustion using a LECO TruSpec CN combustion analyzer. Soil texture was determined using the hydrometer method. Nitrate (NO_3^-) and ammonium (NH_4^+) were extracted with 1 M KCl and analyzed using a continuous flow injection system (QuickChem 8500 series 2, LACHAT). Exchangeable potassium (Exch-K), calcium (Exch-Ca), magnesium (Exch-Mg), and sodium (Exch-Na) were extracted using ammonium acetate (NH_4OAc). Similarly, exchangeable phosphorus (Exch-P) was determined using Mehlich III extraction. Finally, approximate bulk density (BD) was calculated using the cylindrical core method, and soil moisture content was determined by gravimetric analysis (GWC %). Model validation was performed using a Monte Carlo simulation implemented in Python (JupyterLab). Linear regression models were developed using sensor-derived variables to predict soil physicochemical properties. The dataset was randomly split into calibration (60%) and validation (40%) subsets over 1000 iterations. Model performance was evaluated using Root Sum of Squared Errors (RSSE) and Mean Absolute Error (MAE) to assess predictive accuracy across sites, depths, and soil properties.

The results indicate that proximal soil sensors can effectively estimate several key soil physicochemical properties, although their performance varied among sensors and sites. The NIR sensor showed the highest predictive ability for SOC and Exch-P, while VIS performed well for total nitrogen, Exch-P, clay content, and Exch-Mg. The EC_a sensor was strongly associated with pH, exchangeable cations, and clay content, reflecting its sensitivity to ionic composition and soil texture. In contrast, the Force sensor showed limited predictive capacity for chemical properties. Sensor responses also varied across sites, highlighting the importance of accounting for local soil conditions when interpreting sensor data.

Chapter 5

This research study aims were: (i) to estimate the relationships between data obtained from multiple soil sensors and a set of selected physicochemical properties, considering two depths; and (ii) to analyze how physicochemical soil properties influence the intensity of sensor signals and their predictive capacity, aiming to identify the factors that enhance or limit sensor performance in two contrasting experimental fields.

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Chapter 2 - Literature review

Part I. Lead contamination and stabilization mechanisms in urban soils

Lead occurrence, properties, and environmental significance

Lead (Pb) is a bluish-white metal with a bright luster, characterized by being soft, highly malleable, and ductile, exhibits low electrical conductivity and notable resistance to corrosion. Lead has an atomic number of 82 and an atomic weight of 207.19, with a melting point of 327.5 °C, a boiling point of 1,744 °C, and a density of 11.35 g cm⁻³. In nature, Pb commonly occurs in oxidation states of +2 and +4, with +2 the most common (Lindsay, 1979). This element has four stable isotopes: ²⁰⁴Pb (1.48%), ²⁰⁶Pb (23.6%), ²⁰⁷Pb (22.6%), and ²⁰⁸Pb (52.3%) (Boldyrev, 2018; Dudka & Adriano, 1997). Due to these physical and chemical properties, Pb has been used by human societies since ancient times. Archaeological evidence indicates that its use dates to approximately 6500 BC, when the earliest Pb-artifacts were discovered in Turkey (Lessler, 1988). Since then, Pb has been used in pigments, ornamental objects, and utilitarian materials. Additionally, its frequent association with argentiferous minerals enabled the extraction of silver (Ag) through cupellation, a metallurgical technology developed around 4000 BC in Anatolia and Persia, which contributed to the expansion of Pb use in several ancient civilizations (Nriagu, 1983). More recently, the use of Pb increased substantially from approximately 1 million tons per year in the early 20th century to about 4.5 to 5 million tons annually in recent years (averaging 4 to 5-fold increases over time) (Figure 2.1) (U.S. Geological Survey, 2024). For example, the introduction of tetraethyl lead (TEL) as a gasoline additive in 1921 (Sangeetha & Umamaheswari, 2020; Boldyrev, 2018). Commercial production began in 1923 through the Ethyl Gasoline Company, formed by General Motors and E.I. DuPont, and later expanded with Standard Oil in 1924 (Needleman, 2000). Despite early reports of worker poisoning, TEL was

approved for use in gasoline in 1926, allowing its widespread use for several decades (Needleman, 2000). Subsequently, toward the end of the same century, one of the main industrial uses of Pb became the manufacture of lead-acid batteries, while the global production of this metal continued to increase due to the growing demand in this sector (Boldyrev, 2018).

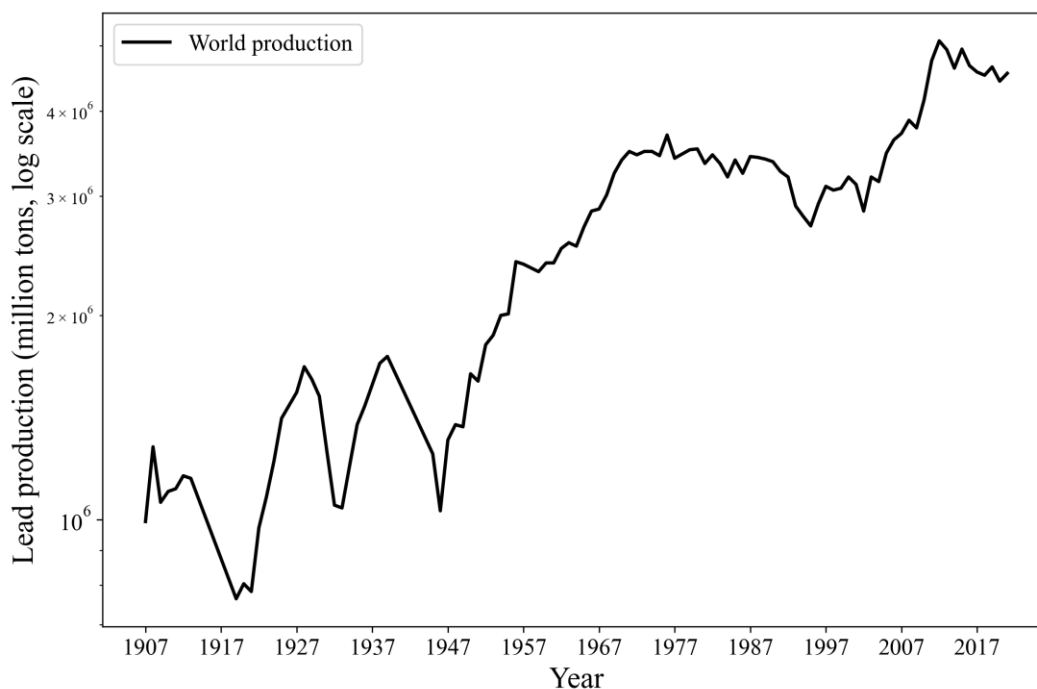


Figure 2.1. Global lead production over time (1907-2020) based on historical data from the U.S. Geological Survey (2024). Values are presented on a logarithmic scale to highlight long-term trends and relative changes in production across more than a century. The data show a sustained increase in global Pb production.

Lead production is generally classified into primary production, obtained from mined ores, mainly galena (PbS), and secondary production, derived from the recycling of Pb scrap (Boldyrev, 2018). Currently, primary Pb production is concentrated mainly in China, Australia, and the United States, followed by Peru, Mexico, Canada, and Sweden. In recent decades, China has experienced significant growth in its mining production, consolidating its position as the world's leading Pb producer (Lopez et al., 2015).

Lead as an environmental contaminant

Despite its extensive use, the utilization of Pb is not without problems and limitations. Lead is a highly toxic metal widely distributed in the environment, with sources that include both natural processes and anthropogenic activities. Among the main human-related sources are metal mining, smelting, and refining, as well as industrial emissions and the historical use of lead gasoline (Sangeetha & Umamaheswari, 2020). In addition, Pb can enter the food chain through surface-deposited soil and dust particles (Attanayake et al., 2014). Where anthropogenic activities in urban environments, particularly the historical use of lead gasoline, the deterioration of lead-based paints in older buildings, and the backfilling of vacant lots with materials derived from industrial or mining activities, have significantly contributed to increase of total Pb concentrations in soils (Ahmad et al., 2021; Scheckel & Ryan, 2004; Zimdahl et al., 1973). As a result, in many cases these levels exceed the reference value established by the U.S. Environmental Protection Agency (EPA, 2024) for residential soils, approximately 200 mg kg⁻¹ (EPA, 2024). For instance, in the Kansas City area, extensive datasets from the Brownfields and Lead Safe KC programs indicate widespread Pb contamination in residential soils. Where, among 262 properties evaluated, 47% exceeded the EPA threshold for child play areas (400 mg kg⁻¹), and 59% surpassed the Missouri Risk-Based Target Level (RBTL) of 260 mg kg⁻¹, with maximum concentrations reaching up to 39,600 mg kg⁻¹. Average maximum Pb concentrations were 1,115 mg kg⁻¹ in driplines and 615 mg kg⁻¹ in yard soils, highlighting the severity and spatial extent of contamination in urban neighborhoods (KCMO, 2016) (Figures 2.2 and 2.3).

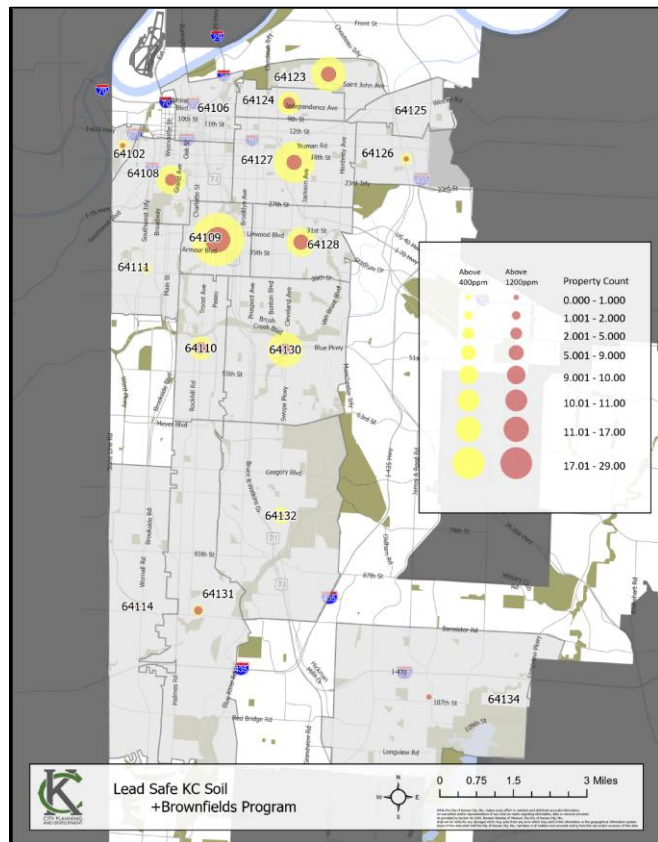


Figure 2.2. Number of properties exceeding lead concentration of 400 and 1200 mg kg⁻¹ (KCMO, 2016)

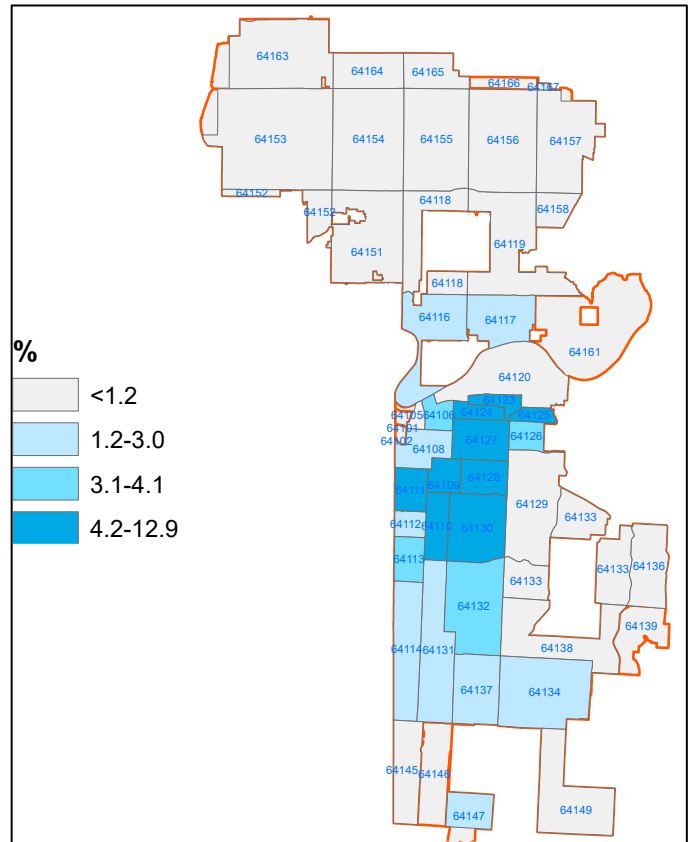


Figure 2.3. Percent of blood lead level at 5 or over µg/dl in children aged under 6 years, by zip code (KCMO, 2016).

The primary pathways for Pb exposure to humans are ingestion and inhalation of contaminated soil or dust (Hettiarachchi et al., 2001b; Chaney et al., 1989; Duggan & Inskip, 1985). Lead has been identified as a toxic element for humans, particularly affecting children whose nervous systems are still in the developmental stage (Centers for Disease Control and Prevention (CDC, 2012). In the United States, young children (≤ 6 years old) living in areas with Pb-contaminated soils have had blood lead levels (BLLs) > 5 µg/dL in approximately 20-60% of cases (Laidlaw et al., 2017; CDC, 2012). However, this proportion could increase significantly due to the recent reduction of the BLLs references value to 3.5 µg/dL (U.S. Department of

Housing and Urban Development (HUD), 2026). As no safe BLLs have been identified for infants or children, Pb contamination remains an urgent global public health concern (CDC, 2012).

Effects of lead on human health

Several studies have demonstrated that Pb primarily affects the central nervous system by binding to sulfhydryl groups in proteins, thereby disrupting the function of enzymes and structural proteins (Sangeetha & Umamaheswari, 2020; Lessler, 1988). In addition, many of its toxic effects arise from its capacity to mimic or compete with calcium (Ca), thereby altering neuronal signaling and various cellular metabolic processes (Sangeetha & Umamaheswari, 2020). Similarly, Pb interferes with heme synthesis and brain development, which can lead to neurological, behavioral, and cognitive impairments (Sangeetha & Umamaheswari, 2020; Needleman, 2004). Long-term exposure further increases the risk of serious neurodevelopmental disorders, including IQ loss, attention deficit disorders, and aggressive behavior, effects that may persist into the second decade of life (Laidlaw et al., 2017, 2005; CDC, 2012).

Effect of lead on soil biological component

In addition to human health risks, soil-Pb contamination adversely affects soil quality by disrupting critical biological processes (Khan et al., 2007; Tejada et al., 2007; Doelman & Haanstra, 1979). One of the most affected functions is enzymatic activity, which serves as a reliable indicator of soil health (Alasmary et al., 2021; Kandziora-Ciupa et al., 2021; Zeng et al., 2007; Tejada et al., 2007; Doelman & Haanstra, 1979). The main findings from previous studies on the effects of Pb contamination on soil enzymatic activity are summarized in Table 2.1. Biological components of soil are generally more responsive to environmental changes than physical or chemical properties, making them particularly sensitive to contamination (Nannipieri

et al., 2011). Extracellular soil enzymes are biomolecules primarily produced by microorganisms, including bacteria and fungi, that catalyze biochemical reactions involved in the decomposition of organic matter and the soil cycling of nutrients (Taggart et al., 2025). Through these processes, they facilitate the breakdown of complex organic compounds and the release of nutrients in forms that can be utilized by plants and other microorganisms (Taggart et al., 2025). In soils, extracellular enzymes may occur in different forms, including soluble enzymes in the soil solution, enzymes associated with microbial or plant debris, or enzymes adsorbed onto clay minerals and organic matter. Their interaction with soil colloids such as clays and humic substances can increase their stability and protect them from degradation (Nannipieri et al., 2011; Alef & Nannipieri, 1995). In addition, extracellular enzyme activities vary depending on soil substrate availability and nutrient conditions. Microorganisms allocate metabolic resources to produce carbon (C)-, nitrogen (N)-, and phosphorus -acquiring enzymes in response to soil nutrient status, thereby optimizing growth (Fujita et al., 2017). According to Alkorta et al. (2003), soil enzymes are commonly classified into several major groups based on the type of reactions they catalyze, including oxidoreductases, hydrolases, transferases, and lyases. For example, β -glucosidase is involved in the carbon cycle by releasing glucose from cellulose, phosphatases release plant-available phosphate from organic phosphorus compounds, and arylsulfatase catalyzes the mineralization of organic sulfur to sulfate. Other enzymes, such as urease, amidase, proteases, and peptidases, play key roles in nitrogen mineralization, while oxidoreductases such as dehydrogenase reflect overall microbial oxidative activity in soils (Alef & Nannipieri, 1995). Soil enzyme activity is highly vulnerable as it reflects even subtle alterations in soil conditions (Kandziora-Ciupa et al., 2021; Nannipieri et al., 2011; Zeng et al., 2007). As a result, changes in enzyme activity could indicate shifts in soil health and reveal the

broader impacts of anthropogenic pollution. For example, Doelman & Haanstra. (1979), found that the soil respiration decreased by 1.19-, 1.53-, and 1.80-fold when the total soil Pb concentration was 375, 750, and 1500 mg kg⁻¹, respectively. Similarly, Alasmary et al. (2021) reported that the activities of acid phosphatase, alkaline phosphatase, arylsulfatase, and β -glucosidase decreased by 1.24-, 1.18-, 1.03-, 1.15-fold, respectively, when the Pb concentration exceeded 1231 mg kg⁻¹. Likewise, Tejada et al. (2007) observed that urease, β -glucosidase, and BBA-protease activities were reduced by 2.08-, 3.52-, and 3.64-fold lower when the Pb concentration ranged from 100 to 1000 mg kg⁻¹.

Total Pb concentration in soils for risk assessment

The total concentration of Pb in soils is commonly reported in mg kg⁻¹ and is determined by acid digestion with concentrated trace grade metal nitric acid (HNO₃), using a hot plate (U.S. EPA. 3050) or microwave-assisted digestion (U.S. EPA, 3051). Commonly, total Pb concentration is quantified by using an inductively coupled plasma optical emission spectrometer (ICP-OES). Alternatively, total Pb concentration can be estimated directly using portable X-ray fluorescence (p-XRF) following the EPA Method 6200 (U.S. EPA, 6200), although this requires thorough and careful sample preparation to get accurate measurements. The total Pb concentration includes both bioavailable Pb and Pb associated with soil minerals, organic matter, and secondary mineral forms, which are not easily bioavailable (Srivastava et al., 2022; Kirkham, 2006; Basta et al., 2005). In this context, soils with similar total Pb concentrations may present very different human and environmental exposure risks, depending on the chemical speciation of Pb and its associations with soil minerals (Srivastava et al., 2022; Basta et al., 2005).

Table 2.1. Summary of reported effects of Pb contamination on soil enzyme activities

Author / Study	Type of study	Enzyme(s) evaluated	Main controlling factors / Key findings
Doelman & Haanstra, 1979	Soil contamination experiment	Soil respiration (microbial activity indicator)	Soil respiration decreased by 1.19-, 1.53-, and 1.80-fold when total Pb concentrations reached 375, 750, and 1500 mg kg ⁻¹ , respectively, indicating progressive microbial inhibition with increasing Pb contamination.
Zeng et al., 2007	Paddy soil contamination experiment	Acid phosphatase	No inhibitory response below 300 mg kg ⁻¹ Pb, but a progressive decline occurred at concentrations > 500 mg kg ⁻¹ , suggesting a threshold-dependent enzymatic response.
Tejada et al., 2007	Soil amendment experiment with heavy metals	Urease, β -glucosidase, BBA-protease	Activities were reduced by 2.08-, 3.52-, and 3.64-fold, respectively, when Pb ranged from 100-1000 mg kg ⁻¹ , highlighting the inhibitory effect of Pb on microbial enzymatic processes.
Kandziora-Ciupa et al., 2021	Mining-contaminated soils (field study, Poland)	β -glucosidase, urease	Elevated heavy metal concentrations (>650 mg kg ⁻¹ Pb) reduced enzyme activities; however, soil organic matter and nutrient availability exerted a positive control on microbial functioning.
Alasmmary et al., 2021	Pb-contaminated soil experiment	Acid phosphatase, alkaline phosphatase, arylsulfatase, β -glucosidase	Enzyme activities decreased by 1.24-, 1.18-, 1.03-, and 1.15-fold, respectively, when Pb concentrations exceeded 1231 mg kg ⁻¹ , demonstrating strong enzymatic sensitivity to high Pb levels.

Lead speciation and lead minerals in soil

Table 2.2 has been constructed to show some of the Pb minerals and complexes presented in urban soils, according to Lindsay (1979). For example, cerussite (PbCO₃) commonly forms as a secondary mineral during the weathering of anthropogenic Pb sources in soils with pH > 8, such as lead-based paints or atmospheric deposition (Hunt, 2016). Under similar environmental conditions, hydrocerussite (Pb₃(CO₃)₂(OH)₂) may also form and is frequently associated with

degraded paint particles and urban dust (Hettiarachchi & Pierzynski, 2004; Hunt, 2016). In contrast, minerals such as galena (PbS) and anglesite (PbSO₄) are typically linked to mining or smelting activities (Scheckel & Ryan, 2004). Where, galena, when sulfur is present in reduced form, is the most stable solid form of Pb. However, when exposed to the air or oxygen-rich waters, galena is readily transformed into anglesite (Hettiarachchi & Pierzynski, 2004; Lindsay, 1979). On the other hand, in phosphate-rich environments, the pyromorphite mineral group (bromopyromorphite, fluoropyromorphite, hydroxypyromorphite and chloropyromorphite) becomes thermodynamically favored due to its extremely low solubility and high stability within the typical soil pH range (Cao et al., 2008; Hettiarachchi & Pierzynski, 2004; Cotter-Howells, 1996). In sum, soils with similar total Pb concentrations may contain very different mineral phases; therefore, the bioavailability of Pb could be markedly different. Speciation of Pb ultimately controls its solubility, mobility, and potential for human exposure.

Bioavailability and bioaccessibility of lead in soils

The human health risk associated with soil Pb contamination is primarily governed by the fraction of Pb that becomes absorbed into the human body, rather than by the total Pb concentration in soil (Sharp & Brabander, 2017; Chaney et al., 1989). In this context, the concepts of bioavailability and bioaccessibility become particularly important for evaluating the potential human health risk associated with soil Pb contamination. Bioavailability (expressed either in mg kg⁻¹ of soil or as % of total Pb concentration in soil) refers to the physical, chemical, and biological processes that determine the exposure of humans, animals, microorganism and plants to chemicals present in soils and sediments (Magrisso et al., 2009; National Research Council (NRC), 2003; Kramer & Ryan, 2000). These processes include the release of contaminants from the solid phase, the transport of dissolved or particle-bound contaminants to

the membrane of an organism, and their subsequent transfer across physiological membranes with eventual entry into the circulatory system (Semple et al., 2004; NRC, 2003; Ruby et al., 1996). Bioaccessibility (express as mg kg^{-1} of soil or as % of total Pb concentration in soil) represents the fraction of a contaminant that can be released from soil particles, dissolve in gastrointestinal fluids (simulated) following soil ingestion, and become potentially available for absorption into the systemic circulation (Kramer & Ryan, 2000; Sharp & Brabander, 2017; Hettiarachchi et al., 2001; Ruby et al., 1996). Therefore, contaminants present in soil to become bioavailable, it must first be bioaccessible in the gastrointestinal environment and undergo the chemical transformations that occur under acidic conditions (Sharp & Brabander, 2017). In some cases, contaminants may become bioavailable within seconds; in other cases, their release may occur over much longer timescales, making them only potentially bioaccessible (Sharp & Brabander, 2017; Kim et al., 2015; Semple et al., 2004). Consequently, the bioaccessibility of toxic elements in soils is strongly influenced by factors such as the mineral speciation of the contaminant, soil chemistry, and the strength of the associations between the contaminant and soil components, which ultimately control the potential exposure and absorption of contaminants in humans (Chaney et al., 1989).

Table 2.2. Relative stability of Pb species in soils

Pb species / Mineral phase	Typical source	Environmental behavior	log K°
PbCO ₃ (cerussite)	Weathering of anthropogenic Pb materials, degradation of lead-based paints, atmospheric deposition	Common secondary mineral in urban soils, soluble under acidic conditions	4.65
PbSO ₄ (anglesite)	Oxidation of PbS from mining or smelting residues	Moderately stable mineral phase	-7.79
PbS (galena)	Mining residues, smelting waste	Stable under reducing conditions but unstable in oxidized soils	-27.5
Pb(H ₂ PO ₄) ₂	Reaction of Pb ²⁺ with soluble phosphate under acidic conditions	Relatively soluble Pb phosphate phase	-9.85
PbHPO ₄	Precipitation of Pb with phosphate in soils	Moderately stable Pb phosphate mineral	-4.25
Pb ₃ (PO ₄) ₂	Formation in phosphate-rich environments	Pb phosphate phase formed under soil phosphate reaction	-5.26
Pb ₄ O(PO ₄) ₂	Secondary Pb phosphate mineral formation	Secondary Pb phosphate phase that may transform into pyromorphite	2.24
Pb ₅ (PO ₄) ₃ OH (hydroxypyromorphite)	Phosphate amendments, natural precipitation	Highly stable mineral with very low solubility	-4.14
Pb ₅ (PO ₄) ₃ Br (bromopyromorphite)	Formation in bromide-containing environments	Highly stable mineral with very low solubility	-19.5
Pb ₅ (PO ₄) ₃ Cl (chloropyromorphite)	Phosphate amendments, natural precipitation	Highly stable mineral with very low solubility	-25.1
Pb ₅ (PO ₄) ₃ F (fluoropyromorphite)	Formation in fluoride-containing environments	Highly stable mineral with very low solubility	-12.9

The primary pathways for lead (Pb) exposure include ingestion and inhalation of contaminated soil and dust (Figure 2.4) (Hettiarachchi et al., 2001; Chaney et al., 1989; Duggan & Inskip, 1985). For example, Pb present in soil and household dust represents a source of childhood exposure at least as important as lead-based paint in many urban environments (Mielkel & Reagan, 1998). In addition to hand-to-mouth contact, the direct ingestion of soil (pica), particularly among young children, can represent a significant exposure pathway and contribute substantially to elevated blood Pb levels (Duggan & Inskip, 1985). Furthermore, studies conducted near lead smelters have shown that children's blood Pb concentrations are more strongly associated with the ingestion of contaminated dust and soil adhered to the hands than with airborne Pb concentrations, indicating that exposure to surface soil/dust represents a major pathway of childhood Pb exposure (Roels et al., 1980). Likewise, indirect pathways also contribute to human exposure to Pb, including the consumption of contaminated food, atmospheric deposition of Pb containing particles onto crops, and the ingestion of contaminated drinking water (Figure 2.4) (Agency for Toxic Substances and Disease Registry (ATSDR), 2020; Sangeetha & Umamaheswari, 2020). For example, Attanayake et al. (2014) conducted a field study in urban community garden contaminated with Pb located in the Washington Wheatley neighborhood of Kansas City, Missouri, reported that the highest Pb concentrations in edible plant portions were found in root/tuber crops ($224 \pm 55 \text{ mg kg}^{-1}$ in carrot), followed by leafy vegetables ($221 \pm 47 \text{ mg kg}^{-1}$ in Swiss chard), and then fruiting crops ($189 \pm 28 \text{ mg kg}^{-1}$ in tomato). However, thorough cleaning and removal of soil and dust particles deposited on edible plant surfaces significantly reduced Pb concentrations. In leafy vegetables, Pb concentrations decreased by approximately 50-60% when vegetables were kitchen-cleaned compared with laboratory-cleaned samples, while fruiting vegetables such as tomatoes showed reductions of

roughly 30-40% following washing. These findings indicate that a substantial fraction of Pb contamination in vegetables originates from surface-deposited soil and dust particles (Attanayake et al., 2014). In addition, ATSDR. (2020) showed that approximately 27,764 pounds of Pb and 163,361 pounds of Pb compounds were released into surface waters from thousands of industrial facilities between 2018 and 2020. The main sectors responsible for these discharges included the chemical, paper, primary metals, electric utilities, and metal mining industries. These data indicate that various industrial activities remain important sources of Pb contamination in surface waters, potentially posing a risk of human exposure through drinking water (ATSDR, 2020). Overall, these studies indicate that although indirect pathways such as contaminated food and drinking water may contribute to human Pb exposure, the ingestion of contaminated soil/dust especially through hand-to-mouth behavior, remains one of the most significant pathways of Pb exposure in young children. In this context, soil and dust particle size plays a fundamental role in human Pb exposure, particularly through the dust-hand-mouth pathway in children. Several studies have shown that Pb concentrations tend to increase as particle size decreases (Chaney et al., 1989; Duggan & Inskip, 1985). This relationship is partly explained by the greater surface-area to volume ratio of fine particles, which enhances their solubilization and therefore increases Pb bioaccessibility and potential bioavailability (Ruby et al., 1996). In addition, smaller particles are more easily transferred to human hands and tend to remain adhered for longer periods, increasing the likelihood of accidental ingestion (Mielkel & Reagan, 1998). Particles smaller than approximately 50-100 μm are those most retained on the hands, while a large proportion of dust present on children's hands consists of even finer particles, typically smaller than 10 μm (Duggan & Inskip, 1985). These fine fractions not only tend to contain higher Pb concentrations

but may also originate from multiple environmental sources, including playground dust, urban surface deposits, and resuspended soil particles (Chaney et al., 1989; Duggan & Inskip, 1985).

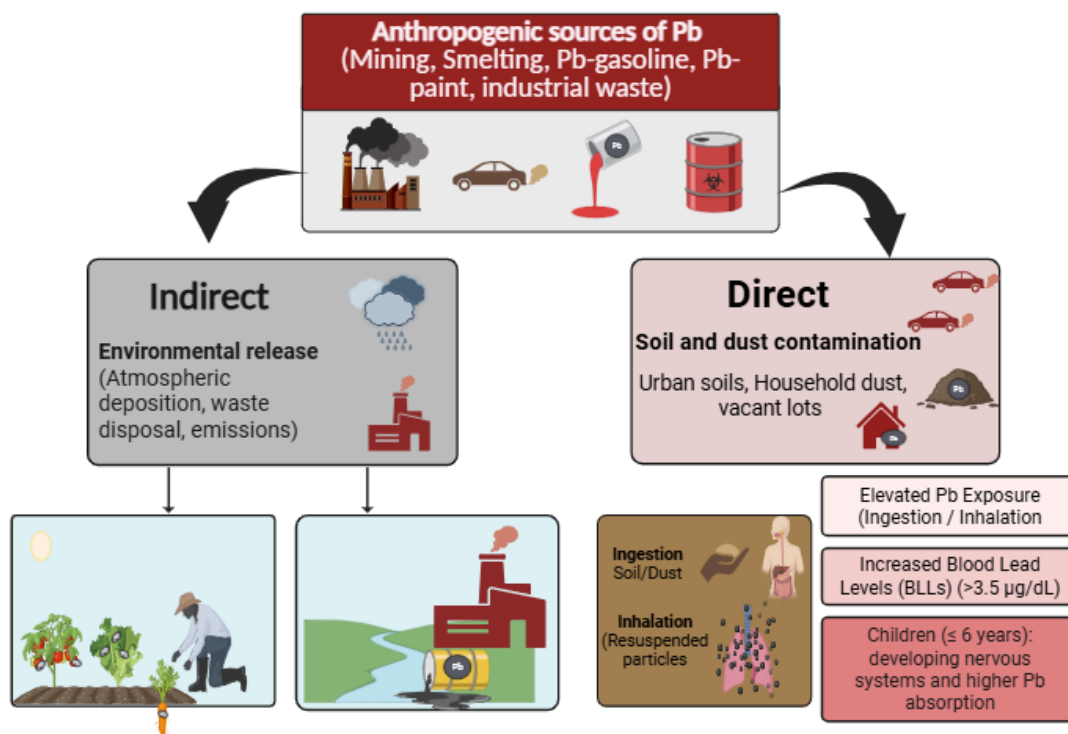


Figure 2.4. Anthropogenic sources, environmental pathways, and human exposure routes of Pb in urban systems, highlighting indirect (atmospheric deposition and emissions) and direct (soil and dust contamination) processes leading to ingestion, inhalation, and elevated blood lead levels, particularly in children.

Methods for assessing Pb bioavailability and bioaccessibility

Gastrointestinal absorption of Pb in humans can vary considerably depending on factors such as age, diet, nutritional status, as well as the chemical species and particle size of the ingested Pb (Ruby et al., 1996). In general, adults absorb approximately 7-15% of Pb ingested from dietary sources, whereas in infants and children absorption may range from 40 to 53%, indicating a greater susceptibility to Pb exposure and its toxic effects during early stages of development (Ruby et al., 1996). In addition, several studies have shown that Pb deposited in the

environment, particularly from the historical use of leaded gasoline and lead-based paint, tends to accumulate and persist for long periods in the surface layers of soils (Sharp & Brabander, 2017; Chaney et al., 1989; Chaney & Mielke, 1986), where it represents an important potential source of human exposure through soil and dust ingestion. In this context, accurately assessing the fraction of Pb that can become available for absorption in the human body is essential for estimating potential human health risks. Consequently, several experimental procedures have been developed to estimate the bioavailability and bioaccessibility of Pb in contaminated soils. These methods include *in vivo* models, which directly measure Pb absorption in biological systems, and *in vitro* bioaccessibility assays that simulate gastrointestinal conditions to estimate the fraction of Pb that may become available for absorption (Brown et al., 2004; Casteel et al., 1997; Freeman et al., 1996; Ruby et al., 1996). The following section summarizes the main methods used to assess Pb bioavailability and bioaccessibility in soils and their relevance for evaluating human exposure risk.

In vivo methods

In vivo experimentation is an essential component of biological research. While these experiments enable scientific advances that benefit human and animal health, ethical and welfare considerations (Kieran et al., 2025). Several studies have used stable isotope dilution techniques in human volunteers to directly quantify Pb bioavailability following oral exposure. These approaches rely on administering isotopically labeled Pb (e.g., ^{206}Pb , ^{207}Pb) along with the exposure material, thereby allowing ingested Pb to be distinguished from background Pb already present in the body. Subsequent measurement of isotopic ratios in blood using mass spectrometry enables the estimation of the fraction of Pb absorbed into systemic circulation. For example, Graziano et al. (1996) administered a controlled oral dose of isotopically labeled Pb to adult

volunteers with low baseline blood Pb levels. Blood samples collected after exposure showed that Pb concentrations increased within 24 hours from approximately 0.10 to 0.18 $\mu\text{mol L}^{-1}$, indicating measurable absorption. Based on isotopic dilution calculations, the fraction of Pb absorbed ranged from 46% to 96%, with an average absorption of about 70%, demonstrating substantial inter-individual variability in Pb uptake (Graziano et al., 1996). Similarly, Maddaloni et al. (1998), used a stable isotope tracer to evaluate the bioavailability of soilborne Pb in adult volunteers exposed to Pb-contaminated soil. Their results showed that Pb absorption was strongly influenced by dietary conditions. Under fasting conditions, blood Pb increased from approximately 0.7 to 1.3 $\mu\text{g dL}^{-1}$, corresponding to a relative bioavailability of 18-36% (average ~26%). In contrast, when soil was ingested with food, the increase in blood Pb was minimal (~1.0 to 1.1 $\mu\text{g dL}^{-1}$), and relative bioavailability decreased dramatically to 0.2-5.2% (average ~2.5%) (Maddaloni et al., 1998). Taking together, these studies demonstrate that although stable isotope methods provide reliable estimates of Pb bioavailability in humans, direct in vivo measurements remain difficult to conduct due to ethical, logistical, and cost constraints (Henry et al., 2015; Maddaloni et al., 1998; Graziano et al., 1996).

The use of animals for scientific purposes is both a long-standing practice in biological research and medicine, and a frequent matter of debate in our societies. The remarkable anatomical and physiological similarities between humans and animals, commonly mammals, have prompted researchers to investigate a large range of mechanisms and assess novel therapies in animal models before applying their discoveries to humans (Good et al., 2024; Barré-Sinoussi & Montagutelli, 2015). In this context, Chiou & Buehler. (2002) compared the oral absorption and absolute bioavailability of numerous drugs between monkeys and humans and found that the fraction of dose absorbed and the time to reach peak concentration were generally similar

between the two species. These results suggest that monkeys may provide a useful model for predicting gastrointestinal absorption in humans. However, absolute oral bioavailability was often lower in monkeys, likely due to greater first-pass metabolism and higher total and nonrenal clearance. Although this study was not conducted with Pb, it highlights an important principle for oral bioavailability research, where animal models may reasonably predict gastrointestinal absorption, but interspecies differences in metabolism can limit direct extrapolation of systemic bioavailability to humans (Chiou & Buehler, 2002). In this context, *in vivo* studies designed to estimate Pb bioavailability commonly compare different Pb species present in contaminated soils with a well-characterized soluble reference compound, typically lead acetate, to determine relative bioavailability. These studies are usually conducted using animal models, such as rats or juvenile swine, which serve as physiological surrogates for the human gastrointestinal system. For example, Freeman et al. (1996) evaluated Pb bioavailability using male Fischer 344 rats exposed to diets containing lead acetate, lead sulfide (PbS), and Pb-contaminated soil from the California Gulch Superfund site. Animals were exposed for 44 days to diets containing 17.6, 42.8, and 127 ppm Pb, with Pb measured in blood, bone, liver, and kidney. Results showed that lead acetate had the highest bioavailability, with body-to-diet Pb ratios of 0.151-0.251 at Day 7 and 0.134-0.143 at Day 44. In contrast, PbS showed much lower ratios (0.012-0.017), while Pb associated with contaminated soil exhibited the lowest bioavailability (0.009-0.087). Similarly, Casteel et al. (1997) used juvenile swine to evaluate the relative bioavailability of Pb in contaminated soils from the Smuggler Mountain Superfund site in Aspen, Colorado. In this study, soils were compared against lead acetate as the reference compound to calculate relative bioavailability values. The results indicated RBA values of approximately 56-58% based on blood Pb measurements, with an overall estimate of 63-64%, corresponding to an absolute

bioavailability of about 32% relative to the soluble reference compound. Together, these studies illustrate how *in vivo* experiments are typically conducted by comparing Pb species present in contaminated soils with a known soluble Pb reference compound, allowing researchers to quantify relative Pb bioavailability and better understand how Pb speciation and soil matrix properties influence metal absorption

In vitro methods

In vivo studies present limitations, including high costs, long-term experiment requirements, and ethical concerns. To solve these issues, indirect methods based on *in vitro* assays have been developed (Ruby et al. 1996). The *in vitro* bioaccessibility assays are widely used as indirect methods for estimating the fraction of Pb that may become bioaccessible following ingestion of contaminated soil. These methods allow quantification of the fraction of Pb present in soil that can be released under simulated physiological conditions of the human gastrointestinal system (Ruby et al., 1996). The *in vitro* methods were initially developed and calibrated using juvenile swine models, which are considered the “gold standard” *in vivo* model closely representing the human gastrointestinal physiology (Deshommes et al., 2012), particularly in children. However, the rat model (along with mouse models) is also accepted and widely utilized as a surrogate for Pb bioavailability studies, particularly when high costs or ethical constraints limit the use of swine (Yan et al., 2017). Ruby et al. (1996) conducted Pb bioavailability studies using Sprague-Dawley rats fed with contaminated soil incorporated into their diet and compared the results with those obtained from lead acetate, a soluble Pb reference compound. Lead acetate administered through the diet was also compared with intravenous administration to determine its absolute bioavailability (ABS). Lead concentrations measured in rat blood were used to estimate the relative bioavailability (RBA) of Pb associated with different

substrates relative to Pb acetate. In parallel, the physiologically based extraction test (PBET) was developed to estimate Pb bioaccessibility in contaminated soils by simulating conditions of the human gastrointestinal system. In this method, soil samples were incubated in a synthetic gastric solution containing pepsin and organic acids at acidic pH (1.3-2.5) and maintained at 37 °C for approximately one hour to determine the gastric phase solubility. Bioaccessibility was calculated as the fraction of Pb released into solution relative to the total Pb in the soil. A strong relationship was observed between in vitro bioaccessibility and in vivo bioavailability when the gastric phase was considered ($r^2 = 0.93$), indicating that gastric PBET results can serve as reliable predictors of Pb bioavailability.

Equation 2.1. Estimation of Pb relative bioavailability (RBA) in untreated soils

$$RBA = 0.878 \times IVBA - 0.028$$

Subsequent studies demonstrated how these relationships can be used to evaluate remediation strategies. For example, Hettiarachchi et al. (2003) evaluated the effectiveness of P-based amendments in reducing Pb bioavailability in contaminated soils by combining in vitro (PBET) and in vivo (rat feeding) approaches. Their results showed that soils treated with triple superphosphate (TSP) and phosphate rock (PR), resulted in significant reductions in Pb relative bioavailability (RBA), with decreases ranging from approximately 10% to 45%, depending on the treatment and biological endpoint evaluated. These reductions were evidenced by decreased Pb concentrations in blood and other tissues (liver, kidney, and bone) compared to untreated soils. Furthermore, the study demonstrated that the addition of manganese oxides in combination with P amendments further enhanced Pb immobilization, likely due to increased sorption and the formation of less soluble Pb phases. Moreover, correlations between in vitro bioaccessibility

factors (BAF) and in vivo RBA values showed that the gastric phase of PBET exhibited stronger relationships ($r^2 = 0.93$) than the intestinal phase ($r^2 = 0.76$), highlighting the importance of gastric extraction in predicting Pb bioavailability. In addition, integrated “point estimates” of bioavailability, calculated as weighted averages across multiple tissues, showed strong correlations with in vitro results (r^2 up to 0.95), supporting the use of PBET as a predictive tool for assessing remediation effectiveness.

Brown et al. (2004) evaluated several in situ amendments, including triple superphosphate (TSP), phosphoric acid (H_3PO_4), phosphate rock, Fe-rich materials, and compost, for reducing Pb, Zn, and Cd availability in a smelter-contaminated soil from Joplin, Missouri. Laboratory screening showed that all amendments reduced Pb bioaccessibility (PBET) relative to the control soil, with reductions ranging from 21% (0.1% P-TSP) to 89% (3.2% P-TSP), and compost and Fe-based treatments also reduced Pb bioaccessibility by approximately 65%. In parallel, the an vivo study in rats using laboratory-incubated soils, the 2.5% Fe + 15 P-TSP treatment had the greatest decrease (~39%), while 10% compost and 1% P- H_3PO_4 reduced Pb bioavailability by about 26% (Brown et al., 2004).

Further work has evaluated different in vitro gastrointestinal extraction methods, including the solubility/bioavailability research consortium (SBRC) method, physiologically based extraction test (PBET), in vitro gastrointestinal method (IVG), unified BARGE method (UBM), and the Deutsches Institut Für Normung (DIN) bioaccessibility procedure. For example, Juhasz et al. (2016) reported wide variability in Pb in vitro bioaccessibility (<4% to >80%) depending on soil properties, Pb mineralogy, and extraction conditions. Soils dominated by soluble Pb minerals, such as hydrocerussite, cerussite, and PbO, showed high bioaccessibility (>60%), whereas soils containing more stable phases, such as hydroxypyromorphite and Pb-

phosphate minerals, exhibited significantly lower values. Similarly, Drexler & Brattin. (2007) developed the relative bioavailability leaching procedure (RBALP) and compared in vitro bioaccessibility results with in vivo bioavailability previously determined in juvenile swine. Their study analyzed 19 contaminated soils and industrial residues with total Pb concentrations ranging from 1,270 to 14,200 mg kg⁻¹. The relative bioavailability leaching procedure used a glycine-buffered solution at pH 1.5 and 37 °C for one hour, simulating gastric conditions, and later renamed as in vitro bioaccessibility test. A strong linear relationship was observed between in vitro bioaccessibility (IVBA) and in vivo relative bioavailability (RBA) ($r^2 = 0.92-0.93$), leading to the empirical model (Equation 2.1).

This relationship allows estimation of Pb bioavailability from in vitro results, with an average predictive error of approximately 10%, and was later adopted by the U.S. Environmental Protection Agency (EPA, 2009) for risk assessment of Pb-contaminated soils. Overall, these studies demonstrate that although in vivo experiments provide the most direct measurement of Pb bioavailability, well-calibrated in vitro bioaccessibility assays can reliably predict Pb bioavailability, offering a practical and cost-effective alternative for evaluating human exposure risk and supporting remediation decision-making at contaminated sites. Overall, in vitro bioaccessibility assays have become widely used tools for assessing Pb exposure risk in contaminated soils due to their practical and methodological advantages compared with in vivo approaches. Although in vivo studies provide direct measurements of Pb bioavailability, they are expensive, time-consuming, and involve ethical concerns associated with animal testing. In contrast, in vitro assays can be conducted at a fraction of the cost and allow the analysis of a much larger number of samples, enabling a more comprehensive characterization of spatial variability in Pb risk across contaminated sites. In addition, when appropriately calibrated using

in vivo data, these methods can provide reasonably accurate estimates of relative Pb bioavailability, with predictive errors typically near 10%. However, the predictive performance of these assays depends on factors such as soil properties, Pb speciation, and extraction conditions, highlighting the importance of careful method selection and interpretation when evaluating Pb exposure risk in contaminated soils. Despite the widespread use of in vitro bioaccessibility assays to estimate Pb exposure risk and evaluate remediation strategies, important knowledge gaps remain. In particular, the mechanisms responsible for Pb immobilization following amendment applications are not fully understood, especially under realistic soil conditions. Most studies have focused primarily on measuring reductions in bioaccessibility, while the mineralogical and geochemical processes that control Pb stabilization in treated soils remain less explored. Moreover, amendments can significantly modify key soil properties, such as pH, phosphorus availability, organic matter content, and mineral surface reactivity, which may influence Pb speciation and, consequently, its bioaccessibility. Understanding how these treatment-induced changes in soil physicochemical properties affect Pb bioaccessibility and stabilization mechanisms is essential for improving the reliability of in vitro assessments and for supporting more informed decision-making in field-scale remediation strategies.

Soil properties controlling Pb bioaccessibility

In addition to the quantification of Pb using in vitro assays in untreated soils, it is necessary to identify the physicochemical properties that control Pb behavior in soils, as these directly influence its bioaccessibility and mobility (Figure 2.5). Soil pH plays a key role in controlling the mobility and availability of Pb. In this context, as soil pH increases, the degree of protonation of functional groups present on mineral surfaces ($\equiv\text{SOH}$) decreases. As a result, the

formation of negatively charged surface sites increases, which favors the adsorption of metal cations such as Pb^{2+} .

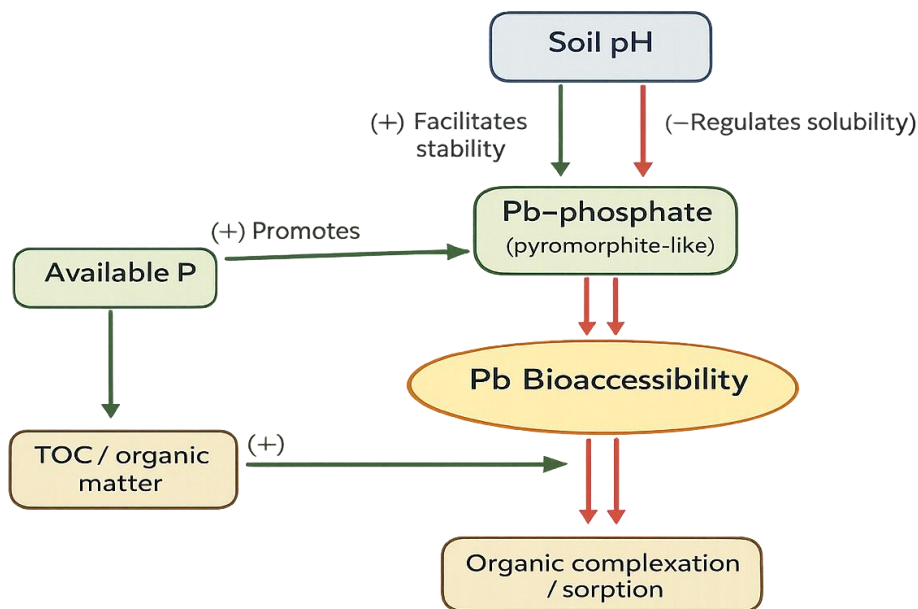


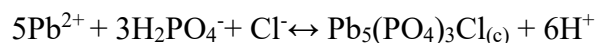
Figure 2.5. Conceptual framework illustrating the main soil factors controlling Pb bioaccessibility. Green arrows indicate positive relationships (enhancement or promotion), whereas red arrows indicate negative relationships (reduction or inhibition). The (+) and (-) symbols represent the direction of the effect of each factor on the associated process. Available P promotes the formation of Pb–phosphate minerals (e.g., pyromorphite-like phases), which reduce Pb bioaccessibility. Soil pH regulates Pb solubility and influences the stability of Pb–phosphate phases. Total organic carbon (TOC) contributes to Pb retention through organic complexation and sorption mechanisms, further affecting Pb bioaccessibility.

Under these conditions, Pb can form surface complexes and precipitate as low-solubility mineral phases, reducing its mobility and contributing to its immobilization in soils (Essington, 2004). At the same time, iron (Fe) and manganese (Mn) oxides play a fundamental role in the retention of trace metals in soils due to their high adsorption capacity relative to other minerals, such as aluminum oxides and many clay minerals (Basta et al., 2005). Surface mineral sorption regulates the partitioning of trace elements between the solid and solution phases of soil and is

highly dependent on pH (Basta et al., 2005). For example, molecular-scale spectroscopic studies have demonstrated that metals such as Pb can strongly bind to the surfaces of Fe and Mn oxides through the formation of inner-sphere surface complexes and the precipitation of metal hydroxides (Basta et al., 2005). Soil organic matter, particularly humic substances, plays an important role in the complexation and speciation of Pb in soils. For example, Steinberg & Hodge. (2022), using competitive ligands such as PAR (4-2-pyridylazo resorcinol), have demonstrated that Pb can form stable complexes with humic acids and other organic compounds. The results indicate that the adsorption of Pb by humic materials occurs rapidly and can be described by Langmuir-type isotherms, suggesting the presence of specific binding sites. For example, Langmuir isotherms showed strong complexation of Pb with humic substances, with stability constants ($\log K_{HA}$) between 6.6 and 8.8 and binding site concentrations (L_t) between 9.66×10^{-5} and $5.46 \times 10^{-3} \text{ mol g}^{-1}$. The high correlation coefficients ($r = 0.96\text{-}0.99$) indicate that the Langmuir model adequately describes the interaction of Pb with these substances. Overall, these organic complexation processes directly influence the speciation, mobility, and bioaccessibility of Pb in the environment, as the formation of complexes with organic matter can control its distribution between the solid and dissolved phases in soil systems. Similarly, precipitation reactions represent an important mechanism controlling Pb bioaccessibility in contaminated soils (Lindsay, 1979). Under favorable geochemical conditions, Pb^{2+} can react with different soil components to form low-solubility mineral phases that significantly reduce its mobility and bioaccessibility. For example, in the presence of P, Pb can precipitate as highly stable Pb-phosphate minerals such as pyromorphite-group ($\text{Pb}_5(\text{PO}_4)_3\text{OH}$; $\text{Pb}_5(\text{PO}_4)_3\text{Br}$; $\text{Pb}_5(\text{PO}_4)_3\text{Cl}$; $\text{Pb}_5(\text{PO}_4)_3\text{F}$), which is considered one of the most stable forms of Pb in soils (Cao

et al., 2008; Hettiarachchi & Pierzynski, 2004; Lindsay, 1979). An example of this reaction is shown in Eq. 6.2.

Equation 2.2. Reaction illustrates the formation of chloropyromorphite ($\text{Pb}_5(\text{PO}_4)_3\text{Cl}$), a stable Pb-phosphate mineral that contributes to Pb immobilization in phosphate-amended soils.



Similarly, interactions with iron (Fe) and manganese (Mn) oxides can promote the formation of Pb hydroxides or Pb-containing surface precipitates through sorption-coprecipitation processes. These Fe and Mn oxides act as strong sorbents due to their high surface area and the presence of reactive sites on their surfaces, promoting the immobilization of Pb in the solid phase of the soil. Consequently, the formation of these secondary mineral phases can considerably decrease Pb solubility and limit its bioaccessibility in soils. For example, Hettiarachchi et al. (2001) found that the presence of Mn oxides, such as cryptomelane, can enhance reactions between applied P and Pb in soil, thereby promoting sorption and coprecipitation. In that study, the combined application of phosphates and cryptomelane reduced Pb bioaccessibility by approximately 40-55% in the gastric phase and up to 60-80% in the intestinal phase compared with the control. In addition, the phosphoric rock (PR) + cryptomelane combination produced reductions in bioaccessible Pb comparable to TSP + cryptomelane, indicating that Mn oxides can enhance Pb stabilization even when the precipitation of pyromorphite-like minerals is limited.

Remediation strategies for lead-contaminated soils

Several remediation strategies have been developed to reduce the environmental and human health risks associated with Pb-contaminated soils. Traditional approaches include soil

excavation and off-site disposal, which involves removing contaminated soil and transporting it to engineered landfills or hazardous waste facilities (U.S. Environmental Protection Agency (EPA), 2007). Another commonly used approach is soil washing and chemical extraction, which removes Pb from soils using chemical agents such as acids, chelating agents, or surfactants. This technique can significantly reduce total Pb concentrations but may generate secondary waste streams that require additional treatment (Dermont et al., 2008). Alternatively, physical containment and capping strategies are frequently implemented to isolate contaminated soils from human exposure. These methods involve covering the contaminated area with clean soil, geotextiles, or other impermeable barriers to prevent direct contact and reduce dust dispersion, thereby limiting Pb exposure pathways (Padhye et al., 2023). Although this method is effective at immediately eliminating exposure risks, it is often costly and disruptive, and it may transfer contamination to another location rather than treating it in situ. On the other hand, remediation of Pb-contaminated soil using chemical stabilization methods is considered one of the most cost-effective mitigation strategies (Hettiarachchi & Pierzynski, 2004; Mayer et al., 2022; Scheckel et al., 2013; Weber et al., 2015). Soil amendments can reduce in-situ Pb bioavailability through various sorption processes, including adsorption onto mineral surfaces, formation of stable complexes with organic ligands, surface precipitation, and modifications in soil chemical speciation (Kumpiene et al., 2008). The use of P amendments to immobilize soil Pb is a widely recognized practice (Henry et al., 2015; Hettiarachchi & Pierzynski, 2002, 2004). The application P sources effectively reduce Pb bioavailability by interacting with free Pb ions to promote the in-situ formation of lead-phosphate compounds, mainly as pyromorphite group. These minerals show significantly lower solubility than other Pb species under typical environmental pH conditions (Baker et al., 2014; Hettiarachchi & Pierzynski, 2002; Lindsay,

1979; Mayer et al., 2022). For example, Weber et al. (2015) demonstrated, using X-ray absorption near-edge spectroscopy (XANES), that soils amended with triple superphosphate (TSP) showed an increase in the formation of chloropyromorphite ranging from 29% to 35% at P: Pb molar ratios of 4:1 and 16:1, which was higher than that of untreated soils. Likewise, Scheckel & Ryan. (2004) using X-ray absorption fine structure analyses (XAFS), showed that pyromorphite was not detectable in untreated soils (0% of total Pb across control samples). Following the addition of 3.2% of P as TSP, Pb speciation shifted significantly, with pyromorphite accounting for up to 41% of total Pb, indicating substantial transformation toward stable Pb-phosphate species. Additionally, 2.5% iron-rich material (IR) mixed with 1% P as a TSP treatment resulted in approximately 41% pyromorphite higher relative to the control. Finally, when 1% of P was added as phosphoric acid (H_3PO_4), pyromorphite content ranged from 43% to 45% of total Pb, as determined by bulk XANES compared to the control over the 3-to 32-month period. Further, Alankarage et al. (2024), observed reductions in soil Pb bioaccessibility of 10.2%, 13.1%, and 15.8%, at Pb:P rates of 0.85, 1.7, and 2.5, using TSP, respectively, following U.S. EPA method 1340 (in vitro bioaccessible assay, IVBA), compared to untreated soil. Likewise, Gu et al. (2020) observed that TSP, struvite, and phosphoric rock (PR) decreased Pb bioavailability by around 10 to 18% compared to the control soil. Additionally, Yang & Mosby. (2006) found significant reductions of ~59% in soil Pb bioaccessibility after H_3PO_4 applications. However, no consensus exists on selecting the most suitable phosphate material for a given contaminated site. Some liquid phosphate sources, such as H_3PO_4 , react quickly but are highly mobile, making them unsuitable for sites where surface water or groundwater contamination is a concern. Alternatively, ammonium polyphosphate (APP) offers a liquid phosphate option that can modify soil pH. On the other hand, solid

phosphates, typically various forms of calcium phosphates, are less soluble, reducing the risk of surface and groundwater contamination. However, they may be less effective in converting soil Pb into pyromorphite (Scheckel et al., 2013).

Combined amendment strategies for Pb stabilization

Combined amendment strategies have gained increasing attention as a promising approach for improving Pb stabilization in contaminated soils. These strategies aim to integrate different materials that promote complementary immobilization mechanisms, including sorption, complexation, and mineral precipitation. For instance, biochar amendments can modify soil physicochemical properties by increasing soil pH, surface area, and reactive functional groups, which promote metal immobilization through sorption and precipitation processes (Beesley et al., 2011; Cao & Harris, 2010). However, combining biochar with other materials such as phosphorus sources or metal oxides may further enhance Pb stabilization by promoting the formation of more stable mineral phases. In addition, the uses of Class A Fe-rich biosolids could be alternative source of in-situ mitigation strategy. For example, Wekumbura et al. (2023) found that Class A Fe-rich biosolids were more effective than other treatments in reducing bioaccessible Pb while maintaining near-natural soil pH conditions. Sequential extraction and synchrotron-based analyses revealed a higher proportion of chemically stable Pb species in biosolids-treated soils, including Pb precipitated as pyromorphite (approximately 30-40%).

Wet-chemical approaches for evaluating Pb solubility in treated soils

Sequential extraction procedures

Sequential extraction schemes consist of a series of chemical extractions (generally 3 to 8 steps) applied consecutively to the same solid sample aliquot. In these procedures, the extractants are applied in order of increasing reactivity, so that the successive fractions correspond to trace

elements associated with different soil phases that exhibit varying degrees of mobility and stability (Du Laing, 2011). The reagents most used in sequential extraction schemes mainly include unbuffered salts, weak acids, reducing agents, oxidizing agents, and strong acids (Adnan et al., 2025; Tessier et al., 1979). This approach allows detailed information to be obtained about the origin, mode of occurrence, mobility, and biological and physicochemical availability of trace elements present in solid samples and has been shown to be a useful tool for identifying the association of these elements with the principal accumulative phases in soils and sediments (Du Laing, 2011). One of the most widely used sequential extraction methods is the scheme proposed by Tessier et al. (1979) in which each fraction is based on the extractability of the applied reagents, and although these fractions are operationally defined, they are generally associated with the relative stability of trace metal species present in the soil. This sequential extraction scheme enables the selective separation of Pb associated with different soil fractions by sequentially applying specific reagents. In this method, the exchangeable fraction is extracted using 1 M MgCl_2 adjusted to pH 7.0 at a soil-to-solution ratio of 1:12.5 (w/v), thereby removing Pb weakly adsorbed on soil exchange sites. Subsequently, the carbonate-bound fraction is obtained using 1 M $\text{NaC}_2\text{H}_3\text{O}_2$ (sodium acetate) adjusted to pH 5.0 at a 1:12.5 (w/v) ratio, targeting metals associated with carbonate minerals that are sensitive to mildly acidic conditions. The Fe-Mn oxide-bound fraction is extracted using 0.04 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 25% (v/v) acetic acid with a ratio of 1:2 (w/v), a reagent designed to reduce and dissolve amorphous Fe and Mn oxides that retain metals through adsorption or coprecipitation processes. The organic-bound fraction is released through an oxidative digestion using 0.02 M HNO_3 and 30% H_2O_2 , followed by successive additions of 30% H_2O_2 to oxidize organic matter. After cooling, 3.2 M $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ in 20% (v/v) HNO_3 and deionized water is added, with soil-to-solution ratios between 1:3 and 1:5,

to extract metals associated with organic compounds and sulfides. Finally, the residual fraction is determined by digestion with 4 M HNO₃ using a ratio of 1:1 (w/v), allowing the quantification of Pb strongly incorporated into the crystalline structure of soil minerals. Together, these fractions represent different levels of geochemical stability of Pb in soils, ranging from potentially mobile forms to species strongly stabilized within the mineral matrix.

Desorption experiments

In general, the desorption phase represents a simpler and faster approach for estimating the fraction of metal that may be remobilized when environmental conditions change and, therefore, may influence heavy metal mobility and bioavailability, as well as for evaluating the long-term stability of immobilization treatments (Ure, 1996). The persistence and mobility of Pb in variable-charge soils are determined by the extent to which metals are adsorbed or desorbed in the solid phase, a process that depends on reactions affecting surface charge (i.e., pH and ionic strength) (Kim et al., 2015). Understanding the mechanisms of metal adsorption and desorption in soils is essential because these reactions govern the strength of interactions between metals and soil surfaces. To access these soil phases (Desorbed), low-concentration saline solutions such as 0.01 mol L⁻¹ NaNO₃ or 0.01 mol L⁻¹ Ca(NO₃)₂, adjusted to pH 5.0 ± 0.1, are commonly used to simulate soil solution conditions and promote ion-exchange reactions that allow the estimation of the fraction of metal potentially desorbed or mobilizable (Hettiarachchi et al., 2003; Kim et al., 2015). These extractants primarily act by displacing weakly adsorbed metals from cation-exchange sites, thereby enabling evaluation of sorption reversibility and the potential remobilization of metals under changing soil geochemical conditions (Sauvé et al., 2000). Finally, extractions are often conducted sequentially to evaluate the kinetics and extent of

desorption, since a single extraction may not completely remove the weakly retained fraction of metals in soils (Sastre et al., 2007), followed by instrumental analysis (GF-AAS, ICP-MS)

Advanced spectroscopic techniques for Pb speciation in soils

X-ray Absorption Spectroscopy (XAS) is a synchrotron-based technique widely used to determine the chemical speciation and coordination environment of elements in complex environmental matrices such as soils. In X-ray absorption spectroscopy, a monochromatic X-ray beam is scanned across the absorption edge of a specific element, and the absorption of X-rays by the sample is measured as a function of photon energy (Lombi & Susini, 2009). When the incident X-ray energy matches the binding energy of a core electron, the electron is excited and ejected from the atom, producing a characteristic absorption edge (Lombi & Susini, 2009). The structure of the absorption spectrum provides detailed information about the chemical environment of the absorbing element, because XAS does not require crystalline samples and can be applied directly to heterogeneous materials, it is particularly valuable for environmental studies where metals occur in poorly crystalline or amorphous phases (Brown et al., 1999; Lombi & Susini, 2009). In soil science, XAS has become one of the most powerful tools for identifying the chemical forms of trace metals such as Pb, Cd, Zn, and As in contaminated soils (Brown et al., 1999; Ryan et al., 2001; Scheckel & Ryan, 2004). The XAS spectrum is commonly divided into two regions based on the energy range relative to the absorption edge (E_0): X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). This division arises from the different physical processes that dominate the interaction between X-rays and matter as photon energy increases beyond the absorption edge (Norman, 1986).

X-ray absorption near edge structure spectroscopy (XANES)

The X-ray absorption near edge structure region of the X-ray absorption spectrum typically extends from approximately 50 eV below the absorption edge (E_0) to about 100 eV above it. The position and shape of the absorption edge are strongly influenced by the oxidation state, coordination environment of the absorbing atom, and the chemical nature of the ligands (Iglesias-Juez et al., 2022). Below the absorption edge, the spectrum is relatively linear and smooth; when the edge is reached, a sharp increase known as the “edge jump” occurs, followed by oscillations at higher energies. In transition metals, hybridization between oxygen p orbitals and metal d orbitals generates accessible electronic states that give rise to pre-edge spectral features (Iglesias-Juez et al., 2022). Because the position of the absorption edge changes systematically with the oxidation state of the element, XANES analysis can be used to identify the valence state and chemical environment of the element under study. Furthermore, due to the high penetration power of hard X-rays, XANES is essentially a bulk analytical technique, where the measured spectrum represents the combined contribution of all chemical species of the selected element present in the sample (Etschmann et al., 2014; Hettiarachchi et al., 2017; Iglesias-Juez et al., 2022). Therefore, quantitative analysis is typically performed by comparing experimental spectra with reference spectra of known compounds, often using approaches such as linear combination fitting (LCF) (Ravel & Newville, 2005)

Extended X-ray absorption fine structure spectroscopy (EXAFS)

The extended X-ray absorption fine structure region of the X-ray absorption spectrum extends approximately from 50 eV to about 1000 eV above the absorption edge (E_0). In this region, photoelectrons emitted from the absorbing atom interact with neighboring atoms, producing scattering effects that generate oscillations in the absorption spectrum (Iglesias-Juez et

al., 2022; Kuzmin & Chaboy, 2014). These oscillations arise from constructive and destructive interference between outgoing and backscattered photoelectron waves. Analysis of the EXAFS signal provides detailed information about the local atomic structure, including interatomic distances, coordination numbers, and structural disorder around the absorbing atom. To simplify interpretation, EXAFS data are typically transformed from the wavenumber domain (k) to the radial distance domain (R) using a Fourier transform, allowing the identification of coordination shells surrounding the absorber. Because EXAFS probes the local structure of elements in complex materials, it is widely used to study trace metal speciation in environmental systems, including their association with minerals, oxides, and organic matter in soils (Iglesias-Juez et al., 2022). Therefore quantitative analysis is typically performed by comparing experimental spectra with reference spectra of known compounds, often using approaches such as linear combination fitting (LCF) (Ravel & Newville, 2005).

Micro-X-ray fluorescence (μ -XRF), micro-X-ray absorption spectroscopy (μ -XAS), and micro-X-ray diffraction (μ -XRD)

These techniques allow to investigate the chemical composition, speciation, and structural properties of materials at micro-to nanoscale resolution, which is essential for studying complex environmental and geological samples (Sutton et al., 2017). These methods enable the identification of elemental distributions, oxidation states, and mineral phases within heterogeneous samples. In environmental and soil science, these techniques are particularly useful for determining the microscale distribution and chemical speciation of trace metals in soils, sediments, and biological materials. These advances have expanded the application of synchrotron techniques in environmental research, allowing scientists to better understand metal-mineral interactions, contaminant speciation, and geochemical processes at the microscale. Such

capabilities are critical for evaluating contaminant mobility, mineral transformation processes, and remediation mechanisms in complex natural systems (Etschmann et al., 2014; Hettiarachchi et al., 2017; Sutton et al., 2017).

Part II. Proximal soil sensors for soil characterization

Importance of soil characterization in agricultural systems

The intensive use of natural resources in agriculture, together with the need to manage them more efficiently, has driven the development of management systems aimed at optimizing agricultural productivity while simultaneously reducing environmental impacts. In this context, essential nutrients such as phosphorus (P) and nitrogen (N) play a fundamental role in crop production; however, their excessive use can generate significant environmental issues. For example, the accumulation of P in agricultural soils can promote its transport to surface water bodies, contributing to eutrophication processes mainly associated with agricultural runoff (Sharpley et al., 2001). Similarly, excessive N applications in agricultural systems can lead to nitrate (NO_3^-) leaching, which results in contamination of groundwater and surface waters when applications exceed crop demand (Di & Cameron, 2002). In addition, soil organic matter (SOM) is a key indicator of soil health, as it is closely related to multiple physical, chemical, and biological processes that regulate nutrient availability, soil structure, and microbial activity. SOM significantly contributes to improving plant available water capacity and increasing the availability of essential nutrients. However, when soil organic carbon (SOC) levels decline below certain critical thresholds, soil functionality may be compromised, negatively affecting soil structure, water retention, and crop productivity (Lal, 2020). Likewise, soil moisture is another critical factor controlling water availability, nutrient transport, and plant growth.

However, soil moisture often exhibits high spatial variability within a single agricultural field due to differences in soil texture, topography, vegetation cover, and management practices (Vereecken et al., 2014). Several studies have shown that soil moisture exhibits high spatial variability, requiring sufficient sampling density to accurately characterize field-scale conditions (Vereecken et al., 2014). This variability is also observed in other physical and chemical properties of soil. For instance, studies conducted at the Gunegal Research Farm have shown that properties such as clay content, bulk density, porosity, and water holding capacity can vary considerably within the same agricultural area depending on land use. Therefore, the accurate characterization of soil properties is essential for guiding land-use planning, improving soil management practices, and implementing effective control measures that support sustainable crop growth and maximize land productivity (Ramesh et al., 2008). Overall, these results demonstrate that soil physicochemical properties exhibit marked spatial variability, which can directly influence water dynamics, nutrient availability, crop productivity, and environmental risks associated with agricultural management

Traditional soil sampling methods for soil physicochemical characterization

Historically, most soil properties have been determined through traditional wet chemical analyses (laboratory test) of collected soil samples, where soil sampling is a key factor in generating field-specific information that support precise agronomic decisions-making. In this context, proper sampling ensures that soil test results are accurate, representative, and reliable (Marcaida et al., 2025). However, numerous studies have shown that many soil properties, such as organic carbon, total nitrogen, nitrate, microbial biomass, and bulk density, exhibit pronounced spatial variability even within the same agricultural field. This variability, which may occur at different spatial scales, is controlled by both inherent soil characteristics and

agricultural management practices (Cambardella et al., 1994; Kuang et al., 2012). Because of this variability, systematic sampling approaches such as grid sampling have been recommended, where samples are collected at regular spatial intervals (e.g., approximately 1 hectare per grid cell) across the field to improve sample representativeness and better characterize soil variability within agricultural systems (Virk et al., 2024). In this context, Mueller et al. (2004) demonstrated that intensive sampling strategies enable the generation of more accurate maps, facilitating variable-rate fertilizer application and more efficient nutrient management at the field scale. Nevertheless, conventional sampling approaches remain limited because the number of samples collected in situ typically represents only a small portion of the total field area, restricting the ability to adequately capture soil spatial variability. This underscores the need for soil characterization methods that provide rapid, accurate, and spatially explicit assessments of soil properties to improve agricultural management and minimize environmental risks.

Precision agriculture and the need for rapid soil assessment

Precision agriculture leverages rapidly evolving information and electronic technologies to enhance soil management and agronomy practices. Soil sensors are practical tools increasingly used for in situ characterization of several chemical and physical soil properties (Ji et al., 2019). Soil sensors generate large datasets across entire fields, providing a significant advantage over traditional soil sampling and laboratory analysis methods. For example, by capturing spatial variability within fields, soil sensors enable the collection of high-resolution data reflecting interactions among physical and chemical soil properties. This enhanced information supports more precise and efficient site-specific soil management and agronomic decision-making (Kuang et al., 2012; Sudduth et al., 2013). Soil sensors have shown several advantages in predicting key physical and chemical properties, such as exchangeable nutrients, pH, soil organic carbon (SOC),

total nitrogen (TN), soil texture (sand, silt, and clay %), cation exchange capacity (CEC), and soil moisture content (Pan et al., 2014). Among these, soil pH is particularly important. Soil pH plays a key role in numerous soil processes, directly influencing the recycling and availability of nutrients for crops, as well as the mobility and distribution of potentially toxic compounds within the soil profile (Neina, 2019). Likewise, assessing accurate real-time data on the availability of major soil nutrients allows for more efficient fertilization and helps to prevent overapplication. For example, in a study conducted by Ji et al. (2019), where using the visible and near-infrared reflectance spectrometer (Spectra) demonstrated a high predictive capability for key variables such as soil organic matter (SOM), as well as exchangeable calcium (Ca), magnesium (Mg), and aluminum (Al), achieving coefficients of determination $R^2 > 0.85$ in all cases. Similarly, the gamma-ray spectroscopy (Gama-ray) sensor showed comparable performance in predicting SOM and available Mg ($R^2 > 0.80$). The DUALEM sensor, based on electromagnetic measurements of apparent soil conductivity, also produced good results for estimating available Ca, Mg, K, and SOC, highlighting its sensitivity to exchangeable cation concentrations ($R^2 > 0.79$). Additionally, the proximal electrical conductivity sensor (ECP) proved useful for predicting Ca, Mg, and Al, although with lower accuracy than Spectra ($R^2 > 0.80$). Similarly, a study conducted by Pinheiro et al. (2017) in the central region of the Brazilian Amazon, using laboratory-based VIS-NIR spectral measurements, found that VIS-NIR spectroscopy was an effective technique that allowed for accurate prediction of clay content (%) and soil organic carbon (SOC), achieving coefficients of determination greater than 0.70 ($R^2 > 0.70$). These results highlight the potential of VIS-NIR spectroscopy as a fast, cost-effective, and reliable tool for characterizing soil properties in complex tropical environments. However, a study conducted by Heiniger et al. (2003) across 16 agricultural fields found that apparent soil electrical

conductivity (EC_a) did not accurately predict nutrient levels such as P, K, Ca, Mg, Zn, and Cu ($R^2 < 0.50$). However, when these nutrients were closely associated with properties that strongly influence EC_a (e.g., texture, CEC, moisture content, and dissolved salts), moderate-to-strong correlations were observed (R^2 values between 0.51 and 0.75). Additionally, subdividing fields by similar soil texture improved prediction accuracy (Heiniger et al., 2003). In a similar approach, Corwin & Lesch. (2005) demonstrated that stratifying agricultural fields into homogeneous texture zones, defined through a combination of EC_a measurements, topographic attributes, and soil sampling, significantly improved the predictive performance of spatial models of soil physicochemical properties (e.g., pH, salinity, nutrient availability, and texture). By reducing within-zone variability, this method enhanced calibration accuracy, minimized the influence of local anomalies, and allowed for more reliable interpolation across management units. However, the use of sensors to estimate soil physicochemical properties is not without limitations. In this context, several studies have shown that soil texture, pH, cation exchange capacity (CEC), salt concentration, and moisture content can significantly affect the signals recorded by technologies such as apparent electrical conductivity (EC_a), visible (VIS), and near-infrared spectroscopy (NIR), among other proximal sensing tools (Corwin & Lesch, 2005). In addition, the effective sensing depth varies depending on the sensor type. Optical sensors (e.g., VIS-NIR) are typically limited to the soil surface and capture information from only the upper few millimeters, whereas conductivity-based sensors (e.g., EC_a) integrate signals over greater depths, commonly ranging from approximately 0 to 30 cm for shallow measurements and up to 90 cm depending on sensor configuration. As a result, these sensors generally provide surface or depth-integrated information rather than resolving discrete soil horizons, which limits their ability to directly characterize detailed soil profiles.

Factors influencing spectral responses

Several soil properties can influence the spectral responses of soil sensors, affecting the accuracy of soil property predictions. Factors such as high clay content, elevated soil pH, and soil compaction can attenuate sensor signals by increasing radiation absorption and reducing reflectance. In addition, soil organic matter and iron oxides strongly influence spectral behavior, as the visible region is particularly sensitive to iron oxides and humic substances, while the near-infrared region responds to functional groups associated with organic compounds such as carbonyl, phenolic, aromatic, carbohydrate, and peptide bonds (Viscarra Rossel & Hicks, 2015). However, environmental and soil physical conditions, particularly soil moisture variability, texture, and bulk density, can significantly reduce model accuracy used to estimate soil physicochemical properties (e.g., pH, SOC, nutrients) from spectral and conductivity-based sensor data during in situ measurements (Hutengs et al., 2019). Changes in soil composition may also affect spectral signals, as variations in organic matter and mineral content across soil horizons can modify reflectance patterns (Zhang et al., 2024). Furthermore, predictions of certain soil nutrients, such as exchangeable P, have shown inconsistent performance across studies, highlighting the strong influence of site-specific conditions on spectral models (Udelhoven et al., 2002; Wenjun et al., 2014). Overall, these findings demonstrate that both soil physicochemical properties and environmental conditions can strongly affect sensor responses, emphasizing the need to account for these factors when interpreting spectral data for soil characterization.

Spectral data preprocessing

Spectral preprocessing is a critical step in proximal soil sensing because raw spectral signals are often affected by measurement noise, non-linearities, and variations in soil physicochemical properties (Stenberg et al., 2010). In diffuse reflectance spectroscopy, factors

such as soil structure, particle size distribution, and surface roughness influence light scattering, meaning that measured reflectance is not always directly proportional to chemical absorbance. To improve data quality and model stability, several preprocessing procedures are commonly applied. These include multiplicative scatter correction (MSC), baseline correction, first-and second-derivative transformations, and standard normal variate (SNV) transformations with detrending. Noise reduction techniques such as moving averages, median filters, and the Savitzky-Golay smoothing algorithm are also frequently applied to stabilize spectral signals (Savitzky & Golay, 1964). For example, Wenjun et al. (2014) applied VIS-NIR spectroscopy combined with Savitzky-Golay smoothing (window size of 11 of spectral data points and polynomial order of 2) and demonstrated that laboratory-based spectra could accurately predict soil properties such as soil organic matter (OM), organic carbon (OC), total nitrogen (TN), available nitrogen (AN), and pH using partial least squares regression (PLSR). Similarly, Pei et al. (2019) evaluated the Veris P4000 sensor for collecting in situ diffuse reflectance spectra, along with apparent electrical conductivity (EC_a) and cone index (CI) measurements, down to 1 m depth. Their results indicated that preprocessing methods such as Gaussian window smoothing and SNV transformations yielded similar prediction accuracies, whereas the choice of modeling methods had a stronger influence on model performance. Finally, Lee et al. (2009) suggested removing measurements at lower visible wavelengths due to their low signal-to-noise ratio and excluding outlier samples prior to model development. Spectral data are then typically transformed from reflectance to absorbance using the $\log_{10}(1/R)$ transformation, followed by normalization and smoothing procedures to reduce scaling effects and spectral noise. Overall, these findings highlight that although spectral preprocessing improves signal stability and model

performance, the effectiveness of prediction models remains strongly influenced by site-specific soil conditions and management practices.

Model validation

Estimating the predictive accuracy of models generated by supervised learning algorithms is essential not only for evaluating their future performance but also for supporting model selection and improving predictive reliability (Wolpert, 1992). An appropriate validation strategy should provide estimates with low bias and variance, which is why cross-validation has become one of the most widely used approaches for evaluating predictive models (Kohavi, 1995). In this method, the dataset was divided into subsets, or folds, which were used sequentially for model calibration and validation. Because model performance can vary with how the dataset is partitioned, repeating the validation process with different random splits improves the robustness of accuracy estimates and can be interpreted as a Monte Carlo approximation to complete cross-validation. At the same time, spectral datasets often contain a large number of highly correlated variables, which can introduce noise and reduce model performance if all wavelengths are included in the calibration process. For this reason, feature selection methods are commonly applied to identify the most informative variables and remove irrelevant spectral information, thereby improving model accuracy, stability, and computational efficiency (Shi et al., 2023). Together, model validation and feature selection constitute essential steps in spectral modeling, ensuring that predictive models are robust, reliable, and capable of accurately estimating soil properties across different environmental conditions. Overall, this result highlights that the performance of proximal soil sensors is strongly influenced by multiple sources of variability, including soil physicochemical properties, environmental conditions, and spatial heterogeneity within agricultural fields. These factors can significantly affect sensor

signals and the accuracy of soil property predictions. Consequently, appropriate spectral data processing and feature selection are essential for reducing noise and enhancing the relevant information in spectral datasets. In addition, robust model validation approaches, such as cross-validation and Monte Carlo methods, are critical for ensuring reliable and transferable predictions of soil properties across different soil and management conditions.

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Chapter 3 - Efficacy of organic and inorganic phosphorus amendments in reducing bioaccessibility of lead in urban soils

Abstract

Lead (Pb) contamination in urban soils is a significant global public health concern, with soil Pb concentrations often exceeding the current screening level for residential properties (200 mg kg⁻¹). In contaminated urban areas, reducing Pb bioaccessibility is critical to mitigating its health risks. This research investigated the effectiveness of organic and inorganic phosphorus (P) amendments on bioaccessibility, chemistry, and soil Pb speciation. Soils were collected from six urban sites in Kansas City, MO, exhibiting varying physicochemical characteristics, and amended with organic P (Class A biosolids at 10%) and inorganic P (commercial fertilizers) at a 1:4 molar ratio of Pb: P. Additionally, a control treatment without P application was included. The results indicated that bioaccessible soil Pb was strongly correlated with soil pH and exchangeable-P. Lead bound to labile fraction was lower in all P-treated soils than the control ($p < 0.05$). Phosphorus treatments facilitated the redistribution of Pb into more stable soil fractions, particularly the Pb-residual fraction, suggesting enhanced Pb immobilization via sorption processes. X-ray absorption near edge spectroscopy analysis confirmed that P treatments increased the formation of pyromorphite minerals by an average of 1.34-fold compared to the untreated control, in which minimal pyromorphite group formation was observed. These findings demonstrate that phosphorus amendments are an effective strategy for reducing bioaccessible Pb and mitigating the associated human health risks in urban soils.

Introduction

Soil lead (Pb) is the most common and widespread environmental contaminant in urban areas (Brown et al., 2016). While Pb occurs naturally in soils with an average concentration of about 10 mg kg⁻¹ (Lindsay, 1979). However, in contaminated urban areas, soil Pb levels can frequently exceed the current U.S. Environmental Protection Agency (EPA) screening level for Pb in soil at residential properties (~200 mg kg⁻¹) (EPA, 2024). In addition, in some hotspots, the total soil Pb concentration can exceed 1000 mg kg⁻¹ (Zahran et al., 2013). Urban soil Pb primarily originates from the past use of leaded gasoline and lead-based paint (Ahmad et al., 2021; Zimdahl et al., 1973). Lead has been identified as a toxic element to humans, and elevated soil Pb concentration is one of the primary pathways of exposure to Pb in humans, especially for children whose nervous systems are still in the developmental stage (Centers for Disease Control and Prevention (CDC), 2012). In the U.S., young populations (> 6 years old) living near Pb-contaminated areas showed elevated blood Pb levels (BLLs) in 20-60% of cases, increasing the risk of adverse neurodevelopmental effects, such as IQ loss, which may persist into the second decade of life (CDC, 2012; Laidlaw et al., 2017). Since no safe BLLs have been identified in infants or children, soil Pb remains an urgent public health concern worldwide (CDC, 2012). Remediation of Pb-contaminated soil using chemical stabilization methods is considered one of the most cost-effective mitigation strategies (Hettiarachchi & Pierzynski, 2004; Mayer et al., 2022; Scheckel et al., 2013; Weber et al., 2015). Soil amendments can reduce in-situ Pb bioavailability through various sorption processes, including adsorption onto mineral surfaces, formation of stable complexes with organic ligands, surface precipitation, and modifications in soil chemical speciation (Kumpiene et al., 2008). The use of phosphorus (P) amendments to immobilize soil Pb is a widely recognized practice (Henry et al., 2015; Hettiarachchi &

Pierzynski, 2002, 2004). The application P sources effectively reduce Pb bioavailability by interacting with free Pb ions to promote the in-situ formation of lead-phosphate compounds, mainly as pyromorphite group. These minerals show significantly lower solubility than other Pb species under typical environmental pH conditions (Baker et al., 2014; Hettiarachchi & Pierzynski, 2002; Lindsay, 1979; Mayer et al., 2022). For example, Weber et al. (2015) demonstrated, using X-ray absorption near-edge spectroscopy (XANES), that soils amended with triple superphosphate (TSP) showed an increase in the formation of chloropyromorphite ranging from 29% to 35% at P: Pb molar ratios of 4:1 and 16:1, which was higher than untreated soils. Likewise, Scheckel & Ryan (2004) using X-ray absorption fine structure analyses (XAFS) showed that, when 3.2% of P as TSP, pyromorphite formation reached up to 41%. Additionally, using 2.5% of iron-rich material (IR) mixed with 1% of P as TSP resulted in approximately 41% pyromorphite formation. Finally, when 1% of P was phosphoric acid (H_3PO_4), pyromorphite formation ranged from 43% to 45% compared to the control over the 3-to 32-month period. At the same time, Alankarage et al. (2024), using TSP at 0.85, 1.7, and 2.5 Pb:P rates, observed reductions in soil Pb bioaccessibility of 10.2%, 13.1%, and 15.8%, respectively, compared to untreated soil. Likewise, Gu et al. (2020) observed that, TSP, struvite, and phosphoric rock (PR) decreased Pb bioavailability by around 10 to 18% compared to the control soil. Additionally, Yang & Mosby (2006) found significant reductions of ~59% in soil Pb bioaccessibility after H_3PO_4 applications. However, no consensus exists on selecting the most suitable phosphate material for a given contaminated site. Some liquid phosphate sources, such as H_3PO_4 , react quickly but are highly mobile, making them unsuitable for sites where surface water or groundwater contamination is a concern. Alternatively, ammonium polyphosphate (APP) provides a liquid phosphate source that can alter soil pH over a relatively long period compared

with other acidulating P sources, owing to its polyphosphate content. On the other hand, solid phosphates, typically various forms of calcium phosphates, are less soluble, reducing the risk of surface and groundwater contamination. However, they may be less effective in converting soil Pb into pyromorphite (Scheckel et al., 2013). Lake & Basta (2024), in a short-term incubation study (3 months), found that when P sources were applied in the form of class A biosolids incinerator ash, biosolids + compost, poultry litter, and TSP, there were no statistical differences in Pb bioaccessibility relative to the control. These results were observed using two different extraction procedures: PBET at pH 2.5 and US EPA 1340 at pH 1.5 (IVBA), showing slight variations in overall IVBA Pb% reductions. The biosolids + poultry litter treatment was the only significant treatment, showing reduction on Pb bioaccessibility from 8.53% in IVBA to 16.1% in PBET. Despite extensive research on Pb mitigation using phosphorus amendments, limited information is available specifically for urban soils and for direct comparison between inorganic P sources (monoammonium phosphate [MAP], diammonium phosphate [DAP], and ammonium polyphosphate [APP] and organic P sources (Class A biosolids) as treatments for Pb-contaminated urban soils. This study evaluates the effectiveness of organic and inorganic P amendments in reducing Pb bioaccessibility, ii) their impact of P amendments on the Pb chemistry, fractionation, and soil Pb speciation, and iii) the relationship between bioaccessible Pb (%) and key soil properties. By integrating these components, this study addresses critical gaps in the literature, where previous research has often examined Pb immobilization, soil physicochemical controls, and amendment performance independently. In contrast, this work provides a unified framework that links amendment type, soil properties, and Pb speciation to bioaccessibility outcomes, particularly in urban soils where such interactions remain poorly understood.

Materials and Methods

Laboratory incubation study

This research was conducted using soils from six urban sites, in Kansas City, MO (Table 3.1), each exhibiting different physicochemical characteristics. At all sites, approximately 15 kg of bulk soil from a depth of 0 to 15 cm was collected from select areas, homogenized in plastic buckets, and placed into plastic bags. Before initial experiments, bulk soil samples were air-dried, ground, and sieved through a stainless-steel mesh (< 2 mm). For the incubation study, soil sub-samples (200 g of < 2 mm bulk soil) were used in triplicate and placed in 250 mL low-density polyethylene (LDPE) containers. Organic amendments in the form of class A biosolids at 10% from three different locations, including Lawrence, KS (BS-LW), Saint Joseph, MO (BS-SJ), and Washington, D.C. (BS-WDC), as well as inorganic P as commercially available fertilizers at 1:4 rate based on molar ratio Pb:P in the form of monoammonium phosphate (MAP), diammonium phosphate (DAP), triple super phosphate (TSP) and ammonium polyphosphate (APP) were applied. There was also a control treatment with no P application. All treatments were applied in a completely randomized design (CRD) with three replicates per site. Treatments were thoroughly mixed, and deionized water was added to bring the soil samples to 30% of their original moisture content. The soils were then thoroughly remixed. Samples were covered with a double layer of parafilm and incubated at 25°C for the desired period (48 weeks). To evaluate the treatment effect over time, ~30 g of soil subsamples were collected at 1, 2, 4, 12, 24, and 48 weeks after treatment application. Before chemical analysis, soil samples were dried at 40 °C, ground, and sieved through two stainless-steel meshes (2 mm and 250 μ m).

Table 3.1. Location and geographic coordinates of sampling sites in Kansas City, MO.

Site	Address	Latitude (N)	Longitude (W)
1	2501 Chestnut Ave	39.08036	-94.55039
2	2703 E 25th St & Montgall Ave	39.08053	-94.5508
3	3200 E 24th St & Terrace Ave	39.08206	-94.54518
4	3928 Garfield Ave	39.05471	-94.55968
5	3620 Wabash Ave	39.0603	-94.55479
6	4205 Brooklyn Ave	39.05017	-94.55801

Physicochemical soil properties

Soil pH was measured using a pH/conductivity meter (Thermo Scientific Orion glass electrode, Beverly, MA) with Milli-Q water ($\sim 18 \text{ M } \Omega \text{ H}_2\text{O}$) in a ratio 1:2.5 soil-to-water, previously calibrated in buffer solutions (Thomas, 1996). Total organic carbon (TOC, %) and total nitrogen (TN, %) were determined by dry combustion method using a LECO TruSpec CN combustion analyzer. Soil texture was measured using the hydrometer method (Sikora & Moore-Kucera, 2014). Cation Exchange Capacity ($\text{CEC cmol}_{(+)}/\text{kg}$) was obtained by displacement method (Peters, et al., 2015). Total soil Pb, iron (Fe), and manganese (Mn) concentrations were obtained using the EPA 3051a method. Finally, exchangeable-P was obtained using Mehlich-3 extraction (Frank et al., 1998).

Mineralogical analysis of soil-clay fraction

Bulk X-ray diffraction (XRD) analysis was conducted on untreated soil samples from 2501 Chestnut Ave, 2703 E 25th St & Montgall Ave, and 3200 E 24th & Terrace Ave to preserve the natural interactions between soil clay minerals and Pb without external chemical modification. The clay-size fraction ($< 2 \text{ } \mu\text{m}$) was separated following the method described by Jackson. (1975). X-ray diffraction analyses were performed using an automated multipurpose powder X-ray diffractometer (XRDynamic 500) with copper (Cu) radiation. To assess clay

mineralogy, layer expansion, interlayer properties, and their influence on Pb bioavailability, soil samples were treated with Mg-saturated (Mg-25) and ethylene glycol (EG) at 25°C. X-ray diffraction spectral fitting was performed using a Python algorithm developed in a Jupyter Lab notebook by the authors, based on an initial mineral database containing the most common soil minerals (Harris & White, 2008). The fitting process involved identifying peak positions (2θ values) and intensities for each mineral phase and matching them against reference positions in the database. The percentage abundance of each mineral phase was estimated using a semi-quantitative approach based on the reference intensity ratio (RIR) method across the three sites. This method calculates mineral percentages based on peak intensities, as shown in equation 3.1. Where I_m is the peak intensity of the mineral, RIR_m is the reference intensity ratio for the mineral, and $\sum(I_i/RIR_i)$ is the sum of all intensity-to-RIR ratios for identified minerals.

Equation 3.1. Equation used to estimate the relative abundance of mineral phases (%) from X-ray diffraction data using peak intensity and reference intensity ratio (RIR) normalization.

$$\text{Mineral (\%)} = \left(\frac{I_m/RIR_m}{\sum I_i/RIR_i} * 100 \right)$$

Bioaccessible soil Pb

The bioaccessible soil Pb was assessed using a modification of the physiologically based extraction test developed by Ruby et al. (1996), as modified by Brown et al. (2003). Briefly, the gastric solution was prepared by dissolving 1.25 g pepsin powder, 500 mg sodium-L-malate, 500 mg citrate, 500 μ L acetic acid, and 420 μ L lactic acid in approximately 500 mL Milli-Q water in a 1-L volumetric flask. After thorough mixing, the pH was adjusted to 2.5 using trace metal-grade concentrated hydrochloric acid (HCl), and the volume was brought to 1 L with Milli-Q water. Soil, sieved to 250 μ m, was mixed with the prepared gastric solution at a 1:100 ratio. For

testing, 1 g of soil was weighed into a 250 mL LDPE bottle, and 100 mL of preheated gastric solution at 37°C was added. The mixture was shaken at 32 ± 2 rpm for 1 hour at 37°C. The pH of the soil-gastric solution mixture was verified every 30 minutes and adjusted as needed using HCl. Subsequently, the final soil-solution pH was measured to confirm that it remained at 2.5 ± 0.15 ; the extract was then filtered through 0.45 μ m syringe filters. Samples were stored in scintillation vials in a cold room at 5°C.

For comparison purposes, the bioaccessible soil Pb was determined using the in vitro bioaccessibility assay (IVBA) based on EPA Method 1340 (pH 2.5). A complete set of samples collected at 48 weeks was used for the assay. The extraction solution was prepared by dissolving free-base glycine in Milli-Q water to a final concentration of 0.4 M, and the pH was subsequently adjusted to 2.50 ± 0.05 using trace metal grade HCl. For each sample, exactly 1.00 ± 0.05 g of sieved soil ($<250 \mu$ m) was weighed into 125 mL HDPE bottles. Then, 100 ± 0.5 mL of the preheated extraction fluid (37 ± 2 °C) was added. The samples were continuously rotated for 1 hour at 37 °C to simulate gastrointestinal conditions. After the extraction period, the bottles were removed and left undisturbed to allow solid particles to settle. The pH of the supernatant was measured to confirm it remained within 2.5 ± 0.5 . Finally, 25 mL of supernatant was filtered through 0.45 μ m syringe filters, and the extracts were stored in scintillation vials at 5 °C.

Bioaccessible soil Pb was quantified by a coupled plasma optical emission spectrometer (ICP-OES, Agilent 5800, Agilent Technologies). Reproducibility was assessed using duplicate samples, and quality assurance was ensured through the systematic inclusion of a certified reference material (NIST 2711a), analyzed in every fourth set of PBET extractions. The results showed a coefficient variation (CV) between duplicates ranging from 0.01% to 25.4%, with a mean value of 6.12%. Similarly, the certified PBET-extractable Pb concentration for the 2711a

material was $50.2\% \pm 0.01$, as reported by Obrycki et al. (2017), and was used to calculate recovery rates, yielding values of $109.9 \pm 20.4\%$ (mean $\pm$ standard deviation).

Total soil Pb concentration

Total soil Pb concentration was determined using a portable X-ray fluorescence (NitonTM XL3t XRF analyzer, Thermo Scientific) connected to a radiation shielding chamber (XL2 Portable Test Stand PN 420-017, Thermo Scientific), following a modified EPA 6200 method. Briefly, soil samples, previously sieved through a 2 mm mesh, were placed into 4 x 4 x 0.02 cm Ziplock plastic bags and compacted to form a uniform sample. Continuous readings were then conducted for 2 minutes, and Pb concentration (mg kg^{-1}) was recorded for each sample. Additionally, to validate the data obtained by p-XRF, the total soil Pb concentration was analyzed using the EPA 3051a method on random samples from each site with a coupled plasma optical emission spectrometer (ICP-OES, Agilent 5800, Agilent Technologies) after microwave-assisted digestion (MARS Xpress 907501, Matthews, NC). Reproducibility was assessed by analyzing duplicate samples every 25 digestions, resulting in a coefficient of variation (CV) between duplicates ranging from 2.33% to 14.1% (mean 6.49%, $n = 17$). Quality assurance was ensured by including certified reference materials in each digestion batch. The certified Pb concentration ($1300 \text{ mg kg}^{-1} \pm \text{uncertainty}$) from NIST 2711a was used to calculate recovery, with measured recoveries of $103.3 \pm 8.78\%$ (mean $\pm$ SD).

Sequential extraction of Pb

Soil Pb was fractionated into five operationally defined pools: exchangeable, carbonate, Fe and Mn oxides, organic, and residual, following the modified Tessier et al. (1979) sequential extraction scheme (Table 3.2). The exchangeable and carbonate fractions were combined to represent the labile fraction.

Table 3.2. Summary of the sequential extraction steps and procedures of soil Pb fractionation.

Step	Target Fraction	Reagent	Soil/solution (w/v)	Experiment Conditions
1	"Exchangeable"	1 M MgCl ₂ at pH 7.0	1:12.5	Shake for 1 hour
2	"Carbonate-bound"	1 M NaC ₂ H ₃ O ₂ at pH 5.0	1:12.5	Shake for 2 hours
3	"Fe-Mn oxide-bound"	0.04 M NH ₂ OH(HCl) in 25% (v/v) HC ₂ H ₃ O ₂	1:2	Shake for 6 hours at 96°C
		0.02 M HNO ₃ – 30% H ₂ O ₂	1:3	Shake for 2 hours at 85°C
		30% H ₂ O ₂	1:5	Shake for 2 hours at 85°C
4	"Organic-bound"	30% H ₂ O ₂	1:3	Shake for 3 hours at 85°C
		After cooling, 3.2 M NH ₄ C ₂ H ₃ O ₂ in 20% (v/v) HNO ₃ and deionized water	1:5	Shake for 30 min
5	"Residual"	4 M HNO ₃	1:1	Shake for 2 hours at 80°C

Between extraction steps, the samples were centrifuged at 2000 rpm for 30 minutes to separate the supernatant, which was then decanted and filtered using Whatman quantitative filter papers (Ashless, Grade 42). The filtered supernatant was stored in scintillation vials at 5°C in a cold room. The residue was subsequently rinsed with 8 mL of Milli-Q water to remove any remaining solution before proceeding to the next extraction step. Lead concentrations in the different soil fractions, except exchangeable-bound, were quantified by an ICP-OES. Soil Pb concentration bound to exchangeable fraction was quantified by Varian GTA 120 graphite tube atomizer w/AA 240Z Zeeman atomic absorption spectrometer (GF-AAS).

Speciation of soil Pb

For speciation, X-ray absorption near edge structure spectroscopy data collection was performed under ambient conditions across the Pb L(III)-edge (12,005 eV) at the Stanford Synchrotron Radiation Lightsource (SSRL), located on the Stanford Linear Accelerator Center (SLAC) National Accelerator Laboratory, located at Stanford University. Using soils sampled at 48 weeks, from site 3 (3200 E 24th St & Montgall Ave) and site 6 (4205 Brooklyn Ave), a composite sample was prepared by combining equal amounts of soil (<250 µm) from three replicates. The combined sub-samples were packed into specific sample holders, covered with Kapton tape, and analyzed at beamline 11-2 using grazing incidence X-ray absorption spectroscopy equipped with collimating and focusing optics, a “double-double” Si(220) LN₂-cooled monochromator, and a 100-pixel monolithic solid-state Ge detector array. The X-ray absorption near edge structure spectra measurements for all samples were repeated at least three times to ensure high reproducibility and smooth spectra. The raw XANES spectra were aligned and calibrated using Athena software (Ravel & Newville, 2005).

Linear combination fitting (LCF) was subsequently performed. The data processing for LCF involved the following steps: spectral background removal, normalization of each spectrum to its edge step, and alignment of sample and standard spectra to correct for energy drifts. The best-fit combination of reference compounds was determined based on the lowest residual value (R-factor).

Statistical analysis

A completely randomized design (CRD) with three replicates was used. All statistical analyses were performed using JMP Student version 19 and JupyterLab notebook software. One-way analysis of variance (ANOVA) was conducted to determine significant differences among the organic and inorganic P treatments. Differences between the treatments were analyzed using Tukey's post hoc test at a significant level of $p < 0.05$. A multivariate correlation analysis was performed to identify the soil physicochemical properties that best correlated with bioaccessible soil Pb. In addition, to validate the total soil Pb concentration obtained using p-XRF, a linear regression analysis was performed by comparing the results with measurements from the 3051a method. Finally, the data were checked to ensure compliance with the assumptions of ANOVA and linear regression.

Results and Discussion

Initial soil physicochemical properties

The initial soil physicochemical properties across the six study sites are summarized in Table 3.3. Total soil Pb concentrations varied considerably, ranging from 419 mg kg⁻¹ (Site 1) to 866 mg kg⁻¹ (Site 3), with this site exhibiting the highest contamination level. The percentage of Pb exceeding the EPA residential soil screening level (200 mg kg⁻¹) followed the same trend. Sites 3 (76%) and 6 (74%) had the highest exceedances, indicating significant Pb contamination.

Additionally, to evaluate the reliability and applicability of p-XRF for determining Pb in urban soils, a comparison was conducted with the standard acid digestion method, EPA 3051a. Figure 3.1 shows the correlation between Pb concentrations obtained by both techniques at six urban sites.

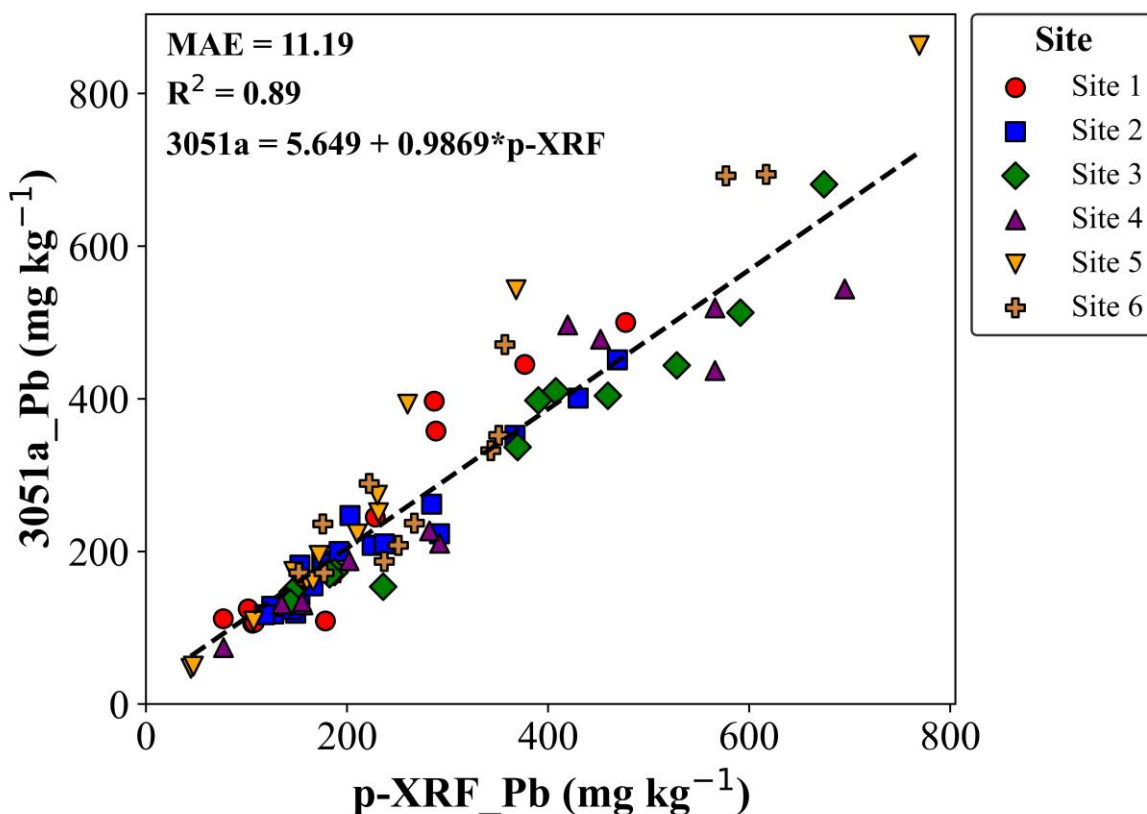


Figure 3.1. Comparison of Pb concentrations (mg kg⁻¹) in urban soils determined by portable X-ray fluorescence (p-XRF) versus the standard acid digestion method EPA 3051a.

Each point corresponds to an individual soil sample, color-coded to differentiate the sampling sites. A strong linear correlation was observed between the two methods ($R^2 = 0.89$), confirming that p-XRF can accurately estimate Pb concentrations in heterogeneous urban soil matrices (ranging from 50 to 800 mg kg⁻¹). Similarly, the low mean absolute error (MAE = 11.19

mg kg⁻¹) demonstrates the reliability and consistency of the p-XRF readings, especially considering the complexity and inherent variability of urban soils (Figure 3.1).

Total Fe concentrations also varied widely, with site 6 exhibiting the highest Fe content, suggesting the presence of Fe-rich minerals that could influence Pb sorption and stabilization. Manganese (Mn) concentrations ranged from 551 mg kg⁻¹ (site 5) to 841 mg kg⁻¹ (site 4), potentially contributing to Pb transformations. Soil pH remained neutral to slightly alkaline across all sites, which could impact the solubility and availability of Pb. Total organic carbon was highest at site 1 (5.79%) and site 2 (5.46%), whereas sites 4, 5, and 6 exhibited lower TOC values (~3.6-3.9%), indicating variations in organic matter content that may affect Pb complexation. Total nitrogen content remained relatively consistent (0.19-0.31%). Cation exchange capacity varied from 14.5 to 23.1 cmol₍₊₎/kg, with site 5 exhibiting the highest CEC (23.1 cmol₍₊₎ kg⁻¹), indicating a greater capacity for cation retention and Pb stabilization. Conversely, site 1 had the lowest CEC (14.5 cmol₍₊₎ kg⁻¹), suggesting potentially less electrostatic attraction, which might lead to higher Pb mobility. The predominant soil textures were loam and silt loam, both of which influenced water retention, nutrient availability, and Pb adsorption (Table 3.3). The variability in Pb contamination and soil properties across the sites underscored the importance of mineralogical composition, organic matter content, Fe/Mn interactions, and CEC in Pb stabilization and remediation strategies.

Mineralogical analysis of soil-clay fraction

The key mineral phases in the clay fractions of the soils were identified by the X-ray diffraction (XRD) analysis, using distinct peaks corresponding to expansible phyllosilicates (montmorillonite, vermiculite) and kaolins (kaolinite) under both treatments: magnesium

saturation at 25 °C (Mg-25 °C) and subsequent verification using solvation with ethylene glycol (EG-25 °C) in three urban soils (Figure 3.2).

Table 3.3. Initial characterization of selected soil properties across different research sites.

Site	Total Pb	Total Fe	Total Mn	Soil pH	TOC [†] (%)	TN [†] (%)	Cation Exchange Capacity (cmol ⁽⁺⁾ /kg)	Soil particle size fraction		
	(mg kg ⁻¹)							Sand (%)	Silt (%)	Clay (%)
1	419	7421	699	7.7	5.79	0.26	14.5	30	57	13
2	534	8996	685	7.9	5.46	0.31	16.6	32	46	22
3	866	6081	744	7.1	4.32	0.29	18.6	23	52	25
4	508	12893	841	7.8	3.87	0.31	20.3	32	48	20
5	483	9179	551	7.3	3.64	0.31	23.1	20	59	21
6	773	27600	-----	8.5	3.65	0.19	15.5	31	48	21

[†]Total organic carbon

[‡]Total nitrogen

The results of the semi-quantitative analysis showed a significant increase in expandable phyllosilicate content following EG-25 °C treatment, with the trend: Site 1 (45.7%) > Site 2 (34%) > Site 3 (28.9%). The presence of a characteristic 14 Å peak, which expanded upon EG treatment, confirms the occurrence of expandable minerals such as montmorillonite and vermiculite (Figure 3.2). Kaolinite was also abundant across all sites, ranging from 29.2% to 35.4%, while micas and oxides/hydroxides were present in moderate proportions. Minor contributions from feldspars and carbonates were observed, particularly at Sites 2 and 3 (Table 3.4). The mineralogical composition of the clay fraction is highly relevant for understanding Pb chemistry in soils. In this context, expandable phyllosilicates play a key role in Pb retention due to their high CEC and reactivity, especially under competitive conditions with other divalent cations such as Ca²⁺ and Mg²⁺. Although kaolinite is non-expandable, it may also contribute to

Pb sorption under near-neutral pH conditions (~ 7.5). When considering soil properties such as pH (5.5-7.5), CEC, TOC (%), and total Pb concentration, Site 3 exhibited the highest potential for Pb immobilization. This site showed elevated total Pb concentrations (866 mg kg^{-1}), relatively high CEC, moderate TOC, and a substantial proportion of reactive clay minerals. In contrast, Sites 1 and 2 contained comparable clay mineral assemblages but lower total Pb concentrations ($\sim 476.5 \text{ mg kg}^{-1}$ on average), suggesting a comparatively lower demand for sorption sites. Clay mineralogical analysis provided additional insights into Pb stabilization, particularly in sites containing vermiculite and montmorillonite, which enhance Pb retention through ion exchange, surface complexation, and interlayer fixation. These findings support previous studies indicating that clay minerals play a crucial role in reducing Pb mobility (Elnajdi et al., 2023).

Table 3.4. Semi-quantitative estimation of major mineral groups in the clay fraction of soils from Sites 1, 2, and 3 under Mg-25 °C. Values represent relative abundances (%) derived from XRD analysis from equation 3.1.

Mineral and Treatment	Site 1	Site 2	Site 3
	(%)		
Expansible phyllosilicates (Mg-25°C)	45.72	34.03	28.9
Kaolins (Mg-25°C)	35.44	29.2	32.1
Micas (Mg-25°C)	12.58	21.3	19.4
Oxides/Hydroxides (Mg-25°C)	6.25	10.9	9.23
Feldspars (Mg-25°C)	0	4.54	6.98
Carbonate (Mg-25°C)	0	0	3.51

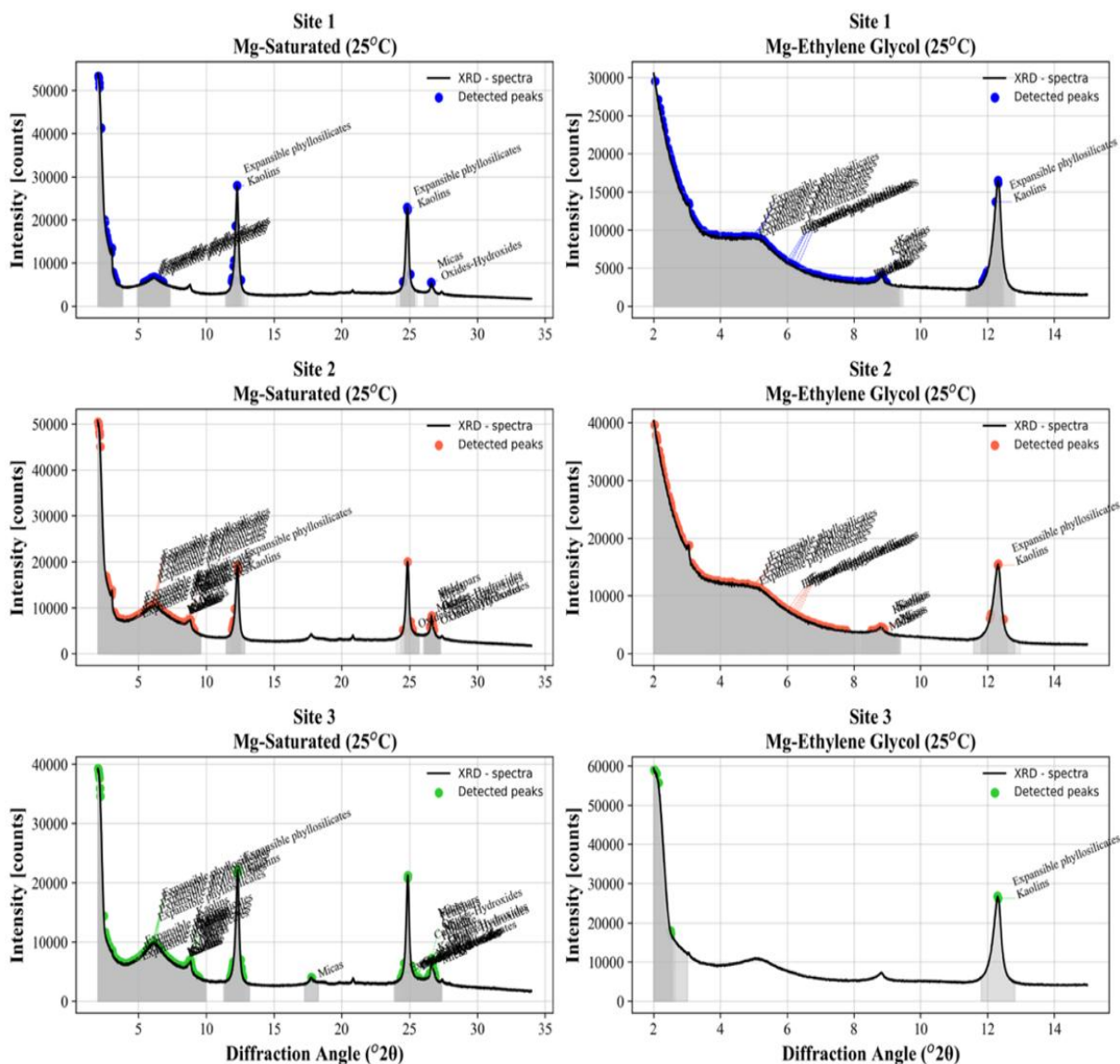


Figure 3.2. X-ray diffraction (XRD) patterns of unamended soils collected from three Pb-contaminated urban sites under Mg-saturated and ethylene glycol at 25 °C treatments. The black curves represent the measured XRD spectra (intensity vs diffraction angle, 2θ), while the colored points (blue for site 1, red for site 2, and green for site 3) indicate the detected peak positions used for mineral identification. The expansion of basal reflection following ethylene glycol solvation confirms the presence of expandable phyllosilicates.

Effect of phosphorus treatments on relevant soil properties

In general, the control soils consistently had a higher pH, particularly at sites 2 and 6, indicating a more alkaline nature than the amended soils (Figure 3.3). Inorganic P treatments

generally moderate soil pH without drastic shifts, while organic amendments exhibited site-dependent pH stabilization effects. However, certain amendments, such as BS-SJ, APP, and BS-WDC, contributed to significant pH reductions, particularly at sites 2 and 3, where pH dropped by up to 1.62 units relative to the control ($p < 0.05$) (Figure 3.3).

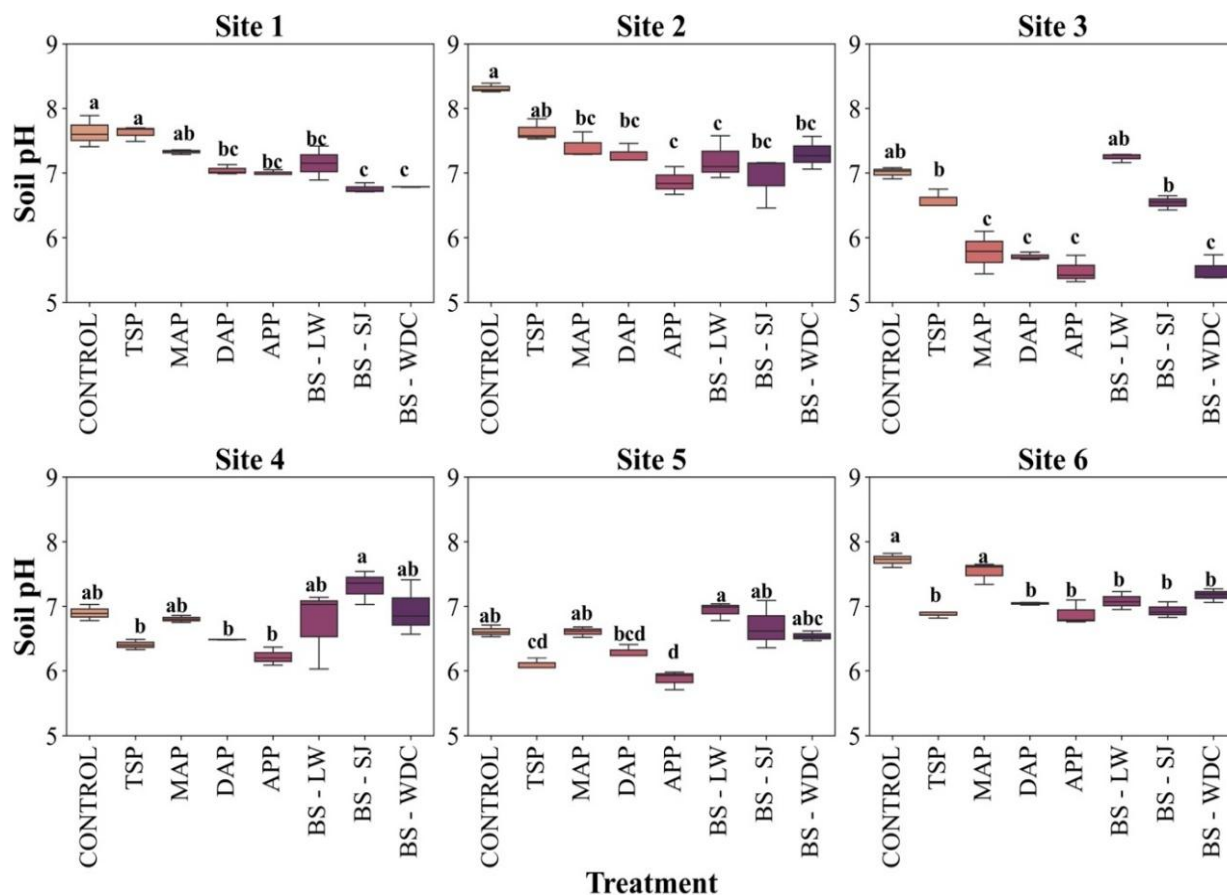


Figure 3.3. Effect of phosphorus amendments on soil pH at 48 weeks across six urban sites. Different letters within each site indicate statistically significant differences between treatments, based on Tukey's post hoc test ($p < 0.05$). Control (untreated soil) TSP (Triple super phosphate); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); APP (Ammonium polyphosphate); BS – LW (Class A Biosolids, Lawrence); BS – SJ (Class A Biosolids, Saint Joseph); BS – WDC (Class A Biosolids, Washington DC)

The observed increase in exchangeable P across amended soils supports this hypothesis, with amendments such as MAP, DAP, and biosolid-based treatments significantly enhancing P availability compared to the control (Table 3.5). Notably, the greatest increases were observed at

site 5, where BS-SJ led to a 6.52-fold rise in exchangeable-P, followed by BS-LW (3.96-fold) and DAP (3.91-fold). Similar trends were evident at sites 3 and 4, where BS-LW, BS-SJ, and APP substantially increased P concentrations.

Table 3.5. Exchangeable phosphorus concentration in six different research sites at 48 weeks.

Treatment	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
CONTROL	46.7±3.99 ^c	94.2±9.97 ^b	57.4±2.53 ^c	105±7.89 ^f	59.8±14.1 ^c	90.3±6.8 ^d
TSP	86.8±13.4 ^{bc}	169±15.6 ^b	212±25.8 ^{ab}	243±3.18 ^{cd}	226±17.3 ^b	232±7.76 ^{ab}
MAP	132±24.6 ^a	141±26.5 ^b	209±13.1 ^{ab}	221±31.7 ^{de}	212±29.2 ^b	198±16.6 ^{bc}
DAP	120±15.6 ^{ab}	169±38.1 ^b	239±33.6 ^a	237±19.3 ^{cd}	234±32.1 ^b	255±8.83 ^{ab}
APP	109±22.6 ^{ab}	151±9.86 ^b	162±10.1 ^b	352±28.4 ^b	198±61.6 ^b	230±33.1 ^{ab}
BS-LW	113±8.98 ^{ab}	116±13.4 ^b	250±9.89 ^a	307±19.8 ^{ab}	237±30.2 ^b	262±41.7 ^a
BS-SJ	110±10.5 ^{ab}	173±65.6 ^b	246±26.5 ^a	447±48.4 ^a	390±93.1 ^a	139±20.6 ^{cd}
BS-WDC	137±3.87 ^a	354±44.3 ^a	108±2.4 ^c	156±36.2 ^{ef}	153±20.1 ^{bc}	118±2.78 ^d

Mean ± Standard Deviations. Different letters indicate significant differences among treatments within each site, based on Tukey's post hoc test ($p < 0.05$). Control (untreated soil) TSP (Triple super phosphate); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); APP (Ammonium polyphosphate); BS – LW (Class A Biosolids, Lawrence); BS – SJ (Class A Biosolids, Saint Joseph); BS – WDC (Class A Biosolids, Washington DC).

The observed increase in exchangeable-P concentrations, particularly under biosolid-based treatments, aligns with the findings of Brown et al. (2012), who reported that biosolids significantly enhance P availability, thereby improving metal immobilization. Similarly, Brown et al. (2003) reported that biosolids amendments increased P concentrations and contributed to Pb stabilization through the formation of Pb-phosphate minerals. Li et al. (2021) demonstrated that phosphate-enriched biosolid amendments effectively reduced the Pb bioaccessibility in contaminated mining wastes.

Effect of phosphorus treatments on bioaccessible soil Pb

In general, inorganic and organic phosphorus amendments had a trend of reducing bioaccessible Pb (%) compared with the untreated control. However, the magnitude of reduction varied depending on the sampling time, amendment type and site-specific soil.

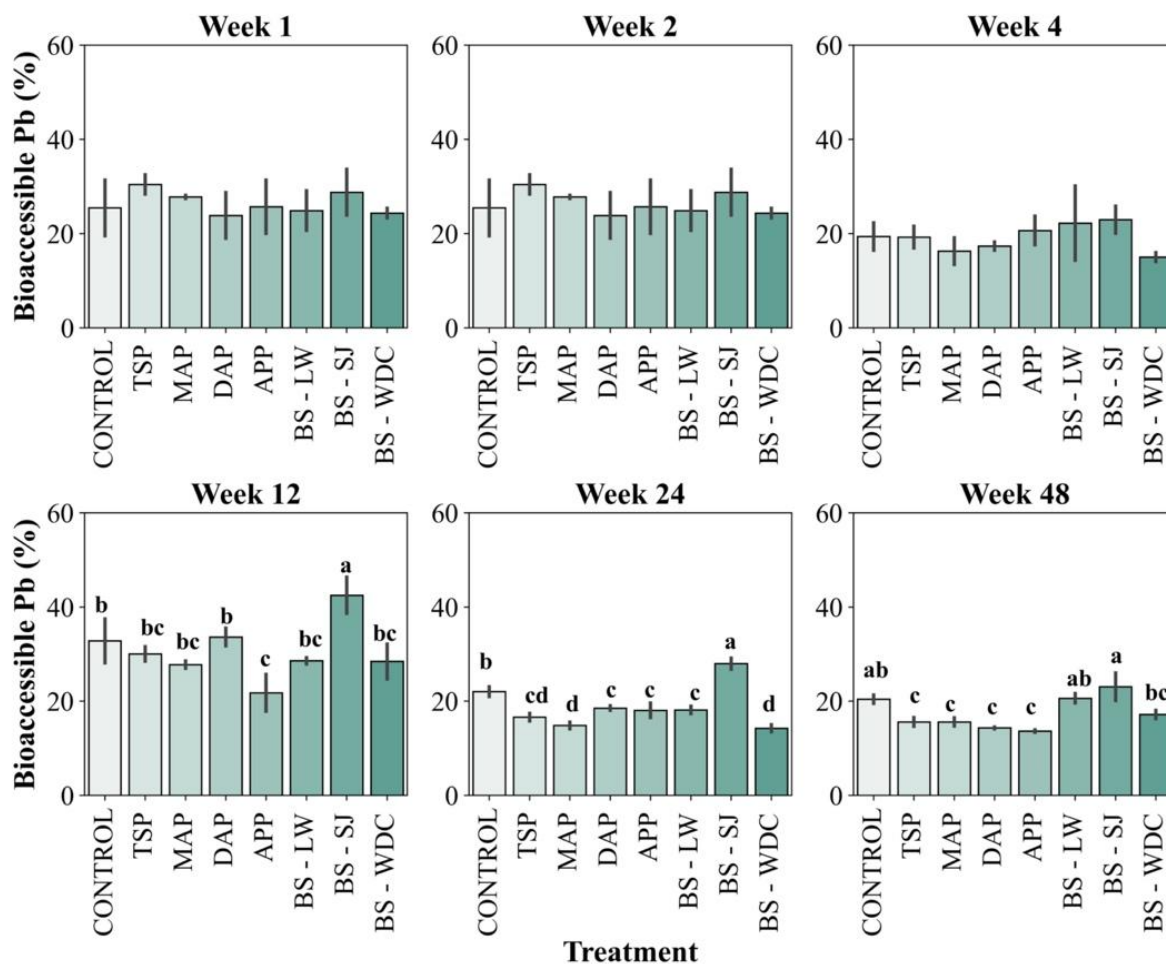


Figure 3.4. Effect of time on reduction of bioaccessible Pb (%) in soils treated with various phosphorus (P) amendments at Site 6, assessed over a 48-week incubation period. Different letters indicate significant differences among treatments within each sampling time, based on Tukey's post hoc test ($p < 0.05$). Control (untreated soil) TSP (Triple super phosphate); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); APP (Ammonium polyphosphate); BS – LW (Class A Biosolids, Lawrence); BS – SJ (Class A Biosolids, Saint Joseph); BS – WDC (Class A Biosolids, Washington DC).

For example, over time, P treatments influenced bioaccessible Pb levels across all sites (Site 6 is presented as a representative example; all sites exhibited similar response trends) (Figure 3.4). In the first four weeks, Pb bioaccessibility remained stable across all treatments, with no significant differences from the control ($p > 0.05$). However, by week 12, some variations emerged, with specific treatments significantly reducing Pb bioaccessibility ($p < 0.05$). Likewise, in week 24, a general decline was observed, showing the most notable reduction, whereas some treatments remained relatively stable ($p < 0.05$). Finally, at week 48, Pb bioaccessibility reached its lowest levels for most treatments, indicating the most consistent long-term reduction in Pb bioaccessibility, with stabilization by week 48 ($p < 0.05$).

The results of Pb bioaccessibility at 48 weeks across six sites are presented in Figure 3.5. At site 1, neither organic nor inorganic P treatments differed significantly from the control. However, in site 2, the highest Pb immobilization potential was observed in BS-SJ, APP, and BS-WDC, with an average reduction factor (RF) of 19.4% ($p < 0.05$). Similarly, in site 3, DAP and BS-SJ reduced Pb bioaccessibility by approximately 24.6%, while BS-WDC exhibited the highest effect, with an RF of 24.9%. At site 4, APP and DAP effectively reduced the bioaccessible Pb by an average of 25.03%. Likewise, in site 5, significant reductions in Pb bioaccessibility ($p < 0.05$) were observed across different treatments, with RF values of 24.7% for MAP and APP. Likewise, BS - LW and DAP had an RF of ~32.3% and TSP of ~34.8%. Finally, site 6 demonstrated an average RF of 27.5% for MAP, DAP, TSP, and APP ($p < 0.05$), indicating strong Pb-stabilization potential (Figure 3.5).

The time-dependent reduction in Pb bioaccessibility highlighted the gradual impact of P amendments. During the first 12 weeks, no significant differences were observed between treatments, consistent with studies indicating that P amendments do not significantly affect

changes in Pb bioaccessibility (Kastury et al., 2019; Lake & Basta, 2024a; Scheckel & Ryan, 2004). However, after 24–48 weeks, bioaccessible Pb levels declined significantly, highlighting the role of aging in Pb stabilization and the transformation of Pb-phosphate minerals. These results align with the findings of Hettiarachchi & Pierzynski. (2004), who concluded that P treatments enhance pyromorphite formation and Pb bioaccessibility reduction within a year of study. The application of P sources, such as TSP, APP, and MAP, resulted in an average reduction of 30.4% in bioaccessible soil Pb, highlighting their strong potential for Pb immobilization. Similarly, Alankarage et al. (2024) observed reductions in Pb bioaccessibility of 10.2%, 13.1%, and 15.8% with TSP application compared to untreated soil. These findings align with the results of Gu et al. (2020), who reported a reduction in Pb bioavailability of approximately 10-18% when using TSP, struvite, and phosphoric rock (PR) compared to control soils. Additionally, Yang & Mosby. (2006) found a significant reduction of around 59% in soil Pb bioaccessibility following the application of H_3PO_4 . These studies collectively support the effectiveness of P amendments in reducing Pb bioaccessibility and emphasize the role of P in promoting Pb immobilization through the formation of stable Pb–phosphate complexes. In addition, Class A biosolids were particularly effective, reducing Pb bioaccessibility by up to 32.3%, consistent with reports that biosolid application rates between 1–7% can reduce Pb mobility in urban soils by 20–35% when applied for at least six months (Brown et al., 2003; Farfel et al., 2005; Hettiarachchi et al., 2001).

For comparative purposes, a dataset from site 3 at 48 weeks was used to assess bioaccessible soil Pb using two in vitro extraction methods, PBET and IVBA, both conducted at pH 2.5. In general, both methods exhibited a similar trend in detecting reductions in the percentage of bioaccessible Pb in amended soils. However, PBET demonstrated a greater ability

to statistically differentiate bioaccessible Pb (%) among treatments, as evidenced by distinct letters across the bars (Figure 3.6). In contrast, IVBA showed lower sensitivity, with more uniform reductions and less pronounced differences between treatments.

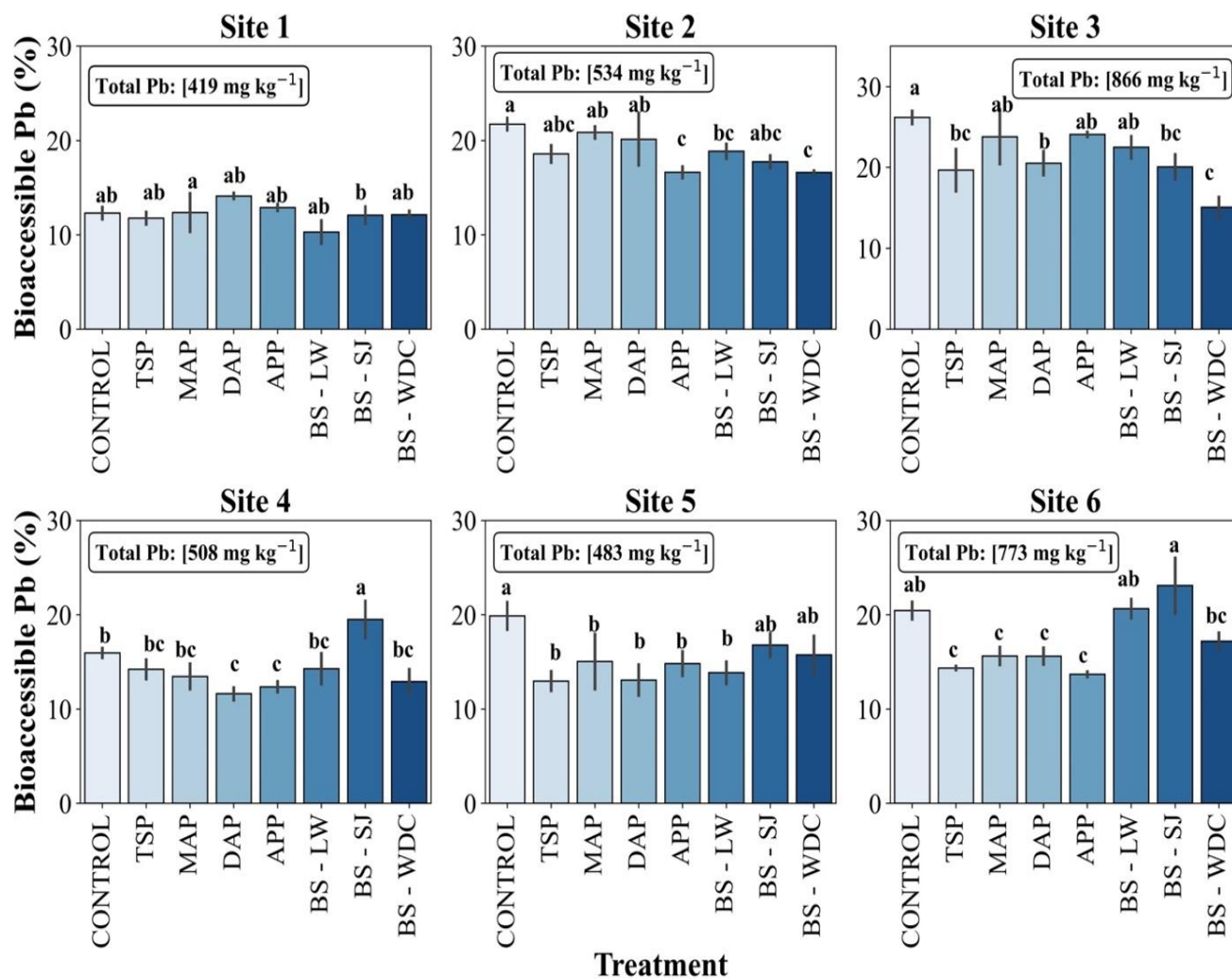


Figure 3.5. Bioaccessible soil Pb at 48 weeks across six urban sites. Different letters within each site indicate statistically significant differences between treatments, as determined by Tukey's post hoc test ($p < 0.05$). Control (untreated soil) TSP (Triple super phosphate); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); APP (Ammonium polyphosphate); BS – LW (Class A Biosolids, Lawrence); BS – SJ (Class A Biosolids, Saint Joseph); BS – WDC (Class A Biosolids, Washington DC).

These results suggest that PBET is more sensitive for detecting subtle changes in the effectiveness of amendments. However, both in vitro methods were effective in identifying the

most efficient treatments. In both cases, the BS - WDC amendment achieved a reduction in bioaccessible Pb of 42.6% using PBET and 44.1% using IVBA (Figure 3.6).

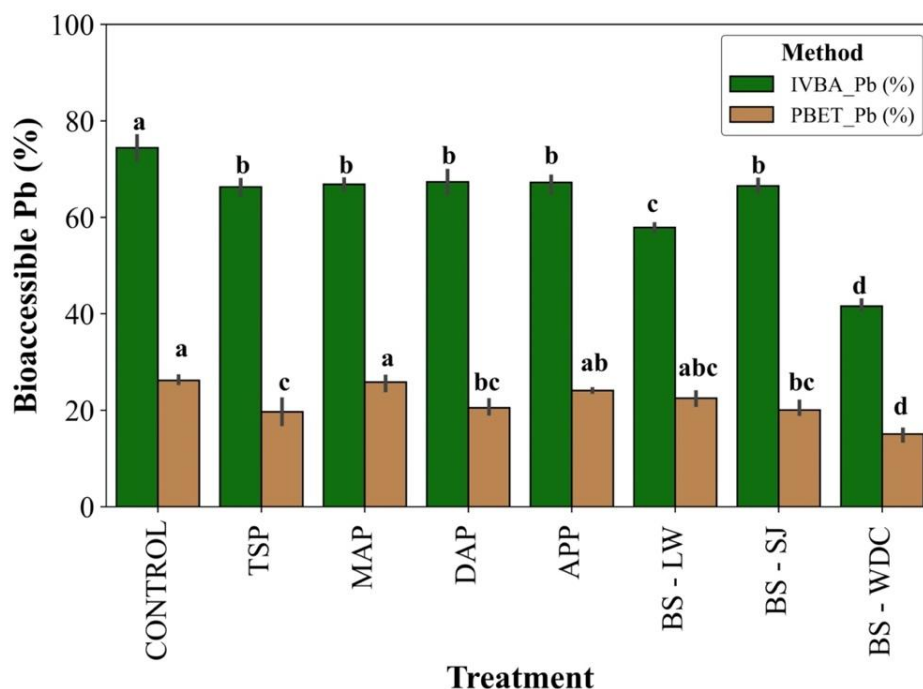


Figure 3.6. Bioaccessible Pb (%) in amended soils as determined by PBET and IVBA extraction methods at pH 2.5 after 48 weeks (Site 3). Bars represent mean values $\pm$ standard deviation. Different lowercase letters indicate significant differences among treatments within each method based on Tukey's HSD test at $p < 0.05$. Control (untreated soil) TSP (Triple super phosphate); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); APP (Ammonium polyphosphate); BS – LW (Class A Biosolids, Lawrence); BS – SJ (Class A Biosolids, Saint Joseph); BS – WDC (Class A Biosolids, Washington DC).

Similarly, the findings reported by Wekumbura et al. (2023) also revealed significant differences between the PBET and IVBA methods when evaluating Pb in dust matrices. In paint dust, IVBA at pH 1.5 achieved the highest total Pb recoveries (89-96%) and nearly 100% bioaccessible Pb, whereas PBET showed lower recoveries (74-76%). In contrast, for soil dust, PBET outperformed IVBA, with total Pb recoveries ranging from 84% to 88%, compared to 77-85% for IVBA at pH 2.5 and 76-77% at pH 1.5. These findings support the conclusion that, while both methods reveal similar trends to evidence the P treatment responses on soil Pb

bioaccessible reduction, PBET at pH 2.5 is more sensitive and robust for evaluating the effectiveness of phosphate-based remediation strategies in Pb-contaminated soils

Lead pools affected by phosphorus treatments

Soil Pb was fractionated into five operational pools according to the sequential extraction scheme of Tessier et al. (1979), where each fraction is based on extractability and, although operationally defined, is generally associated with the relative stability of Pb species in the soil. Generally, the application of P amendments affected the distribution of Pb across different soil fractions (Figure 3.7). At site 1, Pb redistribution followed the order: Pb/Fe-Mn >> Pb/Labile > Pb/Residual > Pb/Organic. However, at sites 2 and 3, the redistribution pattern differed, following the order: Pb/Residual > Pb/Fe-Mn > Pb/Labile > Pb/Organic (Figure 3.7). In this context, organic and inorganic P amendments consistently reduce Pb bound to the labile fraction. For instance, at site 1, Pb in the labile fraction was, on average, 2.12-fold lower in P-treated soils compared to the control ($p < 0.05$). A similar trend was observed at site 2, where DAP and APP treatments reduced Pb in the labile fraction by an average of 1.18-fold compared to the control. Additionally, BS-LW, BS-SJ, and BS-WDC resulted in the highest reduction, lowering Pb in the labile fraction by an average of 2.09-fold compared to the control ($p < 0.05$). At site 3, DAP and APP treatments led to a 1.35-fold reduction in Pb within the labile fraction compared to the control ($p < 0.05$). Similarly, BS-LW, BS-SJ, and BS-WDC reduced Pb in this fraction by an average of 2.01-fold relative to the control (Figure 3.7). Additionally, at site 1, P treatments resulted in the most significant shift of Pb from the labile fraction to the Fe-Mn oxide-bound fraction. Among these treatments, BS-SJ and BS-LW showed the highest Pb accumulation in the Fe-Mn oxide fraction, averaging 1.29-fold higher than the control ($p < 0.05$). In contrast, the shift from the labile fraction to the Fe-Mn oxide fraction was less pronounced at sites 2 and 3.

However, DAP at site 1 and APP at site 3 exhibited an increase in Pb bound to the Fe-Mn oxide fraction, with 1.06- and 1.12-fold increases compared to the control, respectively (Figure 3.7). In addition, at site 1, MAP, DAP, TSP, and APP treatments resulted in Pb concentrations in the organic fraction that were, on average, 2.44-fold higher than those in the control ($p < 0.05$). Surprisingly, Pb bound to the organic fraction was less pronounced at sites 2 and 3, with no significant differences observed.

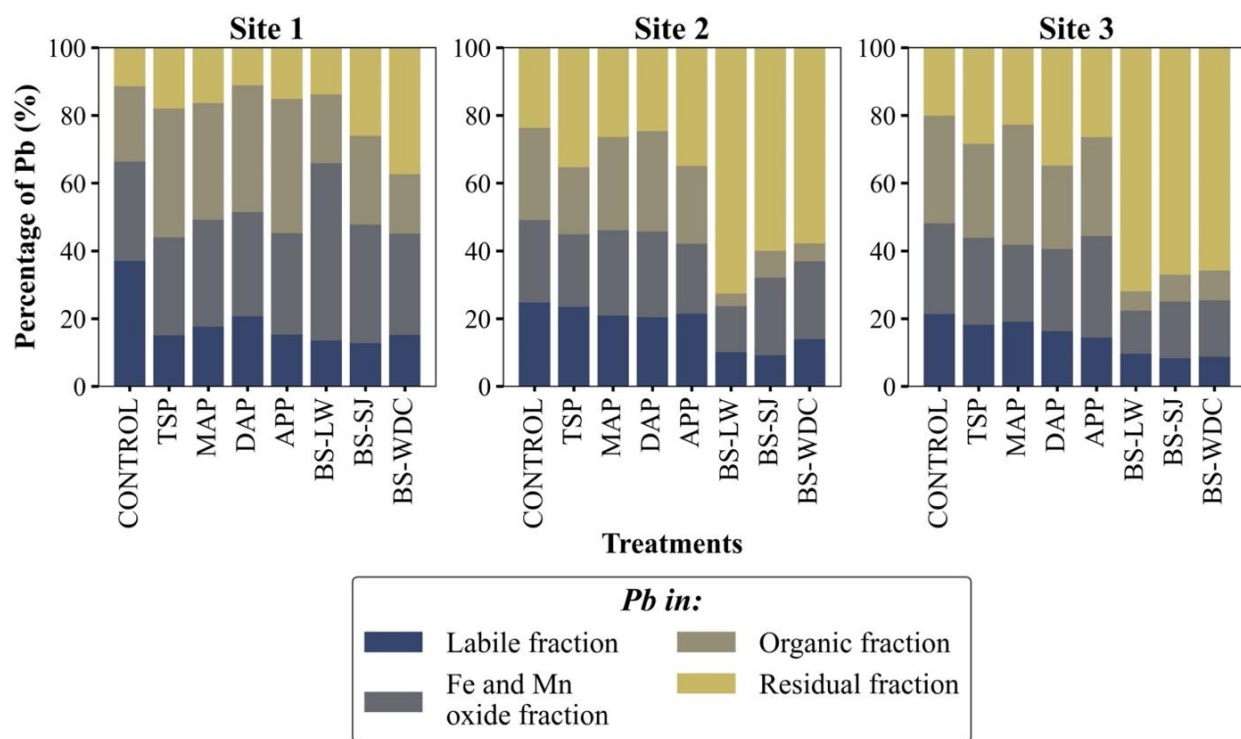


Figure 3.7. Distribution of Pb among operationally defined by sequential chemical extraction across organic and inorganic P treatments and untreated control in soils from three urban sites. Staked bars represent the percentage of total Pb partitioned into four defined fractions: labile, Fe and Mn oxide, organic, and residual. Control (untreated soil) TSP (Triple super phosphate); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); APP (Ammonium polyphosphate); BS – LW (Class A Biosolids, Lawrence); BS – SJ (Class A Biosolids, Saint Joseph); BS – WDC (Class A Biosolids, Washington DC).

Finally, the application of inorganic P amendments led to a notable shift of Pb from the labile, Fe-Mn oxide-bound, and organic fractions into the residual fraction, indicating increased

Pb stabilization. This transformation was particularly pronounced at sites 2 and 3, where APP and TSP treatments significantly increased Pb in the residual fraction by an average of 1.35-fold compared to the control at site 2. Similarly, at site 3, DAP, TSP, MAP, and APP increased Pb in the residual fraction by an average of 1.55-fold relative to the control ($p < 0.05$), suggesting enhanced Pb stabilization through phosphate-induced mineral precipitation. Furthermore, organic amendments (BS-LW, BS-SJ, and BS-WDC) exhibited a distinct Pb redistribution pattern, facilitating the transfer of Pb from the labile and Fe-Mn oxide fractions directly into the residual fraction, with minimal accumulation in the organic fraction. This effect was particularly evident at sites 2 and 3, where Pb bound to the residual fraction increased by an average of 2.81- and 4.35-fold, respectively, compared to the control ($p < 0.05$) (Figure 3.7).

These findings highlight the role of class A biosolids in promoting Pb stabilization, likely through interactions with organic matter and the formation of stable Pb complexes. Overall, both inorganic and organic P amendments play a critical role in Pb redistribution among soil fractions, thereby influencing Pb stabilization. The observed shifts from the labile fraction to more stable fractions, particularly the Fe-Mn oxide and residual fractions, suggest that phosphate-induced Pb precipitation and organic-metal interactions are key mechanisms governing Pb immobilization in amended soils. Notably, the effectiveness of these amendments varied across sites, likely due to differences in soil properties (clay content, total Pb concentration, and pH), amendment composition, and soil Pb speciation. The fractionation analysis showed that inorganic P amendments primarily transferred Pb from the labile fraction to the Fe-Mn oxide and residual fractions. At the same time, biosolids facilitate direct transfer to the residual fraction with minimal accumulation in the organic fraction. This redistribution pattern highlights the

effectiveness of inorganic phosphorus in promoting mineral precipitation and the role of organic amendments in stabilizing lead via organic complexation (Brown et al., 2003).

Influence of soil properties on percentage bioaccessible soil Pb

Soil Pb bioaccessibility across all sites was primarily influenced by the labile Pb fraction and Mn-Fe oxide-bound Pb, while residual Pb fractions significantly reduced bioaccessibility. For instance, the labile Pb fraction, which is easily exchangeable and available for uptake, was positively correlated with Pb bioaccessibility across all sites (Figure 3.8), confirming its direct contribution to bioaccessible Pb. In contrast, residual Pb had a significant negative correlation with Pb bioaccessibility, proving its stability and limited availability for biological uptake. Soil pH also played a crucial role in Pb stabilization, with slightly acidic conditions favoring the formation of Pb: phosphate species. Notably, exchangeable P was significantly and negatively correlated with labile Pb and soil pH, suggesting that increased P availability could reduce Pb mobility by promoting the formation of stable Pb:P minerals (pyromorphite group-like species) (Figure 3.8). Exchangeable-P also had significant positive correlations with residual Pb across sites, supporting the hypothesis that it facilitates the transformation of Pb into more stable, non-bioavailable forms. The correlation between Mn-Fe oxide-bound Pb and Pb bioaccessibility varied by site, with site 1 having a significant negative correlation, indicating that Fe-Mn oxides were more stable, thus reducing the potential for Pb release. Interestingly, a positive correlation was observed between Pb bioaccessibility and organic-bound Pb across all sites, with the strongest effect at site 3 ($r = 0.52$), suggesting that under certain conditions, organic matter can enhance Pb solubility rather than immobilize it (Figure 3.8). The strong correlation between decreasing pH and elevated P availability suggests that soil acidification facilitated Pb immobilization. Specifically, the enhanced availability of P under acidic conditions promoted the

formation of stable Pb-phosphate minerals, such as chloropyromorphite, thereby reducing Pb bioaccessibility.

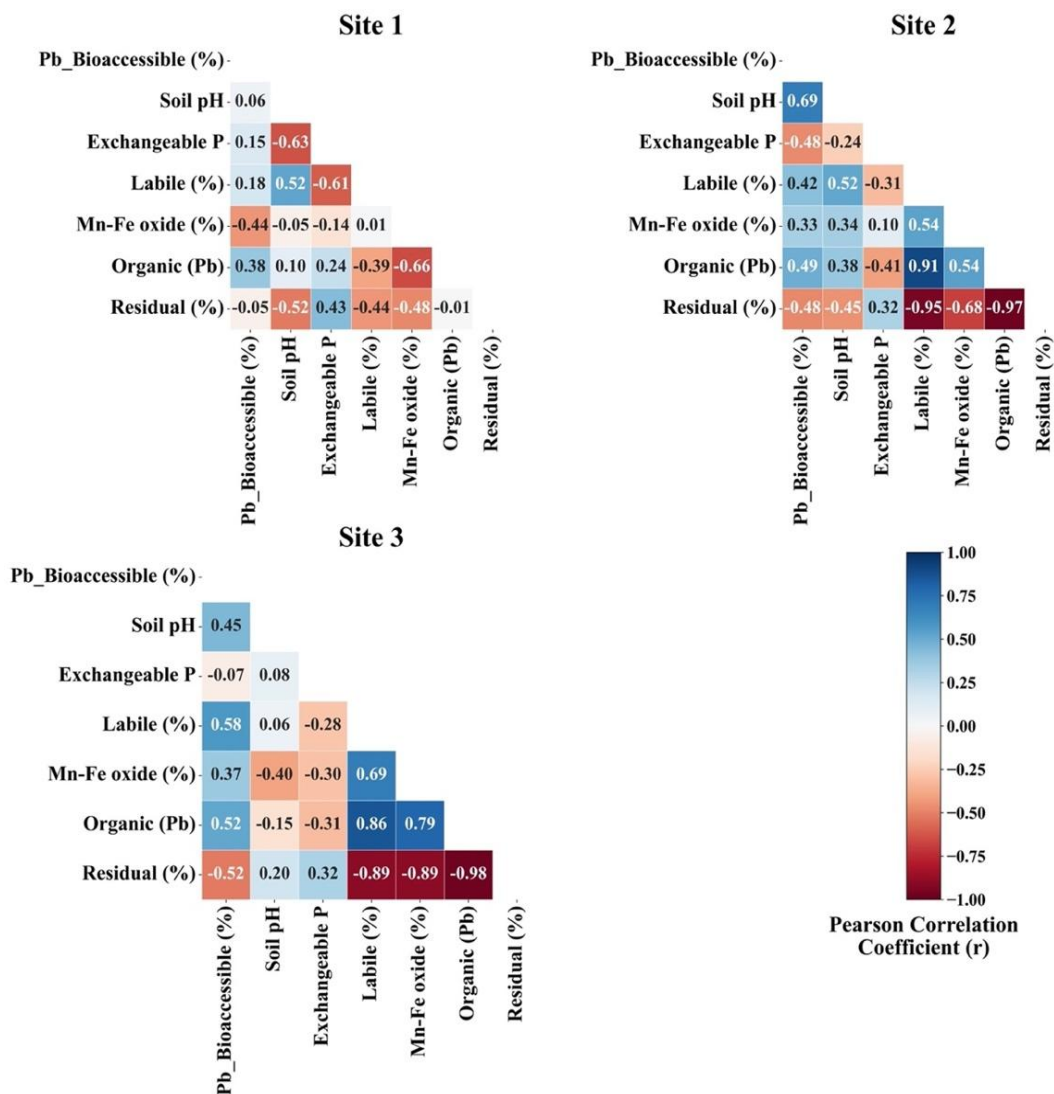


Figure 3.8. Correlation matrix between the bioaccessible soil Pb (expressed as %) and soil parameters at 48 weeks in three different sites.

These findings highlight the dual role of amendments in modifying soil pH and nutrient dynamics to optimize heavy metal stabilization. Several studies support the observed trends in pH reduction and its implications for Pb immobilization. Cao et al. (2002) demonstrated that the

application of phosphate fertilizers enhanced P solubility while simultaneously reducing soil pH, creating favorable conditions for Pb stabilization. Furthermore, Karna et al. (2018) showed that phosphate treatments influence Pb speciation, which plays a critical role in the effectiveness of pH-dependent remediation strategies, as acidic conditions ($\text{pH} < 6$) favor the formation of chloropyromorphite. Additionally, the negative correlation between residual Pb and soil Pb bioaccessibility further confirmed that Pb immobilized in this fraction posed minimal environmental risk. Ryan et al. (2001) demonstrated that the increase in the residual Pb fraction in soil indicates the transformation of Pb into highly stable forms, which can only be extracted through strong acid digestion.

Speciation of soil Pb

The impact of different P amendments on Pb speciation in soils, as compared to untreated control at two locations (site 3 and site 6), is presented in Figures 3.9 and 3.10. At site 3, Pb in the control treatment was primarily associated with Fe-bound forms, with minor contributions from Pb-humic acid and Pb-chloropyromorphite (~21%). However, the application of TSP, MAP, and APP significantly enhanced Pb-chloropyromorphite formation, increasing it by an average of 1.47-fold compared to the control, highlighting their strong potential for Pb immobilization (Figure 3.9). Simultaneously, Fe-bound Pb decreased by an average of 1.55-fold, indicating that phosphate additions facilitate Pb precipitation as chloropyromorphite, effectively reducing Pb mobility. Likewise, class A biosolids amendments, particularly BS-LW, exhibited an alternative and highly effective Pb stabilization mechanism. Where BS-LW showed Pb-chloropyromorphite formation by 1.14-fold higher compared to the control. Additionally, BS-LW significantly enhanced organic complexation, increasing Pb associated with humic acid by 3.5-fold. Likewise, reducing Fe-bound Pb by 1.75-fold relative to the control.

These results suggest that BS-LW not only promotes Pb-phosphate mineral formation but also enhances Pb binding through interactions with organic matter, providing multiple pathways for Pb immobilization (Figure 3.9).

A similar trend was observed at site 6, where Pb in the control treatment was primarily associated with Fe-bound forms, with smaller contributions from Pb-humic acid and Pb-chloropyromorphite (~19%). In contrast, inorganic P treatments significantly altered Pb speciation, reducing Pb bound-Fe by an average of ~1.2-fold compared to the control (Figure 3.10). Notably, TSP, MAP, and DAP increased chloropyromorphite levels by 1.61-fold compared to the control, highlighting their strong Pb-stabilizing effect (Figure 3.10).

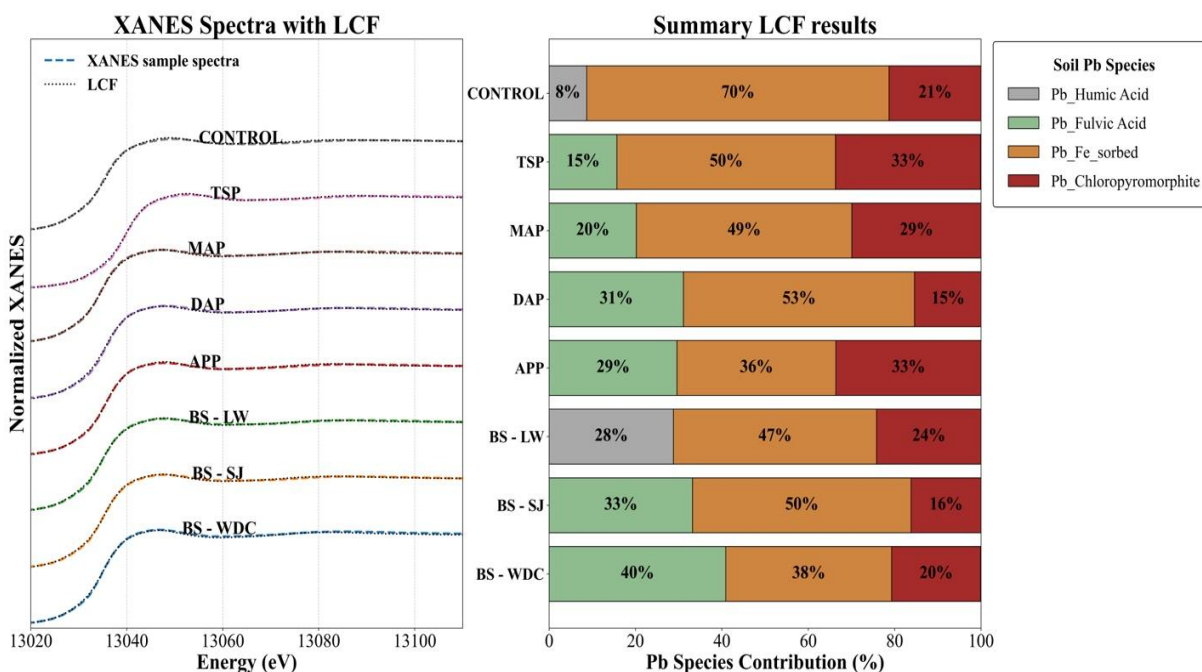


Figure 3.9. XANES Pb spectra (solid lines) for selected phosphorus treatments. The dotted lines represent linear combination fits using standard compounds. Data were collected from Site 3 soils after 48 weeks. Control (untreated soil) TSP (Triple super phosphate); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); APP (Ammonium polyphosphate); BS – LW (Class A Biosolids, Lawrence); BS – SJ (Class A Biosolids, Saint Joseph); BS – WDC (Class A Biosolids, Washington DC).

In addition, APP promoted chloropyromorphite formation (1.1-fold) but retained moderate Fe-bound Pb compared to the other inorganic P sources. Similarly, BS-LW, BS-SJ, and BS-WDC showed elevated chloropyromorphite formation, on average 1.4-fold higher than in the control treatment. In addition, class A biosolids showed a substantial contribution of organically complexed Pb, up to 25% in humic acid (BS-LW) and 23% in fulvic acid (BS-SJ, BS-WDC). Class A biosolids enhanced Pb stabilization through both organic matter complexation and phosphate-induced Pb precipitation (Figure 3.10).

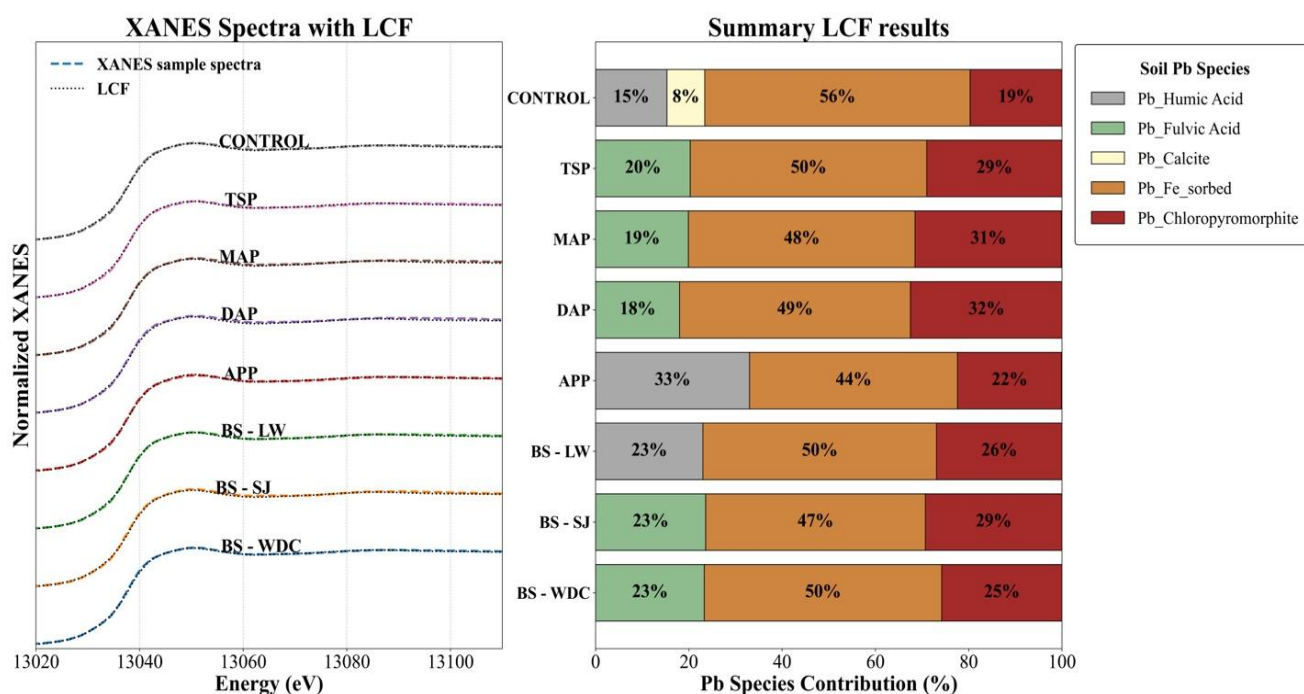


Figure 3.10. XANES Pb spectra (solid lines) for selected phosphorus treatments. The dotted lines represent linear combination fits using standard compounds. Data were collected from Site 6 soils after 48 weeks. Control (untreated soil) TSP (Triple super phosphate); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); APP (Ammonium polyphosphate); BS – LW (Class A Biosolids, Lawrence); BS – SJ (Class A Biosolids, Saint Joseph); BS – WDC (Class A Biosolids, Washington DC).

The observed results from sites 3 and 6 clearly demonstrate that different P amendments, particularly inorganic P sources like TSP, MAP, and APP, as well as class A biosolids

treatments, particularly BS-LW, significantly influence the speciation and stabilization of Pb in soils.

X-ray absorption near-edge structure analysis of soils from two select sites further confirmed that inorganic P sources, such as TSP, MAP, and APP, were particularly effective in promoting the conversion of Pb to chloropyromorphite (~31%) at both sites. The results align with research by Hettiarachchi & Pierzynski. (2002) demonstrated that P amendments facilitate stable Pb-phosphate mineral formation by providing readily available phosphate ions that react with Pb. The conversion of Pb to chloropyromorphite, a highly stable mineral, significantly reduces soil Pb bioaccessibility, thereby mitigating environmental risks and reducing the potential for human poisoning by Pb. Similarly, class A biosolids amendments, particularly BS-LW, exhibited a multifaceted approach to Pb stabilization, enhancing Pb-phosphate mineral formation while also facilitating Pb binding through interactions with organic matter, particularly humic and fulvic acids. This observation aligns with findings that organic amendments significantly reduce Pb bioaccessibility by increasing Pb complexation with organic substances. Humic substances in class A biosolids form stable Pb-organic complexes, further reducing Pb mobility and bioaccessibility. These results suggest that organic amendments, particularly those rich in organic matter, provide an alternative or complementary mechanism for Pb immobilization. The synergy between inorganic and organic P amendments is crucial, as phosphate amendments promote stable Pb-mineral formation, while organic amendments enhance Pb retention through organic complexation. This dual approach strengthens long-term Pb stabilization in contaminated soils, as supported by Brown et al. (2003), emphasizing the role of both phosphorus and organic matter in mitigating Pb contamination and reducing environmental risk.

Conclusions

This research study provides compelling evidence for the effectiveness of both inorganic P amendments and organic treatments, such as class A biosolids, in stabilizing Pb in contaminated urban soils. The findings highlight the importance of long-term monitoring to fully assess the impact of these amendments, as significant reductions in Pb bioaccessibility were observed at 48 weeks, indicating the gradual nature of Pb immobilization processes. The observed shifts from labile Pb fractions to more stable forms, such as Fe-Mn oxide-bound and residual Pb, provide strong evidence for the long-term efficacy of phosphate-induced precipitation and organic-metal interactions in stabilizing Pb. Likewise, soil Pb speciation confirms that in control treatment, Pb remains predominantly adsorbed Pb onto iron oxides and organic matter, making it relatively labile and potentially more bioaccessible. In contrast, phosphate-based amendments, including TSP, APP and MAP, demonstrated strong potential for Pb stabilization, particularly through the formation of chloropyromorphite minerals, which are highly insoluble and less bioaccessible. In addition, class A biosolids exhibited a more multifaceted approach to Pb stabilization, promoting the formation of chloropyromorphite. Also, they facilitated Pb binding through interactions with organic matter, particularly humic and fulvic acids.

By integrating spectroscopy evidence with wet chemical analyses, this research provides mechanistic insight into relative contribution of Pb-phosphate stable minerals and organic matter complexation pathways under different amendments strategies. Furthermore, the results highlight the importance of accounting for site-specific soil properties when designing remediation approaches, as these factors directly influence the formation of stable Pb phases. Future studies should evaluate the long-term stability of Pb immobilization under field conditions and assess

the combined effects of phosphorus amendments, clay mineralogical fractions, organic matter interactions, and microbial activity, which may influence the persistence of Pb-associated phases in soil. Ultimately, this work contributes to the development of more targeted and effective remediation strategies by linking soil physicochemical properties with Pb stabilization for sustained environmental and public health protection.

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Chapter 4 - An integrated chemical-biological pathway for Pb remediation in contaminated urban soils: A long-term field study using phosphorus amendments

Abstract

Soil lead (Pb) is a major source of human exposure, especially for children whose nervous systems are still developing. Phosphorus (P)-based amendments have the potential to transform Pb into stable Pb-phosphate minerals, thereby reducing its bioaccessibility and the risk of human exposure; however, their effects on soil enzyme activity remain poorly understood. This study aimed to address this knowledge gap by evaluating in situ mitigation strategies using P to reduce bioaccessible Pb in contaminated urban soils. Additionally, the long-term effects of organic and inorganic P amendments on soil enzyme activities (acid phosphatase, arylsulfatase, and β -glucosidase) were investigated, along with the main soil chemical properties that control Pb bioaccessibility and their influence on enzyme activity. Finally, the study evaluated whether changes in Pb speciation induced by remediation treatments could affect soil enzymatic activity. The research was conducted at three Pb-contaminated urban sites in Kansas City, MO. Treatments included organic amendments (Class A biosolids) and inorganic P sources (commercial fertilizers), along with an untreated control. Phosphorus-based treatments significantly reduced Pb bioaccessibility over time. Bioaccessible Pb showed a positive relationship with soil pH and a negative relationship with resin-extractable P, indicating that increased P availability and slight soil acidification favored Pb immobilization into more stable Pb-P phases, confirmed by XANES analysis, with pyromorphite proportions of up to 30%. Across the sites, enzyme activity was more strongly associated with soil physicochemical

properties than with total Pb concentration. Acid phosphatase was positively related to total organic carbon (TOC), while β -glucosidase and arylsulfatase activities were mainly controlled by soil pH. These results suggest that P-based amendments effectively reduce bioaccessible Pb through precipitation and organic complexation mechanisms, while changes induced in soil physicochemical properties exert a greater influence on biological processes than total Pb concentration in soil.

Introduction

Soil lead (Pb) contamination in urban areas is a widespread problem that poses significant risks to human health, particularly in places where children play or where food is grown (Zahran, Mielke, et al., 2013). The primary pathways for lead exposure are ingestion and inhalation of contaminated soil or dust (Hettiarachchi & Pierzynski, 2002). Soil Pb contamination poses serious risks to both human health and soil ecosystems. While exposure to Pb from contaminated soil can be as significant as, if not greater than, that other sources such as lead-based paint, its impacts on soil health remain less explored (Mielke & Reagan, 1998). Lead poisoning can affect multiple organs in both children and adults, with long-term exposure leading to accumulation in bones and associated risks during pregnancy, including renal damage (Collin et al., 2022). Elevated blood lead levels (BLLs) above 5 µg/dL have been linked to hypertension, inflammation, and oxidative stress, which disrupts enzyme function (Collin et al., 2022). Since no safe elevated BLLs have been identified for infants or adults, soil-Pb contamination remains an urgent global public health issue (Centers for Disease Control and Prevention (CDC), 2012).

In addition to human health risks, soil-Pb contamination adversely affects soil quality by disrupting critical biological processes (Doelman & Haanstra, 1979.; Khan et al., 2007; Tejada et al., 2007). One of the most affected functions is enzymatic activity, which serves as a reliable indicator of soil health (Tejada et al., 2007). Biological components of soil are generally more responsive to environmental changes than physical or chemical properties, making them particularly sensitive to contamination (Nannipieri et al., 2011). Soil enzyme activity is highly vulnerable as it reflects even subtle alterations in soil conditions (Nannipieri et al., 2011; Zeng et al., 2007). Microorganisms, in response to nutrient availability, strategically allocate resources to

synthesize carbon (C)-, nitrogen (N)-, and phosphorus (P)-acquiring enzymes to maintain productivity (Fujita et al., 2017). As a result, changes in enzyme activity could indicate shifts in soil health and reveal the broader impacts of anthropogenic pollution. Understanding how Pb contamination affects enzymatic processes is crucial for developing effective strategies to mitigate soil Pb contamination and its negative effects on both human and environmental health. For example, Doelman & Haanstra (1979), found that the soil respiration decreased by 1.19-, 1.53-, and 1.80-fold when the total soil Pb concentration was 375, 750 and 1500 mg kg⁻¹, respectively. Similarly, Alasmary et al. (2021) reported that the activities of acid phosphatase, alkaline phosphatase, Arylsulfatase, and β -glucosidase decreased by 1.24-, 1.18-, 1.03-, and 1.15-fold, respectively, when the Pb concentration exceeded 1231 mg kg⁻¹. Likewise, Tejada et al. (2007) observed that urease, β -glucosidase, and protease activities were reduced by 2.08-, 3.52-, and 3.64-fold lower when the Pb concentration ranged from 100 to 1000 mg kg⁻¹. In this context, several studies have identified the in-situ soil chemical stabilization as the most cost-effective strategy for remediating Pb contamination in urban soils (Brown et al., 2012; Henry et al., 2015; Wijesekara et al., 2016). Where the use of P amendments to immobilize soil Pb is a widely recognized practice. The application of P sources could transform Pb into Pb-phosphate minerals transforming the chemical speciation, thereby reducing its Bioaccessibility (Baker et al., 2014; Hettiarachchi & Pierzynski, 2002; Lindsay, 1979; Mayer et al., 2022).

Despite the crucial role of microbial functions in maintaining soil health, our understanding of the effects of Pb contamination under field conditions, particularly in soils treated with amendments, remains limited. In addition, the indirect impacts of remediation treatments on soil enzymatic activity, as well as how changes in soil Pb speciation could influence enzymatic processes, remain poorly understood. This study aims to bridge this

knowledge gap by examining the effectiveness of in situ mitigation strategies (P treatments) for reducing bioaccessible soil Pb in mildly to moderately contaminated urban soil in the field. Additionally, to investigate the long-term effect of organic and inorganic P amendments on soil enzyme activities in Pb contaminated urban soils. Further, to identify the major soil chemical properties that control the bioaccessible soil Pb and the influence on soil enzyme activity, and to determine whether changes in soil Pb speciation induced by the application of P treatments could affect soil enzymatic activity.

Material and Methods

Location and sampling strategy

The research was conducted at three urban sites contaminated with Pb, each in Kansas City, MO (Table 4-1). According to the USDA-NRCS, (2026), the study area is classified as a Knox-Urban land complex, consisting of approximately 65% Knox soil and 35% Urban land. This classification indicates a mixed soil system with significant anthropogenic disturbance, characteristic of urban environments. At all sites, a standardized soil preparation process was carried out prior to the application of treatments. First, the soil was manually tilled with a rotor tiller to a depth of 15 cm. Subsequently, buried objects that were presumably associated with old constructions, such as plastics, metals, glass, pieces of crockery, large rocks, and construction materials, were removed. Once cleared, the soil was leveled, and physical barriers were installed to delineate the experimental plots, using plastic plot dividers. Subsequently, organic and inorganic P amendments were applied.

The organic amendments were applied as class A biosolids from two locations: Saint Joseph, MO (BS-SJ) and Lawrence, KS (BS-LW), at 10%. The inorganic-P treatments were applied as commercial fertilizers, including monoammonium phosphate (MAP), diammonium

phosphate (DAP), triple superphosphate (TSP), and ammonium polyphosphate (APP), at a 1:4 Pb:P molar ratio (Table 4.1). An unamended control plot without P application was also included. All treatments were applied in a completely randomized block design (CRBD) with four replicates per site. Afterward, each treatment plot received an equal amount of water (~6 L) to maintain uniform soil moisture conditions. Finally, the plots were covered with mixed wood chips mulch. The 3928 Garfield Ave and 4004 Olive St sites were implemented in winter (November and December 2023), respectively, whereas the 4205 Brooklyn Ave site was implemented in spring (March 2024).

Soil samples were collected before treatment application (0 months) and 1, 3, 6, and 12 months after treatment applications. Soil samples were obtained as composites from five soil sub-samples at a surface (0 to 15 cm) depth using a soil core sampler per treatment, and washed with deionized (DI) water to prevent cross-contamination prior to the next sampling. Soil sub-samples were placed in a Ziplock bag and mixed thoroughly. Before analysis, soil samples were air-dried, ground, and sieved through stainless-steel meshes of 2 mm and < 250 µm.

Table 4.1. Specific treatment combinations for three urban sites

Urban Site Adress	GPS location	Treatments
3928 Garfield Ave	N: 39.05471, W: - 94.55968	Control, MAP, TSP, APP, BS-SJ, BS-LW
4205 Brooklyn Ave	N: 39.05017, W: - 94.55801	Control, MAP, DAP, TSP, APP, BS-SJ
4004 Olive St	N: 39.05376, W: - 94.55623	Control, MAP, DAP, TSP, APP, BS-SJ, BS-LW

Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

Soil physical and chemical properties

Soil pH was measured with Milli-Q water ($\sim 18 \text{ M } \Omega \text{ H}_2\text{O}$) (1:2.5 soil/water ratio) using a pH/conductivity meter (Thermo Scientific Orion glass electrode, Beverly, MA), previously calibrated in buffer solutions (Thomas, 1996). Cation Exchange Capacity ($\text{CEC cmol}_{(+)}/\text{kg}$) was obtained by displacement method (Peters, et al., 2015). Total organic carbon (TOC) and total nitrogen (TN) were determined by dry combustion method using a LECO TruSpec CN combustion analyzer and soil texture was measured by hydrometer method (Sikora & Moore-Kucera, 2014).

Resin extractable phosphorus

Phosphorus concentration was assessed using an anion exchange resin following the (Myers et al., 2005) procedure. Briefly, soil samples were prepared by weighing 0.5 g of air-dried, sieved (2 mm) soil into 50 mL conical polypropylene centrifuge tubes. To each tube, 40 mL of Milli-Q water was added, followed by a bicarbonate-saturated anion-exchange resin strip (50 mm $\times$ 25 mm). The tubes were capped and placed on a horizontal reciprocating shaker at 100 rpm for 24 hours. After shaking, the resin strips were removed and briefly rinsed with DI water to eliminate any adhering soil particles. The rinsed strips were transferred to clean 50 mL conical polypropylene centrifuge tubes containing 25 mL of eluant solution (0.5 M HCl) and shaken for 90 minutes. After extraction, the eluate solutions were filtered through Whatman #42 filter paper to remove residual soil particles. Finally, the resin-extractable P concentration in the filtered extracts was determined using a DU 800 Spectrophotometer set to 880 nm, with background correction at 400 nm and 420 nm. The quality assurance and quality control (QA/QC) procedures included the routine analysis of spiked samples, which showed recovery percentages ranging from 71.3% to 109.6%, with a mean recovery of 99.7% ($n = 15$).

Total soil lead

Total soil Pb concentration was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES; Agilent 5800, Agilent Technologies) following microwave-assisted digestion. Briefly, 0.5 g of dry soil sample ($> 250 \mu\text{m}$) was digested with 10 mL of SCP Science UN2031 nitric acid (HNO_3) in Teflon vessels (MarsXpress tubes) using a microwave digestion system (MARS X-Press 907501, Matthews, NC). Digestion was carried out over 15.5 minutes in three steps: initially at 165°C for 5.5 minutes, then at 175°C for 4.5 minutes, and finally at 180°C for 5.5 minutes. Reproducibility was assessed by analyzing duplicate samples every 25 digestions, resulting in a coefficient of variation (CV) between duplicates ranging from 0.32% to 10 % (mean 4.42%, $n = 14$). Quality assurance was ensured by including certified reference materials in each digestion batch. The certified Pb concentration ($1400 \text{ mg kg}^{-1} \pm$ uncertainty) from NIST 2711a was used to calculate recovery, yielding a measured recovery of $82.3 \pm 8.5\%$ (mean $\pm$ SD).

Bioaccessible soil lead

Bioaccessible soil Pb was assessed using a modified physiological-based extraction test (PBET) at pH 2.5 (Brown et al., 2003; Ruby et al., 1996). The gastric solution was prepared by dissolving 1.25 g of pepsin, 0.5 mg of sodium L-malate, 0.5 g of citrate, 500 L of acetic acid, and 420 L of lactic acid in Milli-Q water, adjusting the pH to 2.5 with HCl, and bringing the volume to 1 L. Soil samples (sieved to $250 \mu\text{m}$) were mixed with the solution at a 1:100 ratio (1 g soil to 100 mL solution) and shaken at 37°C for 1 hour. The pH was monitored and maintained at 2.5 ± 0.15 . After extraction, the mixture was filtered and stored at 5°C . Bioaccessible soil Pb concentrations were measured using ICP-OES and the absolute bioaccessible Pb concentration (mg kg^{-1}) was divided by the total concentration (mg kg^{-1}) and expressed as percentage (%) (Eq.

4.1). The quality assurance and quality control (QA/QC) procedures included the routine analysis of internal reference material (IRM) and certified reference material, as well as the incorporation of blanks and duplicate samples. The PBET-extractable Pb concentration from the IRM was 205 mg kg⁻¹, while NIST SRM 2711a was total Pb concentration of 1300 mg kg⁻¹.

Quality control samples (IRM, NIST 2711a, blanks, and duplicates) were analyzed every 50 PBET extractions. Measured Pb recoveries were 100 ± 24.1% for the IRM and 45.1 ± 3.81% for NIST 2711a (mean ± SD). These recoveries are consistent with previous reports indicating that approximately 50% of the total Pb concentration in NIST 2711a is extractable using the PBET method, and thus appropriate for QA/QC evaluation of PBET-based bioaccessibility measurements (Obrycki et al., 2017). Analytical reproducibility was evaluated by duplicate analyses, with coefficients of variation (CV) ranging from 6.89% to 18.1%, and an average CV of 12.9%, indicating acceptable precision for PBET Pb determinations.

Equation 4.1. Equation used to calculate the percentage of bioaccessible Pb relative to total Pb concentration

$$\text{Bioaccessible Pb (\%)} = \left(\frac{\text{Bioaccessible Pb mg kg}^{-1}}{\text{Total Pb mg kg}^{-1}} \right) * 100 \quad (\text{Eq. 4.1})$$

Soil enzyme activity

The soil enzyme activities of acid phosphatase, β-glucosidase and arylsulfatase were determined based on the release of *p*-nitrophenol according to Tabatabai (1994). Briefly, replicates (A, B) and a control (C) were prepared by adding 0.5 g of air-dried (2 mm) soil into 20 mL labeled scintillation glass vials for each enzyme. Where 2 mL of the appropriate start buffer and 0.5 mL of the specific enzyme substrate were added to the replicates, while the control vial contained only the start buffer (Table 4.2). All vials were gently swirled to ensure thorough

mixing and the flasks were then incubated at 37 °C for 1 hour. Following the incubation, 0.5 mL of CaCl₂ and 2 mL of the stop solution, were added to both replicates and the control vials. To balance the reaction, 0.5 mL of the substrate was also added to the control vials. The resulting mixture was filtered through Fisher brand Q.2 filter paper and collected in test tubes. Finally, the absorbance of each sample was measured using a DU 800 Spectrophotometer at 400 nm. If necessary, samples were diluted to maintain absorbance values below 1.6. The dilution factors (sample: stop solution ratio) varied according to the enzyme (1:4 for β-glucosidase, 1:2 for arylsulfatase, and 1:10 for acid phosphatase).

The enzyme activity was calculated according to equation 2 (Eq. 4-2), in which the absolute concentration obtained from spectrophotometric measurements was multiplied by the corresponding dilution factor. The average of replicated samples was calculated, corrected by subtracting the control, and enzyme activity was expressed as µg of p-nitrophenol g⁻¹ soil hour⁻¹. The quality assurance and quality control (QA/QC) procedures included the routine analysis of check samples within each analytical batch to ensure data accuracy and precision, with recovery percentages ranging from 95.7% to 104% (n = 49).

Equation 4.2. Equation for enzyme activity determination (µg p-nitrophenol g⁻¹ soil h⁻¹)

$$\text{Enzyme activity } (\mu\text{g } p - \text{nitrophenol g}^{-1} \text{ soil hour}^{-1}) = \left(\frac{\text{Replicate A} + \text{Replicate B}}{2} * \right. \\ \left. \text{Dilution factor} \right) - \text{Control} \quad (\text{Eq. 4.2})$$

Table 4.2. Buffers, substrates, and stop solutions for enzyme activity assay

Enzyme activity	Start buffer	Substrate	Stop Solution
Acid phosphatase	Modified universal buffer (MUB) pH 6.5 (Adjust the pH using 0.1 M of HCl)	0.05 M of p-Nitrophenyl phosphate, Disodium Salt	0.5 M of CaCl ₂ 0.5 N of NaOH
Arylsulfatase	0.5 M of Sodium acetate trihydrate pH 5.8 (Adjust the pH using concentrate Acetic acid)	p-Nitrophenyl sulfate	0.5 M of CaCl ₂ 0.5 N of NaOH
β-glucosidase	Modified universal buffer (MUB) pH 6 (Adjust the pH using 0.1 M of HCl)	0.05 M of p-Nitrophenyl-β-D glucopyranoside	0.1 M THAM, pH 12 (Adjust the pH using 0.5 M of NaOH)

Chemical speciation of soil lead

For speciation, X-ray Absorption Near Edge Structure data collection was performed under ambient conditions across the Pb L_(III)-edge (12,005 eV) at the Stanford Synchrotron Radiation Lightsource (SSRL), located on the Stanford Linear Accelerator Center (SLAC) National Accelerator Laboratory, located at Stanford University, and the Advanced Photon Source (APS) at Argonne National Laboratory. Soils from the 3928 Garfield Avenue and 4004 Olive Street sites collected at sampling times 0- and 12 -months were used for this analysis and a composite sample for each was prepared by combining equal amounts of soil (<250 μm) from all

four field replicates. The combined sub-samples were packed into specific sample holders, covered with Kapton tape, and analyzed at beamline 11-2 using Grazing incidence X-ray absorption spectroscopy equipped with collimating and focusing optics, a “double-double” Si(220) LN₂-cooled monochromator, and a 100-pixel monolithic solid state Ge detector array for SLAC. The X-ray absorption near edge measurements for all samples were repeated at least three times to ensure reproducibility and reduce spectral noise. Additionally, for the 0- and 12-month sampling times, a composite soil sample was prepared by combining equal amounts of soil (<250 µm) from four replicates of each treatment at the 4205 Brooklyn site, and XANES data were collected at the APS at Argonne National Laboratory. The soil samples were mounted in Plexiglas holders. Sample thickness was maintained below 250 µm to minimize self-absorption effects. The XANES spectra were acquired under ambient conditions across the Pb L₃-edge (12,005 eV) at the 5-BM-B beamline of the DuPont–Northwestern–Dow Collaborative Access Team (DND-CAT), using a Si (111) monochromator and a pair of horizontal focusing mirrors to condition and focus the incident beam. The raw XANES spectra were aligned and energy calibrated using Athena software (Ravel & Newville, 2005). Linear combination fitting (LCF) was subsequently performed. The data processing for LCF involved the following steps: spectral background removal, normalization of each spectrum to its edge step, and alignment of sample and standard spectra to correct for energy shifts. The best-fit combination of reference compounds was determined based on the lowest residual value (R-factor).

Statistical analysis

A completely randomized block design (RCBD) with four replicates was used. All statistical analyses were performed using JMP 19 Student Edition and JupyterLab Notebook software. A one-way analysis of variance (ANOVA) was conducted to determine significant

differences among treatments. Differences between the treatments were analyzed by Student's t-test with the Least Significant Difference (LSD) approach ($p < 0.05$). Given the high spatial variability observed in soil Pb concentrations, this method was considered for detecting statistically significant differences among treatments. In addition, multivariate correlation analyses, and principal component analyses (PCA) were also conducted. Finally, the data were checked for compliance with the assumptions of ANOVA and regression.

Results and Discussion

Initial soil characteristics of the urban sites

The initial characterization of the urban sites is shown in Table 4.3. In general, the three vacant lots exhibited a near-neutral soil pH, with an average of 7.16. Cation exchange capacity (CEC) ranged from 16.9 to 23.1 $\text{cmol}_{(+)}\text{kg}^{-1}$ across the sites, reflecting moderate capacity for nutrient retention and metal sorption. Total organic carbon (TOC %) content was relatively low across the three sites ($< 3.97\%$), and the soil texture was classified as loam in all sites. Additionally, the initial resin extractable P concentration did not exceed 141 mg kg^{-1} at any of the three sites, indicating moderate P bioavailability prior treatment application. The total soil Pb concentrations across the three sites ranged from 387 mg kg^{-1} to 727 mg kg^{-1} . Total soil Pb concentrations exceeded the Missouri Risk-Based Corrective Action (MRBCA) of 260 mg kg^{-1} (MRBCA, 2006), by 1.45-fold at 3928 Garfield Ave, 1.65-fold at 4205 Brooklyn Ave, and 2.79-fold at 4004 Olive St. Finally, total soil Pb concentrations had high spatial variability, as reflected by coefficients of variation (CV) of 115%, 59%, and 116% for Garfield, Brooklyn, and Olive St, respectively. Similarly, bioaccessible Pb also had considerable variability, with CV values of 38.9%, 49.7%, and 45.4% at the same sites. This pronounced heterogeneity is

characteristic of urban soils affected by historical, discontinuous anthropogenic contamination sources (Table 4.3).

Table 4.3. Initial physicochemical soil characterization across three urban sites.

Soil properties	3928 Garfield Ave			4205 Brooklyn Ave			4004 Olive St		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Total Soil Pb (mg kg⁻¹)	387	456	2292	432	261	1021	727	861	3171
Bioaccessible soil Pb (%)	19.1	7.2	28.4	16.3	8.1	39.8	23.4	13.2	57.9
Resin-P (mg kg⁻¹)	97	59	282	52.4	19	67.6	141	41	156
Soil pH (1:2.5 soil: H₂O ratio)	7.48	0.3	1.3	7.02	0.2	0.9	7.12	0.4	2
Cation Exchange Capacity (cmol₍₊₎ kg⁻¹)	20.1	-	-	16.9	-	-	23.1	-	-
Total organic carbon (%)	3.16	-	-	3.49	-	-	3.97	-	-
Total Nitrogen (%)	0.22	-	-	0.25	-	-	0.23		
Soil particle size fraction	3928 Garfield Ave			4205 Brooklyn Ave			4004 Olive St		
Sand (%)	21			33			24		
Silt (%)	55			48			51		
Clay (%)	24			19			25		

Effects of phosphorus treatments on soil physicochemical properties

Soil pH

Soil pH was influenced by both organic and inorganic P treatments. The APP and BS-SJ treatments exhibited the strongest acidifying effects, whereas MAP, DAP, and TSP caused moderate pH changes that gradually diminished over time. In contrast, BS-LW had a consistent

alkalinizing effect, while the control treatment remained near neutral to slightly alkaline across all sampling times and locations (Figure 4.1). For instance, at 3928 Garfield Ave, the strongest acidification occurred at 6 months, with pH values ranging from 6.6 to 6.9 for APP and BS-SJ, compared with a control pH of 7.5 (reductions of 0.6-0.9 units). This effect persisted at 12 months, when both treatments showed pH reductions of approximately 1.0 unit, reaching an average pH of 6.6. Similarly, at 4205 Brooklyn Ave, the greatest acidification was observed at 3 months, with pH values of 6.4 and 6.6 for APP and BS-SJ relative to a control pH of 7.4 (reductions of 0.78-1.0 units). At this site, acidification persisted through 12 months, with pH reductions of approximately 0.6 units for BS-SJ (pH~7.0) and 1.0 unit for APP (pH~6.6), compared with the control (pH~7.6). At 4004 Olive St, the greatest acidification was observed at 6 months, when APP and BS-SJ reduced soil pH to approximately 6.1 relative to a control pH of 7.1 (1.0-unit reduction). This effect persisted at 12 months, with pH values of 6.4 and 6.7 for APP and BS-SJ, corresponding to reductions of 0.7 and 0.4 units, respectively. In contrast, BS-LW exhibited a consistent alkalinizing effect, with the greatest increase observed at 3928 Garfield Ave at 12 months (pH~7.5), slightly higher than the control (pH~7.4). At 4004 Olive St, BS-LW maintained pH values equal to or higher than the control at all sampling times (pH~7.1, pH~7.15). Finally, MAP, DAP, and TSP treatments showed only slight pH reductions during the 1-and 3-month sampling periods ($\leq \pm 0.3$ units; $p < 0.05$) and pH values statistically similar to the control at 6 and 12 months ($p < 0.05$) (Figure 4.1). These results suggest that inorganic P amendments and biosolids BS-SJ, which can contain appreciable amounts of NH_4^+ , can induce soil acidification through nitrification processes, which release protons (H^+) and result in a net decrease in soil pH, particularly in soils with limited buffering capacity (Rigby et al., 2016). In the case of APP, the observed acidifying effect could be explained by its intrinsic chemical

behavior in acidic soil conditions, where low pH promotes the hydrolysis of ammonium polyphosphate. This process, combined with ammoniacal N transformations, enhances proton (H^+) release and reinforces soil acidification following APP application (McBeath et al., 2007). Similarly, Kastury et al. (2019), using phosphoric acid (PA), MAP, and TSP, found a pronounced decrease in soil pH from 6.5 to 5.3 after the application of phosphate amendments. Madsen et al. (2024) reported that inorganic P treatments using TSP induced soil acidification, reducing soil pH to 5.76 ± 0.06 , which corresponds to a decrease of approximately 1.40 units relative to the control. In contrast, BS-LW showed a slightly alkalinizing effect, consistent with the liming capacity and buffering effect associated with the stabilized nature of organic amendments. According to Brown et al. (2003), the characteristics of biosolids depend on the stabilization process used to meet pathogen-reduction standards prior to land application. Biosolids can be treated with slaked lime, which can result in increased soil pH. For example, Basta & Sloan (1999) observed that the increase in soil pH induced by the application of alkaline biosolids (LS) was a key factor in reducing the mobility and bioavailability of Cd and Zn. In this context, Cd and Zn concentrations in soil solution under high LS application rates were comparable to those observed in soils treated with agricultural lime without biosolids.

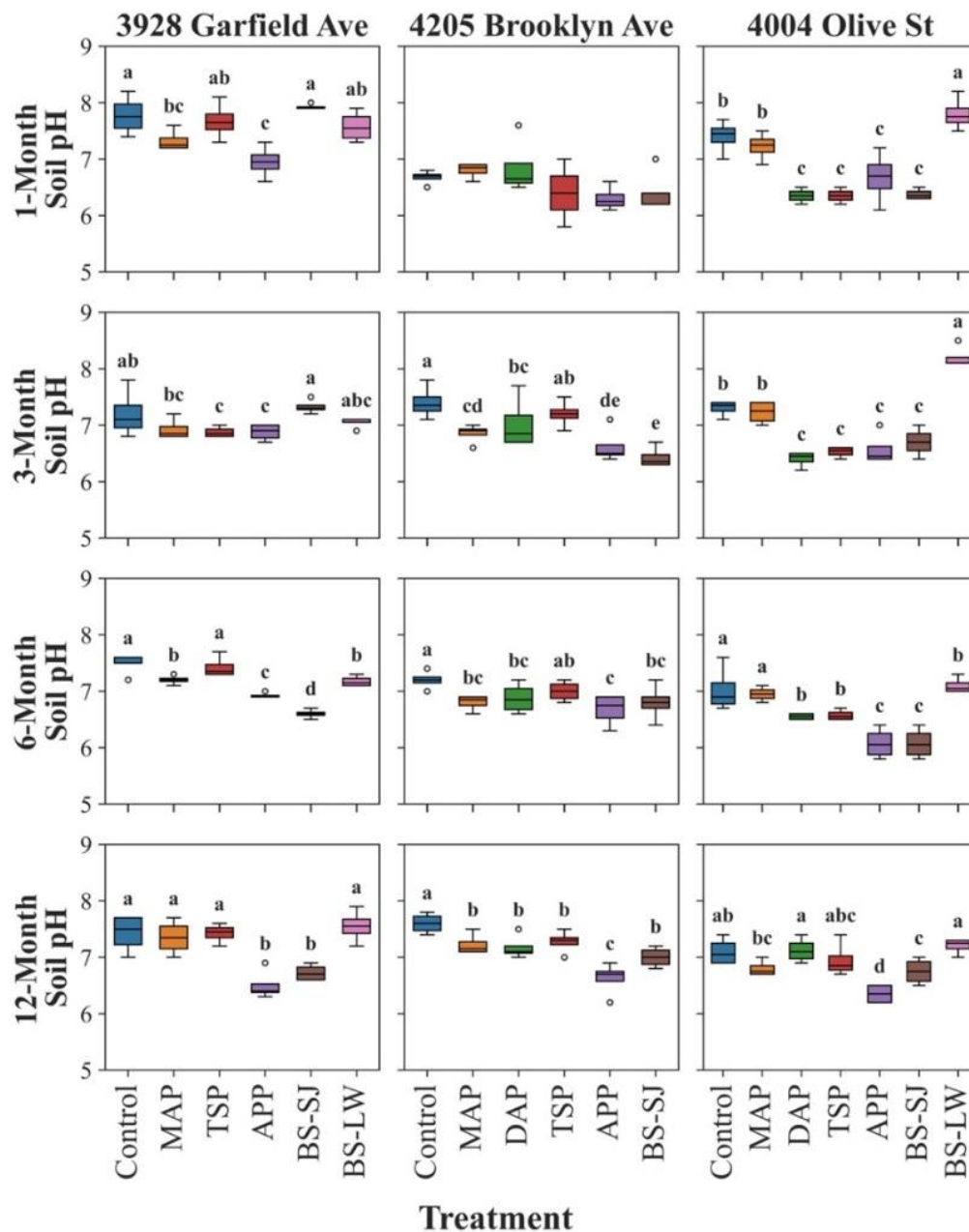


Figure 4.1. Effects of organic and inorganic phosphorus treatments on soil pH across three urban sites over four sampling times (1, 3, 6, 12 months). Different letters within each site indicate statistically significant differences between treatments, as determined by LSD's post hoc test ($p < 0.05$). Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

Resin extractable P

Phosphorus treatments significantly increased resin extractable P (potentially available P) at all sites ($p < 0.05$). The effect of P treatments had the greatest effect at early sampling times, indicating elevated concentration of resin extractable P, which could facilitate interactions with Pb and contribute to Pb immobilization in the treated soils (Figure. 4.2). At 3928 Garfield Ave, during the 3-month sampling, APP had the highest resin extractable P concentration, approximately ~7-fold higher than the control. In addition, TSP and MAP, and BS-SJ and BS-LW, had similar effects, with average of ~5.5-fold highest than the control. Similar results were observed at 6 and 12 months, where MAP and BS-SJ increased resin extractable P by approximately ~6-fold relative to the control, whereas APP, TSP, and BS-LW were on average ~4-fold higher than the control (Figure 4.2). At 4205 Brooklyn Ave, the highest resin extractable P concentrations were observed at the 1-month sampling, when inorganic P treatments (MAP, DAP, TSP, and APP) showed approximately a 10-fold increase relative to the control ($p < 0.05$; Figure 4.2). In addition, BS-SJ showed, on average, a 4.5-fold increase relative to the control. Similarly, at the 3- and 6-month samplings, both inorganic and organic P treatments were on average ~5-fold higher than the control. Finally, at the 12-month sampling, BS-SJ exhibited the largest difference (~7-fold), followed by APP and BS-LW (~5-fold), and MAP and DAP (~4-fold), all statistically higher than the control ($p < 0.05$). Similarly, at 4004 Olive St, at the 3-month sampling, APP, MAP, and BS-SJ significantly increased the resin extractable P concentration, on average, ~6-fold higher than the control ($p < 0.05$), followed by TSP, DAP, and BS-LW, which showed an average increase of ~4-fold higher than the control. This trend persisted at 6 and 12 months, indicating sustained availability of P over a long time (Figure 4.2). Similar results were reported by Malik et al. (2012), who showed that, after pre-incubation, soils

amended with high-rate inorganic P (KH_2PO_4), high-P compost (faba bean-based compost), and low-rate inorganic P exhibited significantly higher resin-extractable P throughout the experiment compared with the unamended control. In that study, resin P concentrations were highest under high-rate inorganic P, reaching approximately $30\text{--}35\text{ mg kg}^{-1}$ at day 0, followed by high-P compost ($15\text{--}20\text{ mg kg}^{-1}$), whereas low-P compost, low-P residue (white lupin), and high-P residue (faba bean residue) showed only minor increases in resin-extractable P ($\leq 5\text{--}7\text{ mg kg}^{-1}$).

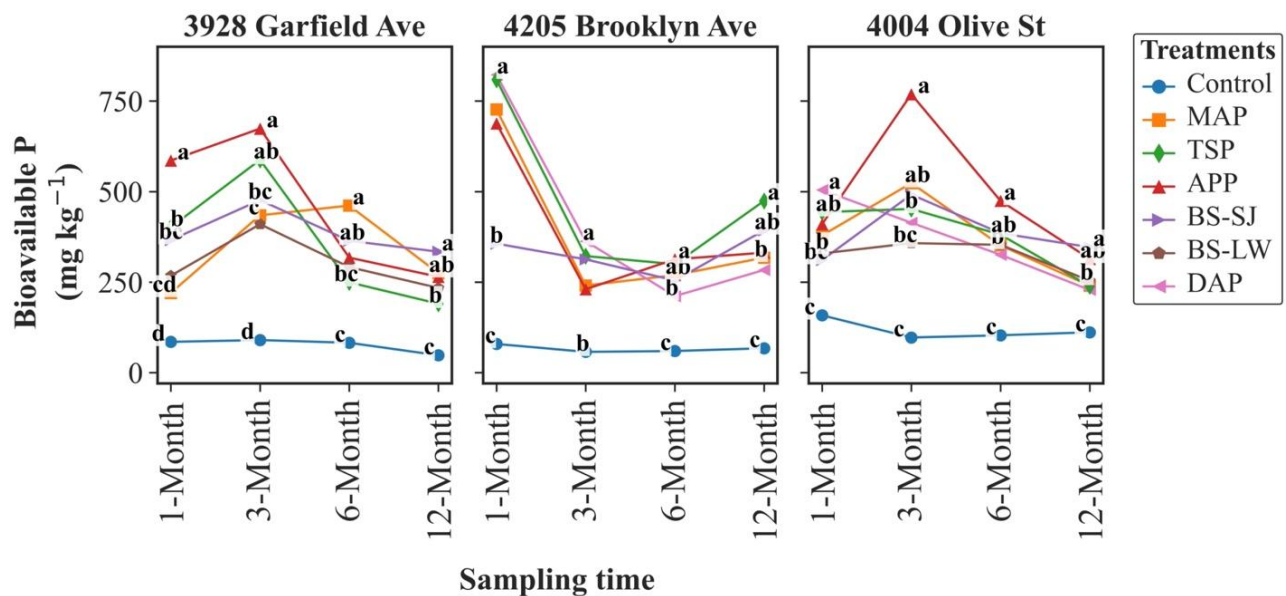


Figure 4.2. Resin-extractable P (mg kg^{-1}) response to organic and inorganic phosphorus treatments across three urban sites. Different letters within each site represent statistically distinct groups among treatments, as determined by LSD post hoc test ($p < 0.05$).

Total organic carbon

For the analysis of total organic carbon (TOC %), measurements were conducted only at the 12-month sampling time, and the geometric mean was calculated for treatments that presented missing data (Figure 4.3). The application of organic phosphorus-based treatments significantly increased TOC (%) at all sites. The BS-SJ treatment showed the largest significant

differences compared with the control, with increases of 4.02-, 2.73-, and 3.27-fold at 3928 Garfield Ave, 4205 Brooklyn Ave, and 4004 Olive St, respectively. Likewise, the BS-LW treatment showed a more moderate effect, with increases of 1.76- and 1.32-fold compared with the control at 3928 Garfield Ave and 4004 Olive St, respectively. Although some differences relative to the control were observed in the inorganic phosphorus treatments, these increases were attributable to the natural variability of TOC in soils with high spatial heterogeneity. Where the TSP treatment increased from 5.0% at 0-month sampling to 5.43% at 12-month sampling at the 3928 Garfield Ave site. Likewise, in the MAP treatment, TOC (%) increased from 4.8% at 0-month to 5.40% at 12-month, with no difference between 0- and 12-month sampling times.

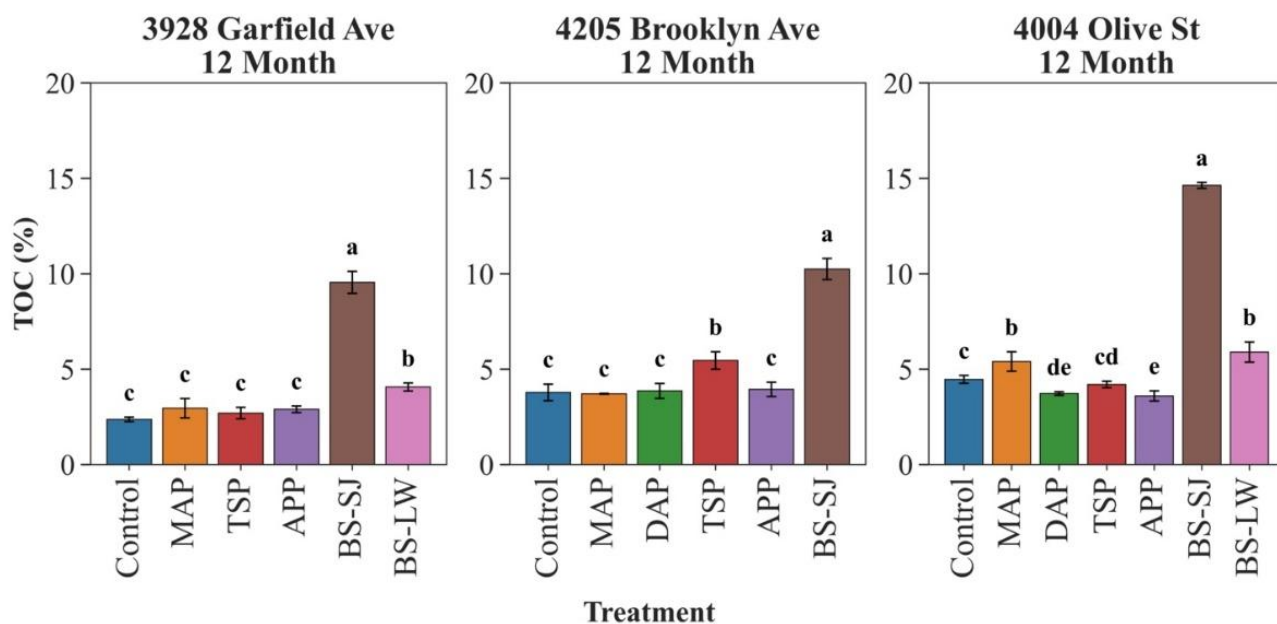


Figure 4.3. Total organic carbon (TOC %) at 12 months as affected by phosphorus-based amendments at three urban sites. Bars represent treatment means and standard deviation. Different letters indicate statistically significant differences between treatments, as determined by LSD's post hoc test ($p < 0.05$). Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

Bioaccessible soil lead

In general, the application of inorganic and organic P treatments showed a consistent trend toward reducing bioaccessible soil Pb (%) compared to the untreated control over time. However, the statistical significance of these reductions depended on the sampling time and the type of treatment applied. Results from the 3928 Garfield Ave site are presented as a representative example, as similar temporal response patterns were observed across all sites (Figure 4.4). For example, at 1-month sampling, soil Pb bioaccessibility (%) were similar among treatments, and no statistically significant differences to the control were observed ($p > 0.05$), indicating a limited short-term effect of treatments. Similarly, at 3-months, greater variability among treatments was observed, however soil Pb bioaccessibility (%) remained statistically similar to the control, suggesting that Pb immobilization processes (precipitation-complexation) were still developing. The treatment effects became evident at 6-months sampling time, when all P-treatments significantly reduced bioaccessible soil Pb (%) compared to the control ($p < 0.05$). At this sampling time, MAP, TSP, APP, BS-SJ, and BS-LW formed a statistically distinct group with lower bioaccessible soil Pb (%), showing an average reduction factor (RF) of 23% relative to the control. At 12-months sampling, P-treatments remained significantly lower than the Control ($p < 0.05$). These results indicate that the effectiveness of both organic and inorganic P treatments increased after 6 months and stabilized by 12 months, highlighting their long-term potential for reducing Pb bioaccessibility (%) in contaminated urban soils.

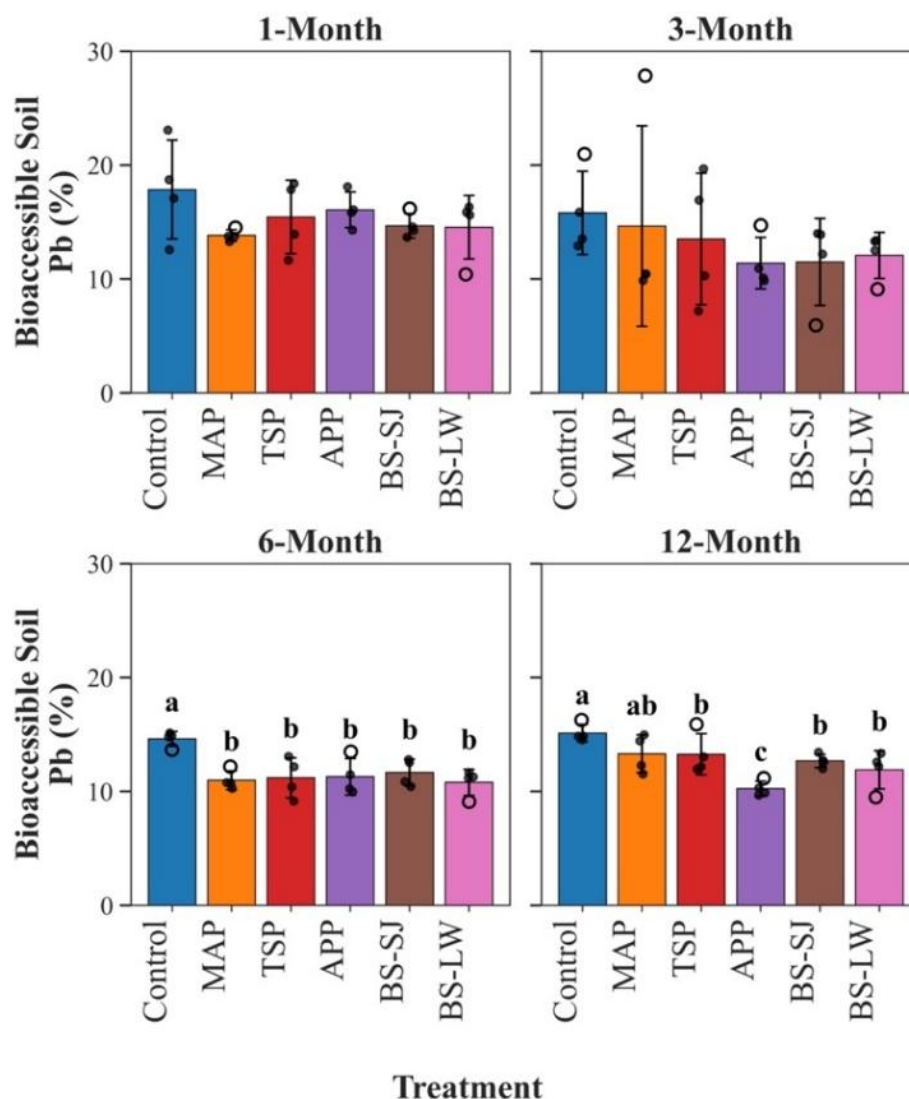


Figure 4.4. Effect of time on reduction of bioaccessible Pb (%) in soils treated with P treatments at 3928 Garfield Ave. Different letters indicate statistically significant differences between treatments, as determined by LSD's post hoc test ($p < 0.05$). °: Open symbols denote observations higher than 3 standard deviations from the group mean; these points were treated as outliers and excluded from statistical analyses. Control (non-P amended); MAP (Monoammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

The effect of P-treatments at 12-month sampling on the bioaccessible soil Pb (%) at the three sites is presented in Figure 4.5. At 3928 Garfield Ave, inorganic-P treatments significantly reduced bioaccessible soil Pb, with TSP and APP showing RF values of 16% and 33%,

respectively, indicating greater Pb immobilization potential than the control. Likewise, BS-SJ and BS-LW showed an average RF of approximately 12% compared with the control. At the 4205 Brooklyn Ave site, the BS-SJ treatment resulted in the largest reduction in bioaccessible soil Pb (%), with an RF of approximately 57% ($p < 0.05$) compared with the control; followed by APP and MAP with an average RF of approximately 31%, whereas TSP exhibited an RF of 23% compared with the control. Finally, at the 4004 Olive St site, all treatments resulted in a significant decrease in Pb bioaccessibility (%) relative to the control. At this site, organic-P treatments (BS-SJ and BS-LW) had an average RF of approximately 20%. Likewise, TSP, MAP, DAP, and APP exhibited the greatest reductions, with average RF values of ~30%, suggesting greater effectiveness in Pb stabilization in this contaminated urban soil. Overall, these results confirmed that Pb bioaccessibility (%) in response to P-based treatments is highly site-dependent, reflecting the influence of soil physicochemical properties on the effectiveness of long-term Pb immobilization treatments. Soil Pb bioaccessibility showed a clear time-dependent response to both organic and inorganic P treatments. During the early sampling periods (1 and 3 months), Pb bioaccessibility remained comparable to the control, indicating no significant short-term reduction in bioaccessibility. Similar results were reported by Lake & Basta (2024), in a short-term incubation study (3 months) using Pb-contaminated soils collected from multiple vacant residential lots in Cleveland, OH. Their short-term evaluation showed no significant reduction in Pb bioaccessibility (IVBA/PBET) for most treatments, including TSP, indicating that Pb immobilization processes were limited during the early stages of incubation. Similarly, Madsen et al. (2024) evaluated the effectiveness of bone meal, triple superphosphate, and potassium hydrogen phosphate, applied at P:Pb molar ratios of 7.5:1, 15:1, and 22.5:1, in reducing bioaccessible Pb in a soil contaminated with Pb-based paint.

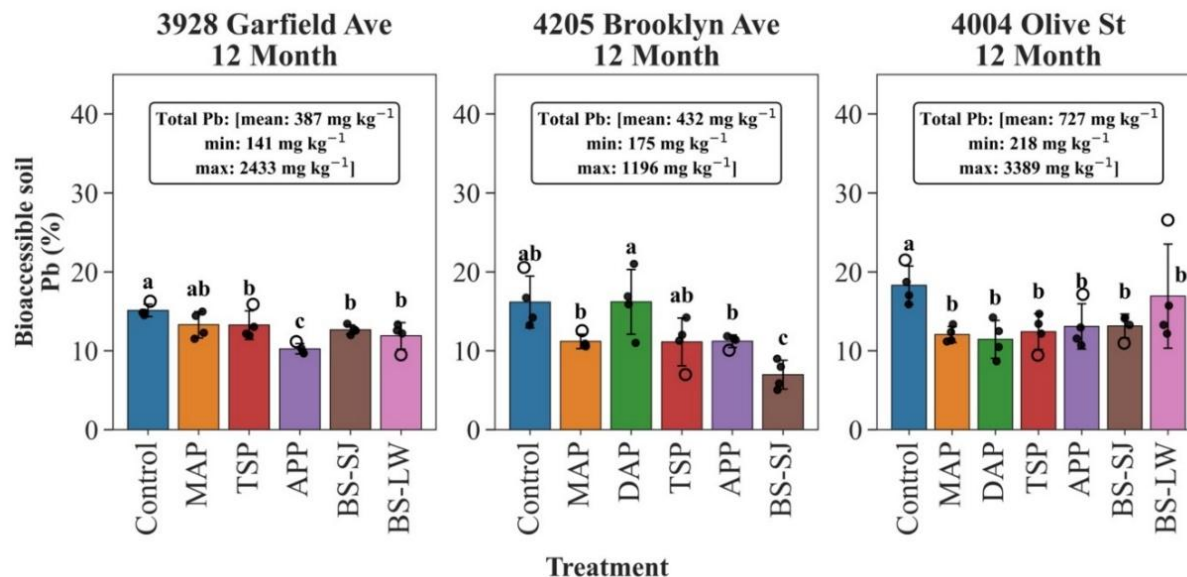


Figure 4.5. Bioaccessible soil Pb (%) at 12-month sampling time across three urban sites. Different letters within each site indicate statistically significant differences between treatments, as determined by LSD's post hoc test ($p < 0.05$). °: Open symbols denote observations > 3 standard deviations from the group mean; these points were treated as outliers and excluded from statistical analyses. Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

The results showed that the effect of time did not result in significant differences in bioaccessible Pb during the early stages of incubation (0–30 days), as all treatments exhibited values comparable to the control. Differences became evident after 99 days, when TSP and KP showed significant reductions in bioaccessible Pb relative to the control soil. In contrast, a pronounced reduction in Pb bioaccessibility was showed after 6 months, when all P treatments significantly decreased bioaccessible Pb relative to the control, and this effect was maintained through 12 months sampling. At the end of the field experiment (12-months) inorganic P treatments achieved reductions factors up to 33% in bioaccessible Pb compared with the control, confirming the strong capacity of inorganic P amendments to reduce Pb bioaccessibility (%) over the long term. Similar results were reported by Hettiarachchi et al. (2001), who conducted an

incubation study using mine waste-contaminated soils to evaluate phosphorus-based amendments, including triple superphosphate, phosphate rock phosphoric acid, and a combined treatment involving pre-acidification with acetic acid (pH 5.0) followed by TSP application, where P treatments promoted significant reductions in Pb bioaccessibility, with average decreases of approximately 20% in the gastric phase and 60% in the intestinal phase relative to the control. Likewise, Laperche et al. (1996) demonstrated that the application of P amendments in the form of hydroxyapatite to a residential soil collected in Oakland, California, contaminated primarily by Pb-based paint spills and exhibiting a high total Pb concentration ($\sim 37,026 \text{ mg kg}^{-1}$) in the surface soil (0–10 cm), significantly reduced Pb bioavailability under acidic conditions (pH 5.0), with treatment efficiency reaching reductions in dissolved Pb ranging from 79% to 99%. In addition, Class A Biosolids were particularly effective, reducing Pb bioaccessibility up to 57%, similar results were presented by Farfel et al. (2005), where using urban soils with average Pb concentrations exceeding 1000 mg kg^{-1} , the application of biosolids resulted in reductions in Pb bioaccessibility, reaching values between 60% and 67% after one year, indicating that biosolids promote progressive Pb stabilization, beyond simple dilution effects, likely through organic complexation and phosphorus-driven stabilization.

The significant reduction in soil Pb bioaccessibility resulting from P treatments observed over the long term was directly related to changes in soil Pb chemical speciation. In general, the treatments affected soil pH and resin-extractable P, resulting in a modification of soil chemistry, with both parameters acting synergistically. In this context, a strong correlation was observed between the increase in resin-extractable P concentration and the decrease in soil pH. The resulting soil acidification could increase Pb^{2+} concentrations in the soil solution, enhancing the

reaction with available P and, consequently, the precipitation of Pb-phosphate minerals (Cao et al., 2008; Cui et al., 2024; Scheckel et al., 2013).

Effects of phosphorus treatments on soil enzyme activities

The effects of P treatments on soil enzyme activity differed between sites and sampling time. Acid phosphatase activity showed that organic-P treatments increased enzymatic activity, particularly at early sampling times, whereas MAP, DAP, TSP, and APP did not differ statistically from the control (Figure 4.6). For instance, at 3928 Garfield Ave, BS-SJ showed increases of approximately 1.8-fold and 2.1-fold relative to the control at 1-and-3 months sampling, respectively. Similarly, the BS-SJ and BS-LW at 6-months showed an average of 1.25-fold higher than the control. Likewise, at 4004 Olive St, the BS-SJ treatment showed increases of 1.6-fold and 1.5-fold compared to the Control at 1- and 3-months sampling times, respectively. However, at 6 and 12 months, no significant responses were observed among P treatments. Finally, at the 4205 Brooklyn Ave site, BS-SJ showed increases of approximately 1.3-fold relative to the control at 3-month sampling (Figure 4.6). β -glucosidase activity did not show a clear effect of treatment application, whereas BS-SJ consistently reduced enzyme activity at specific sampling times. For example, at the 3928 Garfield Ave site, BS-SJ exhibited significant reductions of up to 1.71-and 2.04-fold lower than the control at 3-and 6-month sampling, respectively (Figure 4.7). By 12 months, no statistically significant differences among treatments were observed. Similarly, at the 4004 Olive St site, no significant differences relative to the control were observed during the early sampling periods; however, DAP, TSP, APP, BS-SJ, and BS-LW exhibited a reducing effect, with values on average 1.4-fold lower than the control. Finally, at the 4205 Brooklyn Ave site, the control treatment did not differ statistically from the other treatments at any sampling time, reinforcing the notion that β -glucosidase activity

at this site was primarily governed by soil physicochemical properties rather than by the application of P treatments (Figure 4.7).

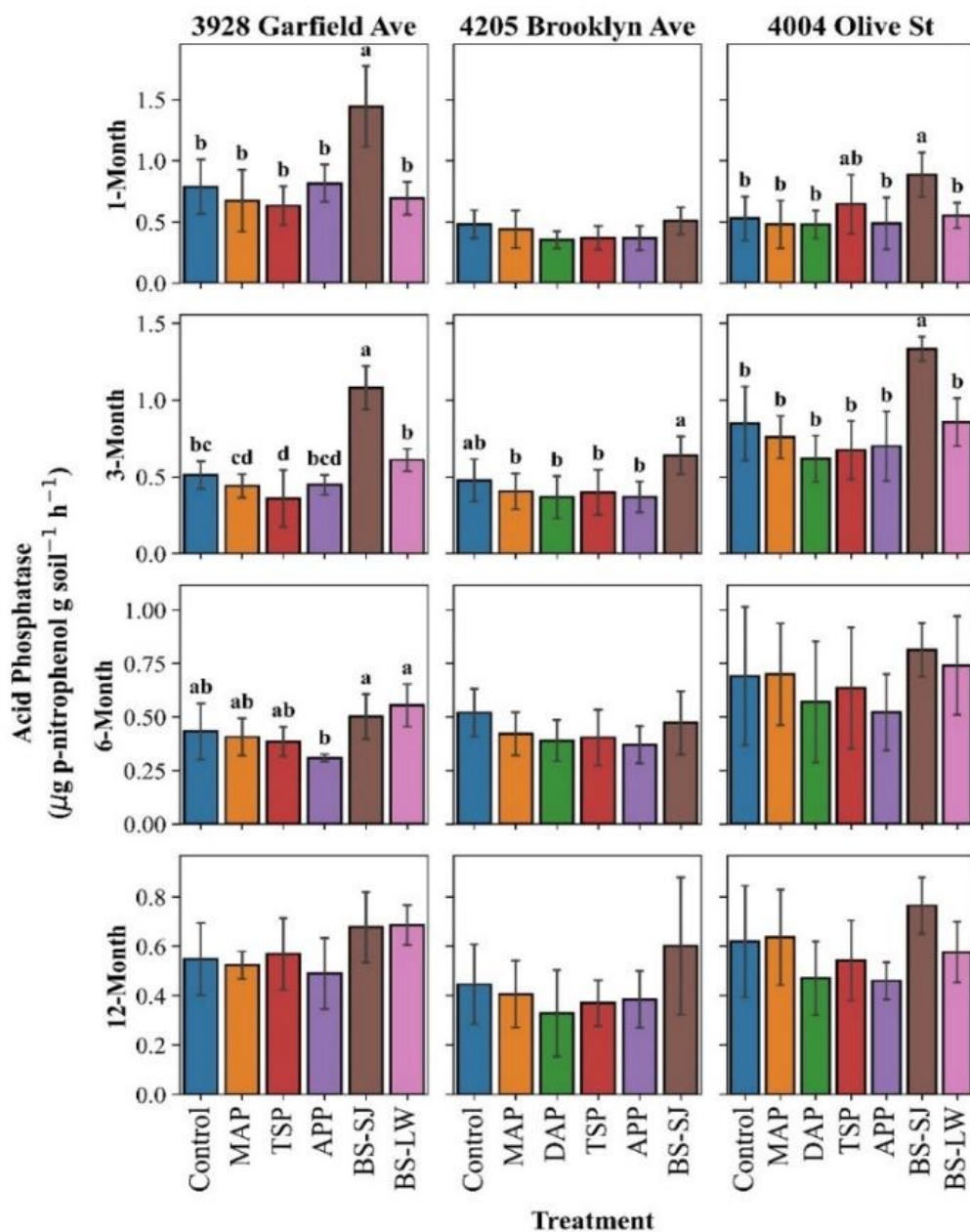


Figure 4.6. Acid phosphatase activity in urban soils amended with different phosphorus treatments over time. Bars represent mean values $\pm$ standard deviation (SD) of acid phosphatase activity. Different letters above bars indicate significant differences among treatments within each site and sampling time according to LSD test ($p < 0.05$). Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

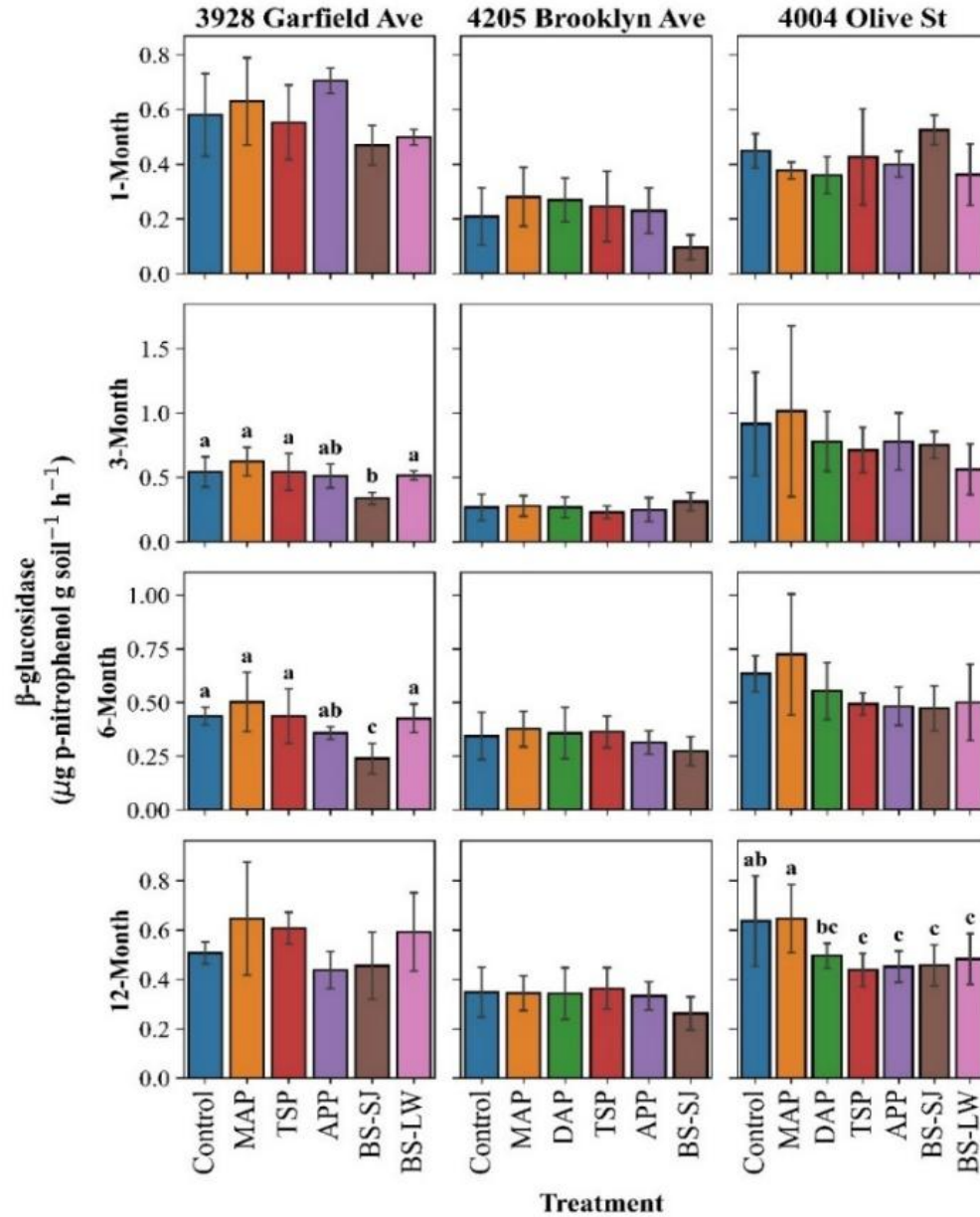


Figure 4.7. β -glucosidase in urban soils amended with different phosphorus treatments over time. Bars represent mean values $\pm$ standard deviation (SD) of acid phosphatase activity. Different letters above bars indicate significant differences among treatments within each site and sampling time according to LSD test ($p < 0.05$). Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

In general, arylsulfatase did not show a constant response across treatments and sampling times, suggesting that this enzyme was less sensitive to the chemical and biological changes induced by the amendments (Figure 4.8). For example, at 3928 Garfield, BS-LW showed a significant increase of up to 1.48-fold compared to the control, at 3-month sampling. However, BS-SJ was 2.2-fold lower than the control, at 6-month sampling. Similarly, at 4004 Olive St, the arylsulfatase activity was 1.68-fold in DAP and 3.01-fold in APP, and BS-SJ were lower than the control, at 6-month sampling. Additionally, at 4205 Brooklyn Ave, APP and BS-SJ decreased by approximately 1.88-fold and 2.66-fold than the control, at 12 month-sampling (Figure 4.8).

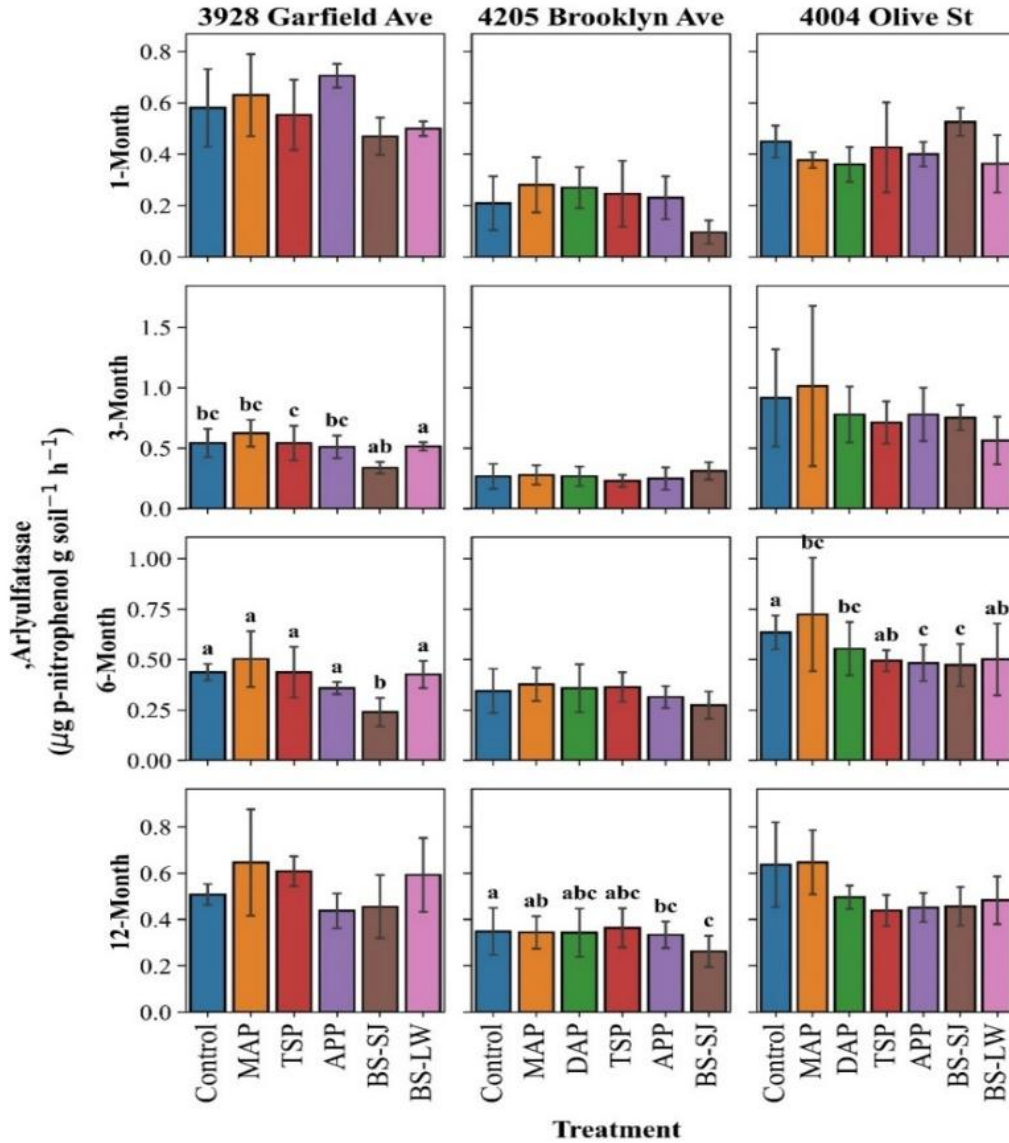


Figure 4.8. Arylsulfatase activity in urban soils amended with different phosphorus treatments over time. Bars represent mean values $\pm$ standard deviation (SD) of acid phosphatase activity. Different letters above bars indicate significant differences among treatments within each site and sampling time according to LSD test ($p < 0.05$). Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

Influence of soil physicochemical properties on bioaccessible Pb (%) and soil enzyme activity

To analyze the effect of P-based treatments on soil physicochemical properties and their relationship with bioaccessible soil Pb (%) and enzyme activity, a multiple correlation analysis was conducted at the 12-month sampling time, when clear reductions in Pb bioaccessibility were observed. The results were distinguished by site and showed in Figure 4.9. In general, a significant positive correlation between soil pH and bioaccessible Pb (%) was observed, with coefficients ranging from 0.20 to 0.54, indicating that under higher pH conditions, a greater fraction of bioaccessible Pb remained in potentially mobile forms. In contrast, resin-extractable P was negatively correlated with bioaccessible Pb, with coefficients ranging from -0.34 to -0.46, suggesting that Pb bioaccessibility was strongly controlled by bioavailable P. Consistently, a negative correlation was also observed between soil pH and resin-extractable P (-0.27 to -0.41), indicating that treatments that increased P concentrations were associated with more acidic soil conditions. In addition, at the 4205 Brooklyn site, a negative correlation between TOC and bioaccessible soil Pb was observed, suggesting that organic matter may contribute to reducing Pb bioaccessibility. Overall, these results suggest that soil acidification associated with phosphorus-based treatments, together with the increase in resin-extractable P, may facilitate interactions with bioaccessible soil Pb, promoting its immobilization by precipitation mechanisms (Pb-P minerals). Similarly, at some sites, an additional effect on Pb immobilization may be attributed to organic matter complexation mechanisms associated with higher organic carbon contents.

The correlations between total Pb and the enzymes were weak ($r = 0.22$), indicating that total Pb concentration was not the main limiting factor for biological activity in the treated soils. In contrast, several soil parameters showed more consistent associations with enzyme activities (Figure 4.9). For example, acid phosphatase showed positive correlations with TOC (%), with coefficients ranging from 0.36 to 0.50, indicating a strong dependence on organic carbon availability. In addition, at the 4205 Brooklyn site, this enzyme showed negative relationships with bioaccessible Pb (-0.30) and pH (0.21), suggesting that its activity tends to increase under conditions acidic pH and lower Pb bioaccessibility. Across the three sites, β -glucosidase was positively correlated with soil pH (> 0.60), indicating a high sensitivity of this enzyme to alkaline conditions. In contrast, a negative correlation with TOC was observed (≤ -0.30), suggesting that the increase in total carbon supplied by the organic sources did not necessarily translate into an effective substrate for β -glucosidase. In contrast, arylsulfatase showed the strongest correlations with pH across the three sites, with coefficients of up to 0.72, and a positive association with β -glucosidase ($r > 0.55$). Conversely, it showed negative correlations with TOC and resin-extractable P ($r > -0.40$), suggesting the activity was mainly controlled by changes in soil pH, presumably as an indirect effect of treatments with acidifying capacity. Overall, the three sites showed a consistent pattern where soil chemical properties modified by the treatments, particularly pH, TOC, and resin-P, showed a greater influence on enzyme activity than total Pb concentration. In particular, the negative correlations with pH and resin-P suggest an indirect effect of the phosphate-based treatments, where soil acidification and increased P availability may create unfavorable conditions for microbial activity, masking the toxic effect of Pb on biological processes (Figure 4.9). In phosphorus treated soils with moderate to high Pb contamination (387 mg kg^{-1} to $> 700 \text{ mg kg}^{-1}$), enzyme activity was primarily associated

with soil physicochemical properties, such as TOC (%), resin-extractable P, and soil pH, rather than total Pb concentration. Similar results were reported by Zeng et al. (2007), who conducted a study in paddy soils and observed that acid phosphatase activity showed no inhibitory response at Pb concentrations below approximately 300 mg kg⁻¹, whereas a progressive decline occurred at higher Pb levels (> 500 mg kg⁻¹). This response pattern indicates a non-linear, soil-dependent threshold behavior in the enzymatic response to Pb contamination. Additionally, Kandziora-Ciupa et al. (2021), working with mining-contaminated soils from Poland, showed that increasing concentrations of heavy metals, including Pb (> 650 mg kg⁻¹), led to reduced activities of key soil enzymes such as β -glucosidase and urease, whereas soil organic matter and nutrient availability exerted a positive control on enzymatic activity. In this study, acid phosphatase activity responded positively to organic P treatments, particularly during the early sampling times, whereas inorganic P sources (MAP, DAP, TSP, and APP) generally did not differ significantly from the control. The inhibitory effects of inorganic P additions on acid phosphatase activity have been widely reported. For instance, Netherway et al. (2020) documented reductions exceeding 50% relative to the control soil, consistent with metabolic feedback mechanisms regulating phosphorus acquisition and with a reduced microbial demand for organic P mineralization under conditions of high inorganic P availability. In contrast, Nannipieri et al. (2011) and Tejada et al. (2007) highlighted that organic amendments promote higher phosphatase activity by increasing soil organic matter, which enhances the stabilization of extracellular enzymes and sustains active microbial communities, thereby attenuating the strong enzymatic repression induced by inorganic P sources. In contrast, β -glucosidase activity exhibited a weak reduction following the P-treatments. This pattern indicates that β -glucosidase activity was only weakly influenced by P addition and was primarily regulated by carbon

availability, microbial biomass dynamics, and intrinsic soil physicochemical properties (e.g., soil pH). In this context, Mora et al. (2005) reported that β -glucosidase activity is closely linked to inputs of labile carbon and microbial activity and may display limited sensitivity to changes in nutrient availability or metal-induced stress when sufficient organic substrates are present and this enzyme may increase following organic amendment inputs and plant growth but is also highly sensitive to soluble metal concentrations. This sensitivity complicates the discrimination of whether the observed increases result from reduced metal toxicity, enhanced microbial biomass and enzyme synthesis, or a combination of both processes. Similarly, arylsulfatase exhibited the lowest response to P treatments. The application of inorganic treatments did not show consistent, significant effects at any site, although treatments that reduced soil pH may have affected its activity. Likewise, arylsulfatase showed a limited and variable response to organic treatments. In some cases, the BS-SJ treatment, characterized by a slightly acidic pH, was associated with reduced enzymatic activity, whereas the BS-LW treatment, with a slightly alkaline pH, tended to increase arylsulfatase activity. Nevertheless, in neither case was a clear or consistent effect of organic amendments on the activity of this enzyme observed. These results are consistent with those reported by Jat et al. (2021), who found that higher soil organic carbon content was associated with greater arylsulfatase activity, as evidenced by significant positive correlations between SOC and arylsulfatase (>0.90). Similarly, Mora et al. (2005) reported increases in arylsulfatase activity associated with increases in total organic carbon and soil pH in soils treated with organic amendments, such as municipal waste compost, in soils contaminated with Zn, As, Cd, Cu, and Pb. Overall, these results indicate that enzyme activities are more sensitive to inorganic P availability and soil physicochemical properties, particularly TOC (%) and soil pH, than to Pb (Total or bioaccessible) concentration alone (Lemanowicz et al., 2016).

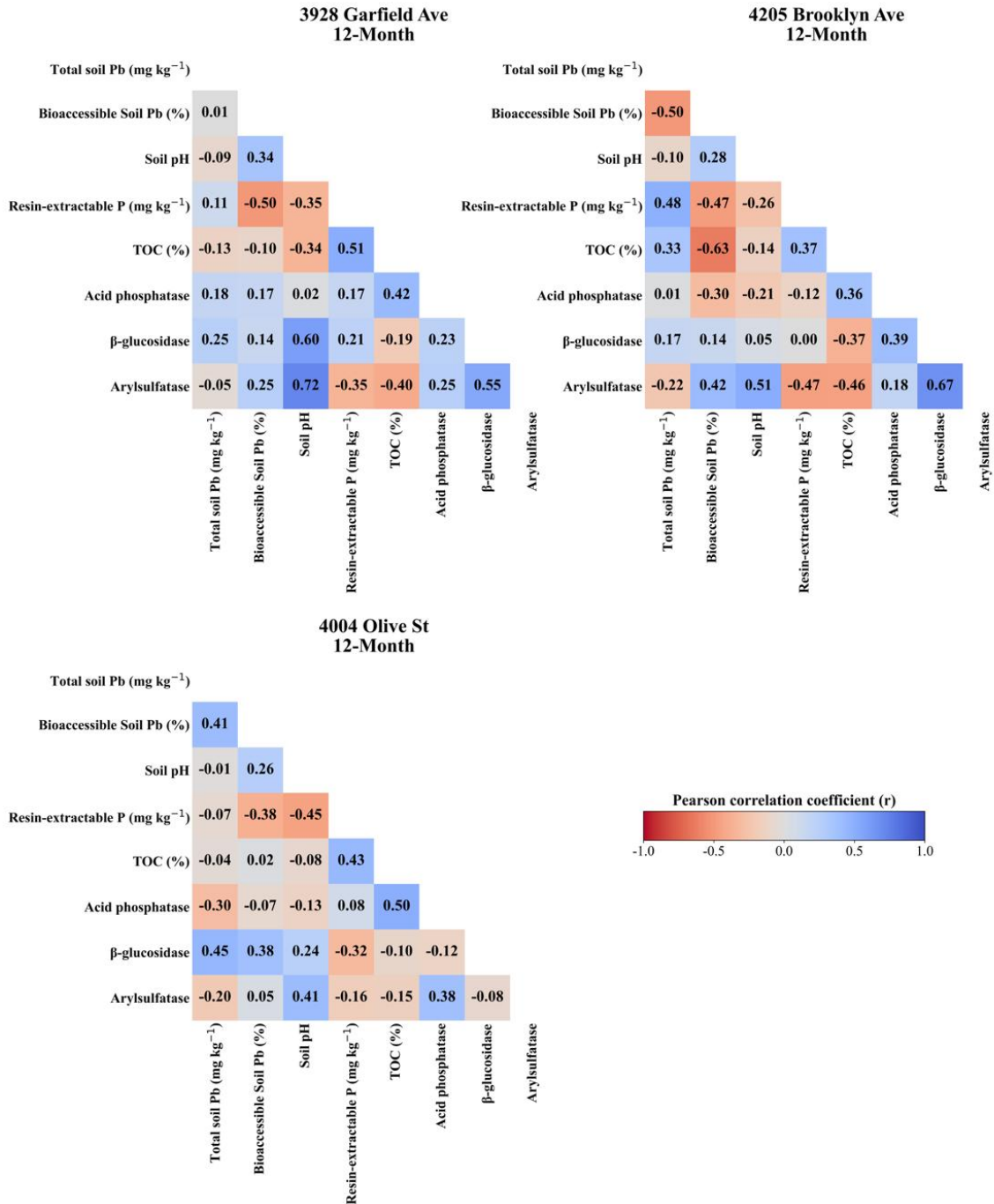


Figure 4.9. Pearson correlation matrices showing the relationships among soil physicochemical properties, bioaccessible soil Pb (%), and enzyme activities (acid phosphatase, β-glucosidase, and arylsulfatase) at the 12-month sampling time for the three study sites.

Effects of phosphorus amendments on soil Pb speciation

The synchrotron-based X-ray absorption analysis of Pb speciation at the 0-month sampling showed that, at 3928 Garfield Ave site, across all treatments, Pb was predominantly associated with Fe oxides (Pb sorbed-Fe-like species) ranged from 54 to 72%, followed by Pb associated with sulfide- and sulfate-type mineral phases, ranging from 6-29% for galena-like species and 21-25% for anglesite-like species. In addition, Pb associated with fulvic acid-type organic matter showed 6-21% of total Pb speciation (Figure 4.10). Additionally, at the 12-month sampling, the control and MAP treatments showed similar Pb speciation patterns, dominated by Pb sorbed-Fe and Pb bound to fulvic acid. Relative to 0 months, Pb associated with Fe-sorbed phases increased by 1.51- and 1.08-fold in the control and MAP treatments, respectively. Likewise, Pb associated with fulvic acid species increased by 1.23- and 1.36-fold. In contrast, the TSP treatment exhibited a distinct response, with a 1.16-fold increase in Pb sorbed-Fe, while Pb associated with fulvic acid species decreased by 1.11-fold, indicating time-dependent redistribution within existing soil pools or enhanced Pb-organic associations over time without necessarily implying increased chemical stability. The APP treatment showed an approximately 1.1-fold reduction in Pb sorbed-Fe, while Pb associated with fulvic acid increased by 1.87-fold relative to the initial sampling (0 months). In addition, Pb associated with Pb-phosphate mineral (hydroxypyromorphite-like species) was observed, approximately 7% of the total Pb speciation. The observed speciation patterns suggest that APP application could promote Pb immobilization through mineral precipitation processes, based on the formation of stable Pb-P species. Finally, BS-SJ showed a 1.28-fold reduction in Fe-sorbed Pb compared with the 0-month sampling, whereas in BS-LW, Pb sorbed-Fe remained the predominant phase (~70%). However, both organic-P treatments exhibited elevated contributions of Pb associated with stable organic matter

species (Pb bound to humic acid-like), with BS-SJ accounting for 44% and BS-LW for 30% of total Pb speciation, indicating enhanced Pb organic complexation relative to the initial sampling. At 12 months sampling time a strong reduction in sulfide- and sulfate-type mineral phases, was observed in all treatments (Figure 4.10).

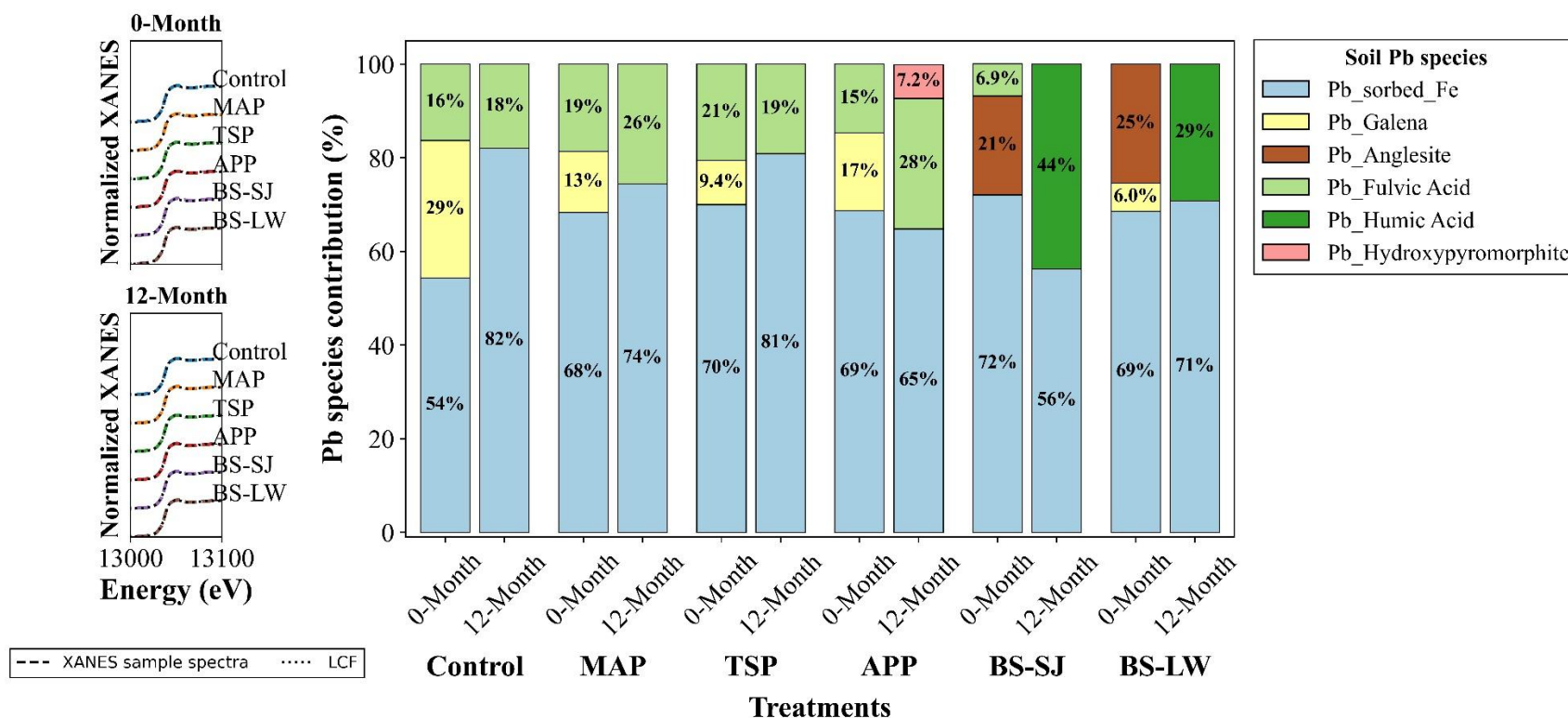


Figure 4.10. Normalized Pb L_{3} -edge XANES spectra with linear combination fitting (LCF) results (left) and relative contribution (%) of Pb species derived from LCF analysis (right) as affected by inorganic and organic phosphorus treatments at 0 and 12 months at the 3928 Garfield Ave site. Stacked bars represent the proportional contribution of individual Pb species for each treatment at both sampling times. Control (non-P amended); MAP (Monoammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

At the 4205 Brooklyn Ave site, Pb speciation prior to treatment application (0 months) was dominated by Pb sorbed-Fe species and fulvic acid-like Pb, ranging from 66 to 77% and 22 to 34%, respectively (Figure 4.11). After 12 months, only minor treatment dependent changes in Pb chemical speciation were observed compared with the initial sampling time. For example, in the Control, MAP, DAP, and TSP treatments, Pb speciation remained primarily associated with sorbed-Fe phases and fulvic acid-type organic matter, averaging 65.5% and 35%, respectively. In contrast, the APP treatment was characterized by a redistribution of Pb away from Fe sorbed species, evidenced by a 2.15-fold decrease in Pb sorbed-Fe, accompanied by only a slight increase (1.13-fold) in Pb bound to fulvic acid, and by the formation of pyromorphite-like Pb-P species, which accounted for approximately 33% of total Pb speciation. On the other hand, the BS-SJ treatment showed a substantial contribution of organically complexed Pb, reaching up to 55% associated with Pb bound to humic acid, along with a reduction in Pb associated with sorbed-Fe species (1.68-fold) (Figure 4.11).

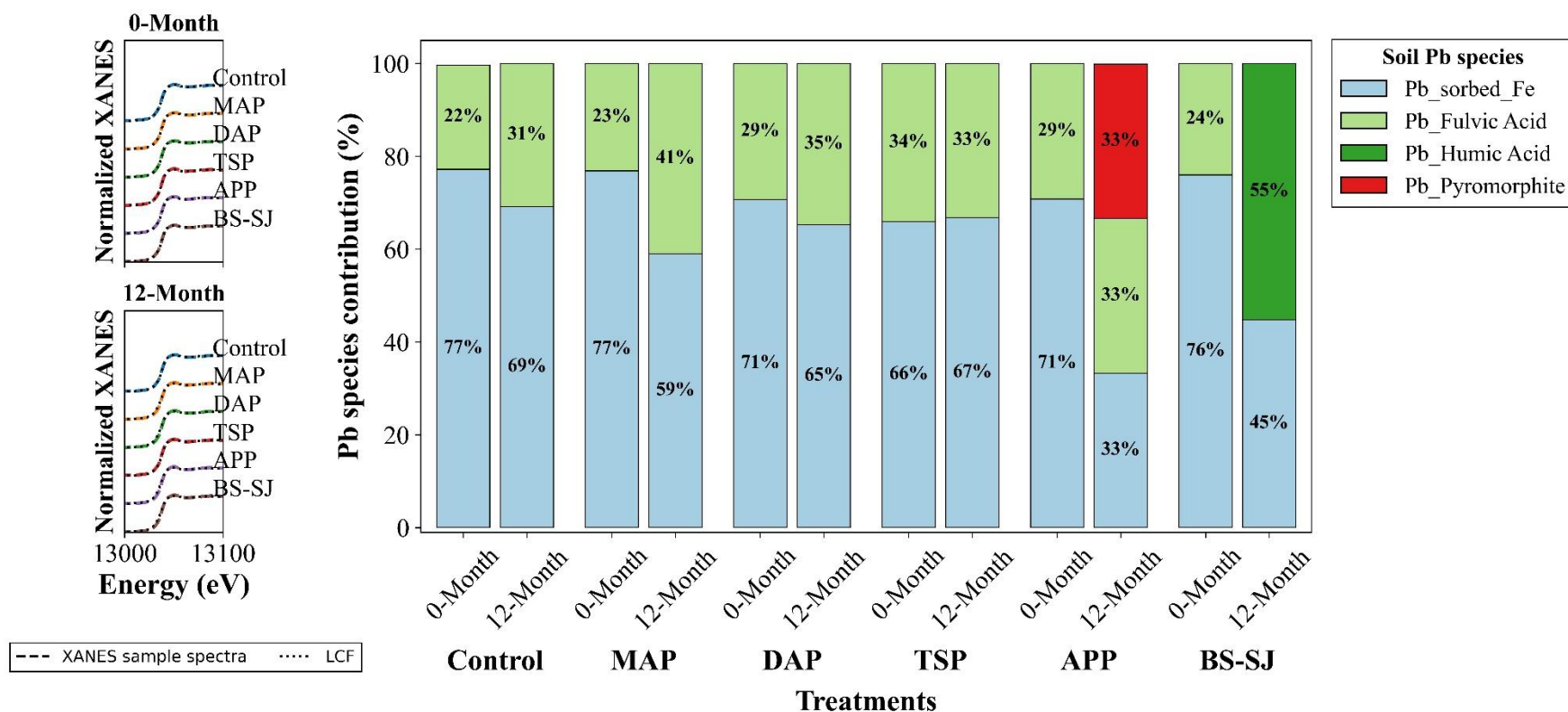


Figure 4.11. Normalized Pb L₃-edge XANES spectra with linear combination fitting (LCF) results (left) and relative contribution (%) of Pb species derived from LCF analysis (right) as affected by inorganic and organic phosphorus treatments at 0 and 12 months at the 4205 Brooklyn Ave site. Stacked bars represent the proportional contribution of individual Pb species for each treatment at both sampling times. Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph).

Finally, at the 4004 Olive St site prior treatment application (0 months) the distribution dominated by Pb associated with sorbed-Fe species was observed. Additional contributions included hydrocerussite-like, as well as anglesite-like and galena-like and, Pb associated with fulvic acid-type species (Figure 4.12). After 12 treatment-dependent changes were observed in Pb chemical speciation. In the control treatment, Pb was mainly associated with Pb sorbed-Fe species and Pb bound to fulvic acid-type organic matter, showing a 1.27-fold and 2.58-fold higher relative to the initial sampling, respectively. The inorganic P treatments promoted the formation of pyromorphite-like species. The APP treatment induced the most pronounced modification of Pb speciation, characterized by reductions in carbonate- and sulfate-associated Pb species and a high portion of pyromorphite-like. The MAP treatment showed a pronounced reduction in Pb bound to fulvic acid (3.28-fold), while DAP exhibited a 1.10-fold increase in Pb sorbed-Fe relative to the initial sampling. Likewise, MAP and DAP promoted the formation of pyromorphite-like Pb-P species in around 26% of total Pb speciation. While, TSP treatment was dominated by Pb redistribution toward sorbed-Fe forms, evidenced by a 1.26-fold increase in Pb sorbed-Fe, with only minor changes in organic-associated Pb and limited formation of hydroxypyromorphite-like phases (~11%). The Class A biosolid treatments exhibited a similar redistribution of Pb chemical speciation, in which both BS-SJ and BS-LW induced a highly reduction in carbonate-bound Pb. This shift was accompanied by an increased contribution round ~15% of Pb bound to humic acid-like species. In addition, both Biosolids treatments promoted the formation of pyromorphite-like phases, representing about 20% and 27% of total Pb speciation in BS-SJ and BS-LW, respectively. In general, although all amendments modified Pb speciation over time, immobilization mechanisms differed markedly among treatments, which was directly reflected in reductions in Pb Bioaccessibility (Figure 4.12).

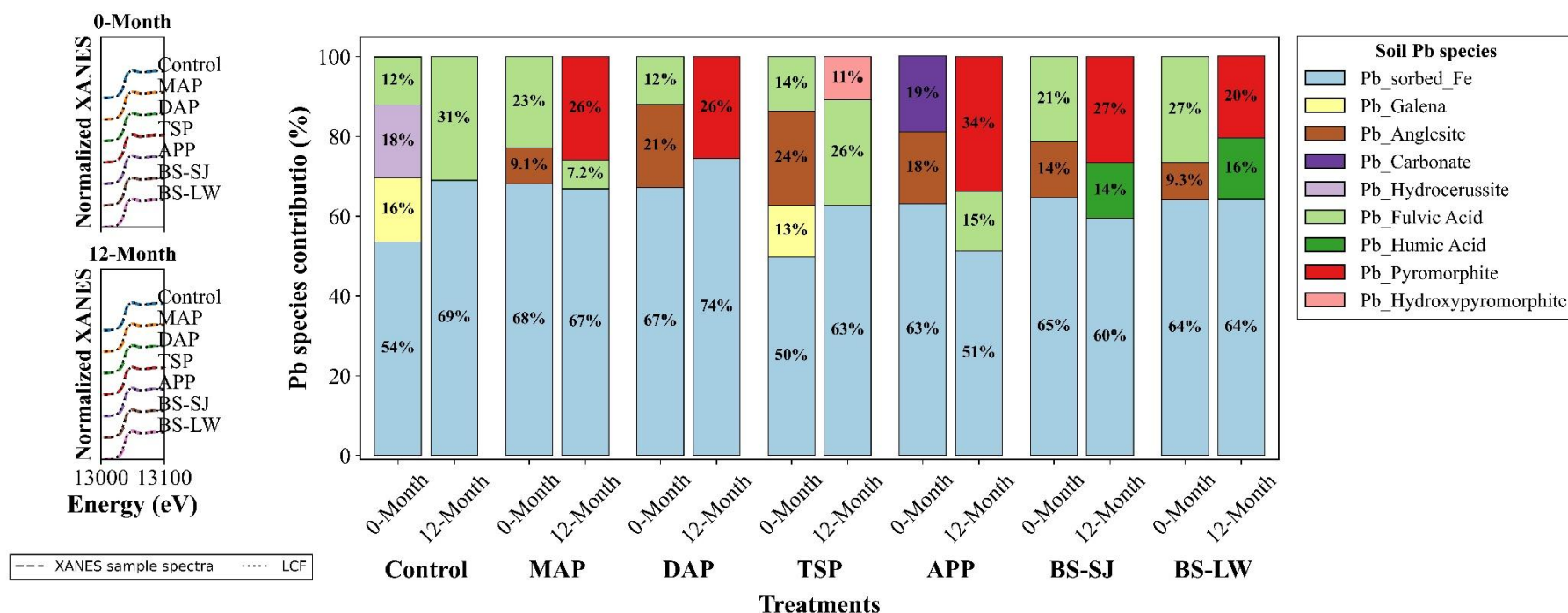


Figure 4.12. Normalized Pb L₃-edge XANES spectra with linear combination fitting (LCF) results (left) and relative contribution (%) of Pb species derived from LCF analysis (right) as affected by inorganic and organic phosphorus treatments at 0 and 12 months at the 4004 Olive St site. Stacked bars represent the proportional contribution of individual Pb species for each treatment at both sampling times. Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

The Synchrotron-based X-ray absorption analysis at the 0-month sampling showed that Pb speciation was dominated by Pb associated with Fe oxides, followed by Pb bound to fulvic acid-type organic matter, with additional contributions from carbonate-associated Pb, anglesite-like, and galena-like species. This distribution was consistent across all treatments and is characteristic of urban soils impacted by heterogeneous anthropogenic Pb sources. Comparable results were reported by Scheckel & Ryan (2004), who showed that in a Pb-contaminated soil impacted by smelter emissions, with total Pb concentrations ranging from 2000 to 3000 mg kg⁻¹, Pb chemical speciation can be dominated by Pb associated with organic matter, galena, anglesite, and Pb adsorbed through outer-sphere mechanisms. Lead distribution can be explained by the atmospheric deposition of particles derived from Pb ores and smelting processes, or berried residue material which contain galena that is susceptible to progressive oxidation to anglesite under environmental conditions (Scheckel & Ryan, 2004). After 12 months of treatment application, Pb associated with Fe oxides and fulvic acid-type organic matter remained a significant fraction of total Pb speciation, whereas carbonate-associated Pb, galena-like, and anglesite-like species were consistently reduced. Inorganic P treatments were distinguished by the appearance of Pb-phosphate phases, which contributed approximately 7–34% of total Pb speciation. Similar results were reported by Cao et al. (2008), who demonstrated that under conditions controlled by low pH (<5) and P availability, Pb minerals such as carbonates and galena-like phases dissolve and are progressively transformed into geochemically stable chloropyromorphite, resulting in a significant reduction in Pb solubility in contaminated soils. Scheckel & Ryan (2004) reported that phosphate treatments were highly effective at promoting pyromorphite formation. Where the relative effectiveness of phosphate amendments followed the order phosphoric acid > triple superphosphate > phosphate rock, highlighting the strong

influence of P solubility and availability on Pb immobilization processes. Accordingly, the proportion of Pb precipitated as pyromorphite increased substantially with more soluble P sources, reaching values of up to ~45% with phosphoric acid applications. Similarly, organic P treatments exhibited higher proportions of Pb associated with stable organic matter, particularly Pb bound to humic acid-like species (15-44%), along with the presence of Pb-phosphate species (~25%). The presence of pyromorphite in the BS-LW treatment, characterized by a slightly alkaline pH, indicates that Pb-phosphate formation can also occur under alkaline conditions when sustained P availability exists, together with additional retention mechanisms such as organic matter complexation and the redistribution of Pb into more stable solid phases (Hashimoto et al., 2009). This combined strategy enhances the long-term stabilization of Pb in contaminated soils, as supported by previous studies by Wekumbura et al. (2025), who found that Class A Fe-rich biosolids were more effective than other treatments in reducing bioaccessible Pb while maintaining the natural soil pH. Sequential extraction and synchrotron-based analyses revealed a higher proportion of chemically stable Pb species in biosolids-treated soils, including Pb precipitated as pyromorphite (30-40%) and Pb sorbed to Fe (hydr)oxide phases. Similarly, Hashimoto et al. (2009) demonstrated that the addition of an incinerated poultry waste amendment led to an increase in soil pH and P-availability, enhancing Pb adsorption using organic matter surfaces complexation (fulvic acid-like, 4%) and promoted the formation of pyromorphite-like, accounting for approximately 30–35% of the total Pb. Overall, these results confirm the effectiveness of phosphorus amendments, both organic and inorganic, in reducing Pb bioaccessibility through the precipitation of Pb-phosphate species and/or through a dual mechanism that combines Pb-phosphate formation with the association of Pb with stable

forms of organic matter, particularly humic acids. Soil enzyme activity associated with phosphorus-induced Pb speciation changes

To evaluate how P treatments induced changes in soil Pb speciation were associated with soil enzyme activity, principal component analysis (PCA) was applied using Pb chemical species determined by XANES-LCF on composite samples by treatment, site, and sampling time (12-months). Soil enzyme activities (acid phosphatase, β -glucosidase, and arylsulfatase) measured at the 12-month sampling were averaged across the same field replicates. Finally, enzyme activity was subsequently projected as an external variable to assess its relationship with the main gradients of variation associated with changes in Pb chemical forms (Figure 4.13). In this context, each PCA observation represented a unique combination of site and treatment, incorporating both Pb speciation and soil enzyme activity.

The three sites showed a consistent multivariate pattern in the relationship between Pb speciation and acid phosphatase activity, with vector orientations in the biplots indicating the presence of common geochemical mechanisms. Where the PC1 was dominated by the separation between Pb sorbed-Fe and Pb linked to organic matter (fulvic- and humic-acid-like species), whereas PC2 showed secondary variations related to Pb-phosphate species and treatment-induced redistributions (Figure 4.13). For example, acid phosphatase showed consistent positive associations with Pb bound to humic acid-like across the three sites, as evidenced by the proximity and similar orientation of their vectors, suggesting that Pb complexation into relatively stable organic forms was linked to more favorable conditions for microbial activity. In contrast, negative associations were observed between acid phosphatase and Fe-associated Pb species, as well as Pb bound to fulvic acid-like, which were positioned in opposite quadrants within the multivariate space. In addition, pyromorphite and hydroxypyromorphite-like exhibited weak,

null, or negative relationships with enzymatic activity, generally reflected by perpendicular or opposite orientations relative to the acid phosphatase vector.

These results indicate that, regardless of site, soil enzymatic response was more strongly associated with Pb organic complexation mechanisms (particularly humic acid species) than with the precipitation of Pb-phosphate phases or with potentially more available Pb species (Pb sorbed-Fe or Pb bound to fulvic acid-like). In this context, chemical stabilization of Pb does not necessarily translate into a uniform improvement in soil biological activity (Figure 4.13).

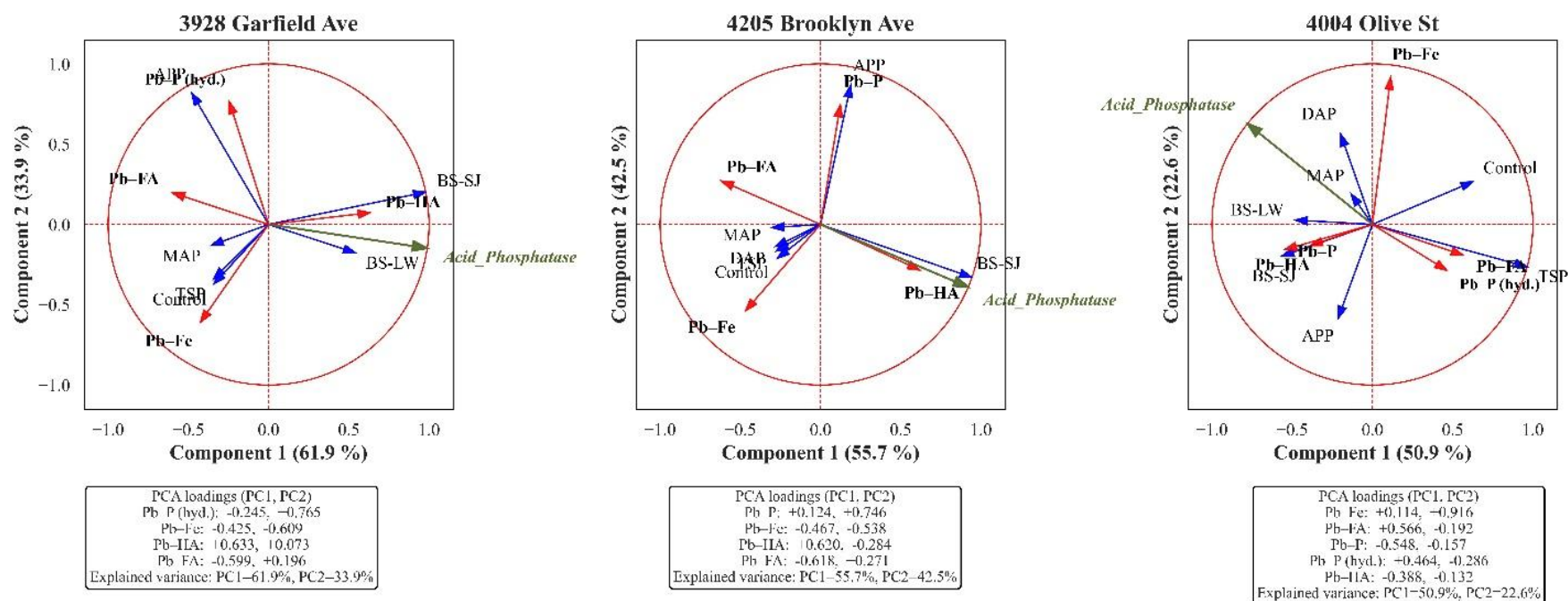


Figure 4.13. Principal component analysis (PCA) showing associations between Pb chemical species and acid phosphatase activity in two urban soils. PCA biplots are presented for the 3928 Garfield Ave, 4205 Brooklyn Ave and 4004 Olive St sites 12 months after treatment application. Red arrows represent Pb chemical species determined by XANES-LCF, blue arrows indicate treatments, and the green arrow represents acid phosphatase activity projected as an external variable. Percent variance explained by each principal component is shown on the axes. Pb-P(Hyd): Hydroxypyromorphite; Pb-P: Pyromorphite; Pb-FA: Fulvic Acid; Pb-HA: Humic Acid; Pb-Fe: Pb sorbed-Fe. Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

The enzyme activity of β -glucosidase showed a differentiated response to treatment-induced changes in Pb speciation, suggesting that β -glucosidase was primarily controlled by specific mechanisms associated with soil physicochemical conditions (Figure 4.14). Across the three sites, β -glucosidase activity showed a consistent positive association with Pb sorbed-Fe, as indicated by the proximity and similar orientation of the vectors within the multivariate space. This positive association was particularly evident along PC2, which was strongly influenced by Pb sorbed-Fe species in all sites. In contrast, β -glucosidase exhibited negative or weak relationships with Pb associated with organic matter species (fulvic- and humic acid) and with Pb-phosphate species (pyromorphite and hydroxypyromorphite-like) promoted by phosphate amendments, as reflected by their location in opposite quadrants or by near-perpendicular orientations relative to the enzyme vector. Overall, treatments that favored organic complexation of Pb or the formation of highly stable Pb-phosphate mineral phases were not associated with increased β -glucosidase activity (Figure 4.14).

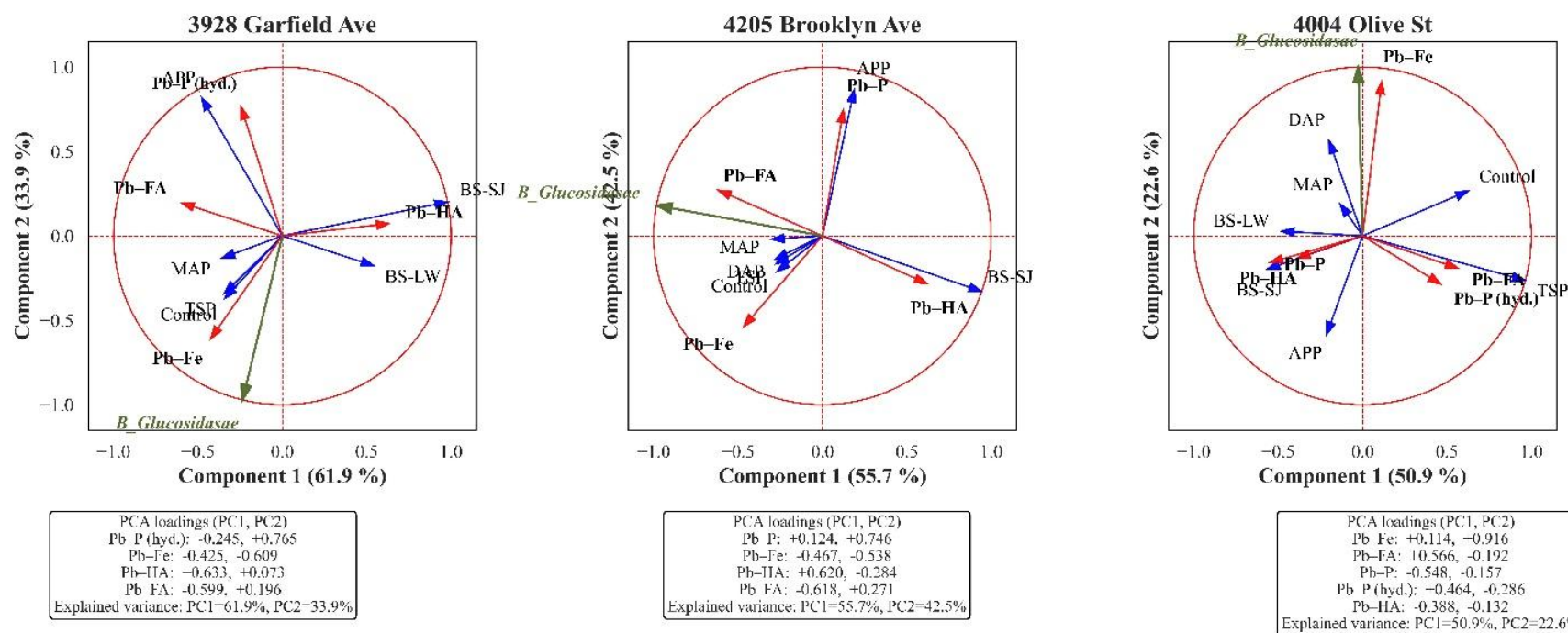


Figure 4.14. Principal component analysis (PCA) showing associations between Pb chemical species β -glucosidase activity in two urban soils. PCA biplots are presented for the 3928 Garfield Ave, 4205 Brooklyn Ave and 4004 Olive St sites 12 months after treatment application. Blue arrows represent Pb chemical species determined by XANES-LCF, blue arrows indicate treatments and the green arrow represents β -glucosidase activity projected as an external variable. Percent variance explained by each principal component is shown on the axes. Pb-P(Hyd): Hydroxypyromorphite; Pb-P: Pyromorphite; Pb-FA: Fulvic Acid; Pb-HA: Humic Acid; Pb-Fe: Pb sorbed-Fe. Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

Arylsulfatase activity exhibited a similar response to treatment-induced changes in Pb speciation than β -glucosidase (Figure 4.15). Across the three sites, arylsulfatase showed a consistent positive association with Pb sorbed Fe, as indicated by the proximity and similar orientation of their vectors within the multivariate space. In contrast, arylsulfatase exhibited weak or negative relationships with Pb associated with organic matter (fulvic and humic acid-like) and with stable Pb-phosphate mineral phases (pyromorphite and hydroxypyromorphite-like), as reflected by near-perpendicular orientations or by their location in opposite quadrants relative to the enzyme vector. Overall, these results suggest that arylsulfatase activity preferentially responded to mineral-associated fractions rather than to organically complex forms or highly stable Pb-phosphate phases, a pattern that was consistent across the three urban sites (Figure 4.15).

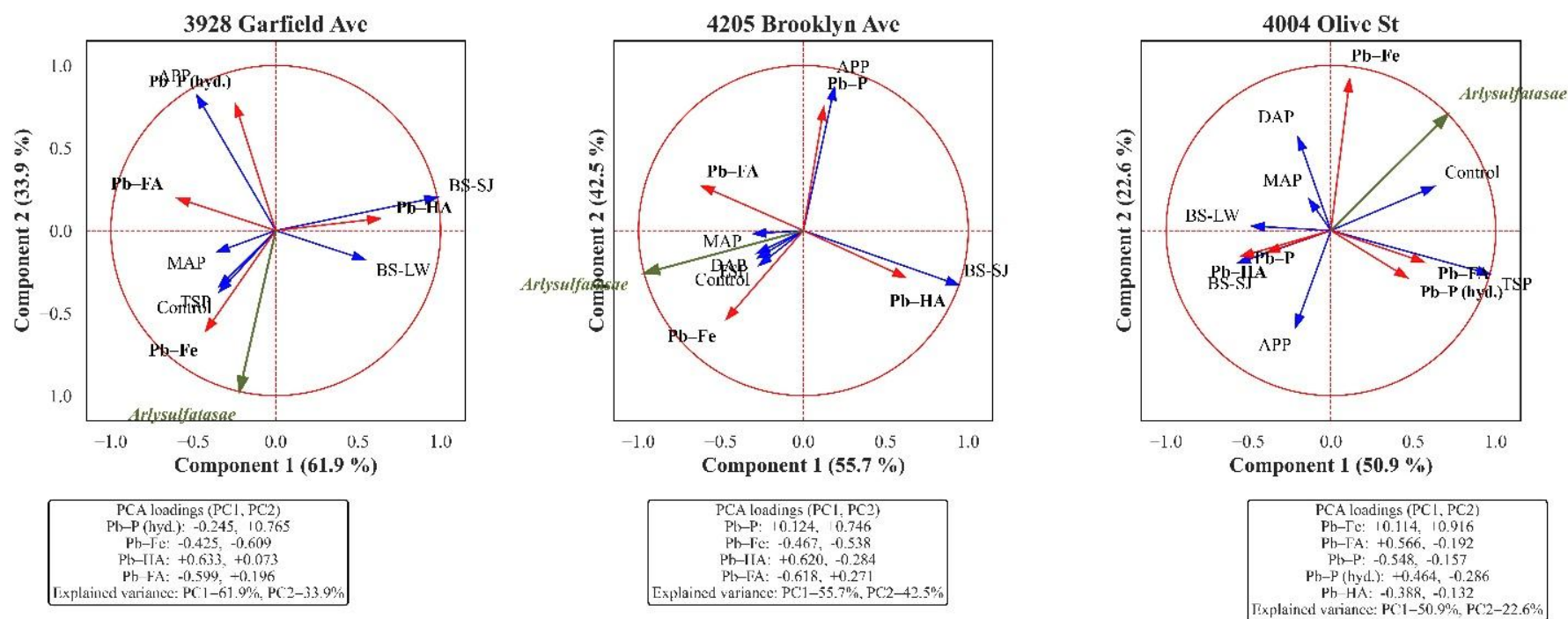


Figure 4.15. Principal component analysis (PCA) showing associations between Pb chemical species Arylsulfatase activity in two urban soils. PCA biplots are presented for the 3928 Garfield Ave and 4004 Olive St sites after 12 months after treatment application. Blue arrows represent Pb chemical species determined by XANES-LCF, blue arrows indicate treatments, and the green arrow represents arylsulfatase activity projected as an external variable. Percent variance explained by each principal component is shown on the axes. Pb-P(Hyd): Hydroxypyromorphite; Pb-P: Pyromorphite; Pb-FA: Fulvic Acid; Pb-HA: Humic Acid; Pb-Fe: Pb sorbed-Fe. Control (non-P amended); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence).

In general, these results suggest that soil enzymes exhibited different responses to the various Pb stabilization mechanisms. The synchrotron-based X-ray absorption analysis results at the 12-month sampling interval confirm that the geochemical immobilization of Pb does not necessarily translate into uniform recovery of soil biological activity, and that enzymatic responses depend on the type of enzyme, the Pb stabilization mechanism, and the physicochemical conditions induced by the treatments. Where Pb speciation determines how P treatments can modify soil chemistry and indirectly influence enzymatic activity. For example, acid phosphatase showed consistent associations with changes in Pb speciation, particularly with increases in Pb associated with stable organic fractions (humic acids). The phosphatases are closely linked to microbial phosphorus demand, enzyme stabilization within organo-mineral complexes, and metal complexation with organic matter (Nannipieri et al., 2011; Tejada et al., 2007). The complexation of Pb into organic forms could reduce chemical reactivity and biological toxicity, creating favorable conditions for acid phosphatase activity, despite the addition of inorganic P, which may repress its synthesis through metabolic feedback mechanisms. In contrast, β -glucosidase and arylsulfatase did not show positive associations with organically complexed Pb or with highly stable Pb–phosphate phases. This pattern is consistent with their functional roles in the carbon and sulfur cycles, respectively, where enzymatic activity is primarily controlled by organic substrate availability, microbial biomass dynamics, soil pH, and mineral surfaces (Mora et al., 2005; Tabatabai, 1994). The positive association observed between these enzymes and Pb sorbed Fe suggests that their enzymatic signal may partially reflect processes of chemical stabilization and enzyme adsorption onto mineral phases, rather than a direct biological response to reduced Pb toxicity. In addition, multiple studies have documented that arylsulfatase is particularly sensitive to changes in soil pH and metal-induced

stress, which may mask any potential benefits of Pb chemical immobilization (Moreno et al., 2003; Tabatabai, 1994).

Conclusions

The application of inorganic and organic phosphorus treatments effectively reduced Pb bioaccessibility, albeit via distinct immobilization mechanisms. Inorganic P treatments primarily promoted Pb stabilization by increasing resin-extractable phosphorus and inducing localized soil acidification, thereby favoring the precipitation of thermodynamically stable Pb-P mineral phases, such as pyromorphite- and hydroxypyromorphite-like species. In contrast, organic P treatments induced Pb immobilization mainly through increases in soil organic carbon and the formation of stable Pb-organic complexes, particularly humic-acid associated Pb, with more moderate or contrasting effects on soil pH. These mechanisms were consistently reflected in site-specific reductions in Pb bioaccessibility and were confirmed by synchrotron-based XANES analyses, which showed pronounced, treatment-dependent shifts in Pb chemical speciation over time. In sum, these findings confirm that long-term Pb stabilization in urban soils is strongly controlled by coupled changes in soil pH, phosphorus availability, and organic matter content, and different amendments immobilize Pb through different chemical processes, mainly precipitation and organic matter surface complexation. Additionally, this study demonstrates that chemical stabilization of Pb induced by phosphorus treatments does not necessarily translate into a uniform recovery of soil biological activity. Speciation analyses revealed substantial transformations in Pb speciation, including the formation of stable Pb-phosphate phases and increases in Pb associated with organic fractions, which contributed to reductions in Pb bioaccessibility. However, enzymatic responses were clearly dependent on enzyme type and on the physicochemical in situ soil properties generated by the treatments, rather than on Pb

chemical immobilization alone. Among the enzymes evaluated, acid phosphatase showed the most consistent associations with Pb speciation, particularly with increases humic acid complexation, suggesting that organic complexation of the metal creates more favorable conditions for microbial activity by reducing its biological toxicity. In contrast, β -glucosidase and arylsulfatase exhibited weaker or more variable responses, primarily controlled by organic substrate availability and soil pH, rather than by the specific chemical form of Pb. Overall, these results highlight that Pb speciation represents a key link between soil chemistry and biological functioning (enzyme-specific). The integration of synchrotron-based techniques with biological indicators provides a mechanistic understanding of the processes governing the remediation of Pb-contaminated soils. This approach provides a novel perspective for evaluating the effectiveness of phosphorus-based management strategies; however, additional research is needed to better understand the influence of amendment application timing, to distinguish among seasonal effects, and the role of vegetative cover should be considered, supporting an integrated remediation perspective that connects soil chemical and biological controls and their relevance for mitigating human Pb exposure.

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Chapter 5 - Mechanisms of lead bioaccessibility reduction induced by phosphorus and biochar amendments

Abstract

Lead is one of the most toxic and persistent contaminants in urban soils, posing a significant risk to human health, particularly for children. Among in situ stabilization strategies, phosphorus-based amendments are widely used due to their ability to promote the formation of sparingly soluble Pb-phosphate minerals. Biochar has recently attracted attention as an alternative remediation material because of its high porosity, large surface area, and capacity to modify soil physicochemical properties; however, its consistency of performance and long-term field effectiveness remain uncertain. This study investigated the mechanisms by which Biochar and phosphorus-based amendments reduce Pb bioaccessibility and compared their relative effectiveness under controlled laboratory and field conditions. Incubation results indicated that phosphorus-based treatments and Biochar significantly reduced soil Pb bioaccessibility relative to the control (~1.34-fold), although the magnitude and consistency of the response depended on amendment type. Under field conditions, treatments significantly modified soil pH, total organic carbon, and extractable P concentrations. Treatment was the primary factor controlling variation in bioaccessible Pb, with reductions following the order triple superphosphate (TSP) ~ ammonium polyphosphate (APP) >> Biochar+APP > Biochar > class A biosolids Lawrence (BS-LW). Similarly, amended soils showed substantial decreases in desorbable Pb by 0.01 M $\text{Ca}(\text{NO}_3)_2$ (~1.91-fold) compared with the control. The principal component analysis revealed distinct Pb immobilization pathways among treatments. Inorganic phosphorus amendments were

primarily associated with Pb-phosphate precipitation, whereas Biochar and BS-LW favored surface organic complexation. In addition, bulk-XANES and μ -XANES-LCF analyses performed on Pb-rich spots identified by μ -XRF confirmed these mechanisms, involving inorganic P treatments, which were dominated by the formation of pyromorphite-like species. Micro-XANES showed Pb-rich spots (“hot-spots”) with high predominance of pyromorphite-like species (>80%), whereas bulk XANES showed only up to 34%. In contrast, Biochar and BS-LW exhibited greater contributions from organic-associated and Pb sorbed Fe phases, indicating dominance of surface complexation and sorption processes.

Introduction

Urban soils contaminated with lead (Pb) pose a significant risk to human health, particularly for children. Lead is one of the most toxic and persistent contaminants, while Pb occurs naturally in soils with an average concentration of about 10 mg kg⁻¹ (Lindsay, 1979). However, anthropogenic activities, including the historical use of leaded gasoline, lead-based paints, aging lead pipes, and mine-derived materials in urban environments, have increased total soil Pb concentrations, often exceeding the current U.S. Environmental Protection Agency (EPA) residential soil screening level of approximately 200 mg kg⁻¹ (EPA, 2024). Lead poisoning mainly affects children whose nervous systems are still in the developmental stage (Brown et al., 2012). Long-term exposure increases the risk of neurodevelopmental effects, such as IQ loss, which may persist into the second decade of life (Centers for Disease Control and Prevention 2012; Laidlaw et al., 2017). The primary pathways for lead exposure are ingestion and inhalation of contaminated soil or dust (Hettiarachchi et al., 2001b). In situ chemical stabilization is one of the most cost-effective techniques for mitigating Pb contamination in urban soils. Among the available approaches, the application of phosphorus (P) amendments has been widely demonstrated as an effective remediation strategy, primarily through its ability to reduce bioaccessible soil Pb (Hettiarachchi & Pierzynski, 2004; Mayer et al., 2022; Scheckel et al., 2013). Bioaccessible soil Pb represents the fraction of the contaminant that is readily available for absorption by the human body following ingestion (Kramer & Ryan, 2000; Sharp & Brabander, 2017; Hettiarachchi et al., 2001; Ruby et al., 1996). Phosphorus treatments promote the transformation of labile and reactive Pb species into highly stable Pb-phosphate minerals, such as pyromorphite-like phases, which are characterized by low solubility across a wide range of soil pH conditions (Baker et al., 2014; Hettiarachchi & Pierzynski, 2002; Mayer et al., 2022;

Lindsay, 1979). For instance, Weber et al. (2015) demonstrated using X-ray absorption near-edge spectroscopy (XANES) that soils amended with triple superphosphate (TSP) exhibited increased formation of chloropyromorphite, ranging from 29% to 35% at P:Pb molar ratios of 4:1 and 16:1, compared with untreated soils. Similarly, Scheckel & Ryan (2004), using X-ray absorption fine structure (XAFS) analyses, reported that applying 3.2% P as TSP resulted in pyromorphite formation up to 41%. They also observed that combining 2.5% iron-rich material with 1% P as TSP produced a comparable level of pyromorphite formation (approximately 41%).

On the other hand, biochar has recently attracted attention as an alternative material for remediating trace-metal contaminated soils. Biochar possesses unique characteristics that depend on the pyrolysis temperature and the feedstock, including increase soil pH, high porosity, large surface area, and the capacity to modify soil physicochemical properties (Ye et al., 2025). In this context, Manzano et al. (2020) found that, in mine-impacted, contaminated soil, the application of biochar pyrolyzed at temperatures above 500 °C at a rate of 5% (w/w) during a one-month incubation period reduced the labile Pb fraction by 79% compared to the untreated soil. The authors attributed these results to several biochar-induced immobilization mechanisms, including its liming effect, which promotes the precipitation of (hydro)metals, and the formation of inner-sphere complexes with carboxylic and phenolic functional groups. Similarly, Pai et al. (2026) demonstrated that biochar amendments can significantly reduce heavy-metal mobility and plant uptake under real agronomic conditions. In a two-year field study conducted in a Cd-contaminated paddy soil, reductions of up to 61.9% in grain Cd concentrations were reported.

Netherway et al. (2019) evaluated poultry litter-derived (PL) and biosolids-derived (BS) biochar produced at 300 and 400 °C and applied at a 1% rate, showing consistent reductions in Pb bioaccessibility (IVBA Pb at pH 1.5) relative to the control, even under relatively low molar

P/Pb ratios. In this study, the biochar was evaluated alongside conventional phosphate treatments and achieved reductions in Pb bioaccessibility ranging from 1.26- to 1.39-fold compared with the control, suggesting a meaningful immobilization effect even at low application rates and moderate P/Pb molar ratios in mining-impacted soils. Despite this, biochar has shown considerable potential for Pb immobilization; the specific physicochemical interactions responsible for reducing Pb bioavailability under laboratory incubation studies remain insufficiently resolved, and realistic evaluations under field conditions remain scarce. Similarly, despite extensive literature supporting the effectiveness of phosphorus-based amendments, significant knowledge gaps persist regarding the dominant immobilization pathways operating under long-term field conditions. This study investigated the mechanisms by which P and biochar-based amendments reduce Pb bioaccessibility and compared their relative effectiveness under both controlled laboratory and field conditions. The assessment combined conventional wet-chemistry analyses with spectroscopic techniques, including bulk X-ray absorption near-edge structure and microscale approaches such as micro-X-ray fluorescence mapping (μ -XRF) and micro-X-ray absorption near-edge structure (μ -XANES) spectroscopy, to better understand the processes controlling Pb immobilization and transformation in treated soils.

Materials and methods

Study Location

This research was conducted on urban site contaminated with Pb, located at 4322 Brooklyn Ave in Kansas City, MO, USA (39.047553° N, 94.558858° W). The site is characterized by a flat topography and a parcel size of approximately 0.16 acres (50 × 133 ft). Historical and urban development records indicate that the site is located within a neighborhood where most parcels previously contained residential structures built before the 1950s, many of

which likely included basements (Accomack Development Group, 2023). Construction materials from this period commonly contained lead-based paint and other hazardous compounds, and demolition practices often lacked proper abatement, resulting in the burial of construction debris within former foundations. These conditions represent potential recognized environmental concerns and are consistent with legacy Pb contamination in urban soils. The initial screening of the site using in-situ p-XRF measurements showed that total soil Pb concentrations ranged from 66 to 749 mg kg⁻¹, indicating substantial variability across the study area. Additional elements, including arsenic (As), chromium (Cr), and barium (Ba), showed lower concentrations, ranging from 11 to 42 mg kg⁻¹, 44 to 74 mg kg⁻¹, and 119 to 512 mg kg⁻¹, respectively. Finally, no detectable concentrations of selenium (Se), mercury (Hg), cadmium (Cd), or silver (Ag) were observed in the soil core samples during the initial field screening. At this location, treatments included inorganic and organic P amendments, Biochar and their combinations were tested in two phases: (i) laboratory incubation study, and (ii) field study.

Laboratory incubation study

To identify treatments that achieved the greatest reduction in bioaccessible soil Pb (%), a long-term laboratory incubation study was conducted using bulk soil samples collected from selected areas, with approximately 15 kg of soil obtained from the 0-15 cm depth. The collected soil was homogenized in plastic buckets, transferred to polyethylene bags, and transported to the laboratory. Prior to the incubation experiment, bulk soil samples were air-dried, ground, and passed through a stainless-steel sieve (< 2 mm). The bulk soil showed a total Pb concentration of 967 mg kg⁻¹, total organic carbon (TOC) of 6.43%, and a cation exchange capacity of 26.7 cmol(+) kg⁻¹. Based on particle size distribution, the soil was classified as sandy loam textural class. Soil subsamples (200 g of <2 mm bulk soil) were prepared in triplicate and placed into 250

mL low-density polyethylene (LDPE) containers. Organic amendments consisted of Class A biosolids applied at a rate of 10% (w/w), sourced from two locations: Lawrence, KS (BS-LW) and Saint Joseph, MO (BS-SJ). The BS-LW was collected on August 1, 2023, from the Wastewater Treatment Plant in Lawrence, Kansas. The material was manually collected in plastic bags, and approximately 2 Mg was transported to Kansas State University. Due to odors, the biosolids were stored outside the greenhouse and covered with three layers of plastic to prevent any increase in moisture content due to precipitation prior to its application. This material had an approximate moisture content of 60%. Similarly, BS-SJ was collected on March 20, 2024, from the St. Joseph Water Protection plant in Saint Joseph, Missouri. The material was loaded into approximately 1Mg bulk bags and transported to Kansas State University. Subsequently, the biosolid was stored under greenhouse conditions until application and was characterized by having a low moisture content (dry material). The results of the chemical characterization of the biosolids showed differences between both sources. The total P concentrations differed between the two biosolids, with BS-LW exhibiting a concentration of 2509 mg kg⁻¹, whereas BS-SJ presented a concentration of 2338 mg kg⁻¹. Inorganic P amendments were applied as commercially available fertilizers at a molar Pb:P ratio of 1:4, including monoammonium phosphate (MAP), diammonium phosphate (DAP), triple superphosphate (TSP), and ammonium polyphosphate (APP). In addition, a native mixed-wood Biochar was applied at a rate of 1% (w/w), either alone or in combination with inorganic P sources at the same molar ratio were applied. A control treatment with no P or Biochar addition was also included. Treatments were thoroughly mixed and Milli-Q water (~18 M Ω H₂O) was added to adjust soil moisture content to 30%. The soil was then remixed, transferred to plastic cups, covered with a double layer of parafilm to minimize moisture loss, and incubated at 25 °C

for the designated incubation period. All treatments were arranged in a completely randomized design (CRD) with three replicates per treatment. To assess treatment effects over time, approximately 30 g of soil was collected from each container at 1, 3, 6, and 12 months after treatment application. Prior to chemical analyses, collected soil samples were oven-dried at 40 °C, ground, and sieved through stainless-steel meshes of 2 mm and 250 µm.

Field Study

The field experiment was implemented during fall 2024 (September 2024). Prior to treatment application, the soil was mechanically tilled using a rototiller to a depth of approximately 15 cm. Buried debris, including remnants of old construction materials, plastics, metals (including automobile parts), glass, ceramic fragments, and large rocks, were manually removed. The soil surface was then leveled, and physical barriers were installed using plastic plot dividers (Dig-Free 20 ft x 6 in black plastic poundable scalloped landscape edging) to delineate individual experimental plots. Phosphorus and biochar-based treatments were subsequently applied to experimental plots measuring 2 m² each. Organic P amendments consisted of Class A biosolids, BS-LW, was applied at a rate of 10% (w/w). Inorganic P treatments included TSP and APP, applied at a molar Pb:P ratio of 1:4. In addition, locally available, mixed-wood biochar was applied at a rate of 1% (w/w) either alone or in combination with APP at the same Pb:P molar ratio (1:4). All treatments were incorporated and mixed in with the top 15-cm soil. A control treatment without P or biochar application was also included. All treatments were arranged in a completely randomized block design (CRBD) with four replicates. Following the amendment application, each plot received a uniform volume of water (~6 L) to maintain consistent soil moisture conditions. Finally, each plot was subdivided into 1 × 1 m subplots, where two different soil cover treatments were applied to evaluate the effect of surface

cover on Pb stabilization. This design aims to determine whether commonly used cover materials in remediation strategies can modify in situ conditions of applied amendments, thereby influencing the effectiveness of Pb stabilization in reducing bioaccessibility. The soil cover treatments consisted of (i) a grass seed mixture and (ii) mixed-wood chips mulch. Finally, soil samples were collected at 0 -months (prior to treatment application) and at 1, 6, and 12 months after treatment application. Soil samples were obtained as composite samples from five soil cores collected at the surface (0-15 cm) using a soil core sampler. The sampler was rinsed with deionized water (DI) between plots to prevent cross-contamination. Composite soil samples were placed in Ziplock bags and thoroughly homogenized. Prior to analysis, soil samples were air-dried, gently ground, and sieved through stainless-steel meshes of 2 mm and <250 μm .

Chemical analysis

Biochar characterization

Biochar used in this study was purchased from Missouri Organic Recycling, Kansas City, MO. Biochar was produced from native woody biomass pre-sized to ≤ 3.8 cm, and pyrolyzed in a rotating drum reactor at average temperatures of approximately 450-600 °C under limited-oxygen conditions, with a residence time of 30-45 min. Following pyrolysis, the Biochar was quenched with clean water in the exit auger to rapidly reduce temperature and prevent oxidation. No chemical additives or post-pyrolysis treatments were applied, as the thermal process alone was sufficient to achieve full activation (J. Phillips, personal communication, 2025).

The biochar pH was determined using a pH/conductivity meter (Thermo Scientific Orion glass electrode, Beverly, MA), after shaking for 10 min with Milli-Q water (1:10 biochar: water ratio), total organic carbon (TOC, %) and total nitrogen (TN, %) were determined by dry combustion method using a LECO TruSpec^{CN} combustion analyzer and cation exchange capacity

(CEC $\text{cmol}_{(+)}\text{ kg}^{-1}$) was obtained by displacement method (Peters, et al., 2015). Total elemental concentrations of Pb and P were determined using aqua regia digestion ($\text{HCl}:\text{HNO}_3$, 1:3) (Premarathna et al., 2010). Biochar samples were digested in a block digester, and the resulting extracts were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES; Agilent 5800). Functional groups were identified using Fourier-transform infrared (FTIR) spectroscopy with a Spectrum Two FTIR spectrometer (Spectrum TwoTM, PerkinElmer). Finally, the surface morphology and pore structure of selected areas were analyzed using scanning electron microscopy (SEM) (Hitachi FlexSEM 100-II) operated in low-vacuum mode, which allowed imaging without conductive coating and preserved the native surface morphology and pore structure of the biochar. Elemental distribution and qualitative estimates of elemental composition (atomic %) were characterized by using energy-dispersive X-ray spectroscopy (EDS) with an Oxford EDS system coupled to a scanning electron microscope (SEM) through elemental mapping (SEM-EDS).

Soil physicochemical properties

Soil pH was measured using a pH/conductivity meter (Thermo Scientific Orion glass electrode, Beverly, MA) with Milli-Q water (1:2.5 soil-to-water ratio), previously calibrated in buffer solutions (Thomas, 1996). Total organic carbon (TOC, %) and total nitrogen (TN, %) were determined by the dry combustion method using a LECO TruSpec^{CN} combustion analyzer, cation exchange capacity (CEC $\text{cmol}_{(+)}\text{ kg}^{-1}$) was obtained by the displacement method (Peters, et al., 2015). Soil texture was measured using the hydrometer method (Sikora & Moore-Kucera, 2014). Finally, extractable-P was obtained using 0.01 M $\text{Ca}(\text{NO}_3)_2$ extraction (Hettiarachchi et al., 2003; Houba et al., 2000).

Total soil Pb concentration

Total soil Pb concentration was determined using a portable X-ray fluorescence (Nilton™ XL3t XRF analyzer, Thermo Scientific) connected to a radiation shielding chamber (XL2 Portable Test Stand PN 420-017, Thermo Scientific), following a modified EPA 6200 method. Briefly, soil samples, previously sieved through a 250 µm mesh, were packed in scintillation vials measuring 4.5 cm in height and 2 cm in diameter, covered with one layer of Kapton tape to form a uniform sample. Continuous readings were then conducted for 2 minutes, and Pb concentration (mg kg^{-1}) was recorded for each sample. Reproducibility was assessed by analyzing duplicate samples every 30 samples, resulting in a coefficient of variation (CV) between duplicates ranging from 0.34% to 25.7% (mean 4.49%, $n = 78$). Quality assurance was achieved by including certificate reference material (NIST 2711a) and internal reference material (IRM) in each site-sampling-time batch. The reference Pb concentration for NIST ($1300 \text{ mg kg}^{-1} \pm \text{uncertainty}$) and IRM ($529 \text{ mg kg}^{-1} \pm \text{uncertainty}$) were used to calculate recovery, with measured recoveries of $103.7\% \pm 6.07$ and $97.4\% \pm 2.88$ (mean $\pm$ SD), respectively.

In vitro Pb bioaccessibility

In vitro Pb bioaccessibility was evaluated using a modified physiologically based extraction test (PBET) at pH 2.5, originally developed by Ruby et al. (1996) and modified following Brown et al. (2003). A simulated gastric solution was prepared using pepsin, organic acids (malate, citrate, acetic, and lactic acids), and Milli-Q water, with the pH adjusted to 2.5 using concentrate trace metal-grade HCl. Soil samples ($<250 \text{ µm}$) were extracted at a 1:100 soil-to-solution ratio by mixing 1 g of soil ($<250 \text{ µm}$) with 100 mL of preheated (37 °C) gastric solution in 125 mL LDPE bottles. Extractions were conducted for 1 h at 37 °C under constant agitation ($30 \pm 2 \text{ rpm}$), with pH monitored and maintained throughout the extraction. Following

extraction, suspensions were filtered through 0.45 µm syringe filters, and the final pH was confirmed to be within 2.5 ± 0.15 . At the final of the extraction if the final pH deviated from the target value (2.5 ± 0.15) sample were excluded from further analysis and repeated to ensure consistency and reliability of the bioaccessibility measurements. PBET extracts were stored at 5 °C prior to analysis. Bioaccessible Pb concentrations were quantified using ICP-OES. The absolute bioaccessible Pb concentration (mg kg^{-1}) was divided by the total concentration (mg kg^{-1}) and expressed as percentage (%) (Eq.5.1).

Equation 5.1 Equation used to calculate the percentage of bioaccessible Pb relative to total Pb concentration in soil samples

$$\text{Bioaccessible Pb (\%)} = \left(\frac{\text{Bioaccessible Pb mg kg}^{-1}}{\text{Total Pb mg kg}^{-1}} \right) * 100$$

Quality assurance and quality control procedures included certified reference materials (NIST 2711a), method blanks, and duplicates samples in each analytical batch. Measured Pb recoveries were 585 ± 49.6 from NIST 2711a (mean $\pm$ SD). These recoveries are consistent with previous reports indicating that approximately 50% of the total Pb concentration in NIST 2711a is extractable using the PBET method, and thus appropriate for QA/QC evaluation of PBET-based bioaccessibility measurements (Obrycki et al., 2017). Analytical reproducibility was evaluated through duplicate analyses, with coefficients of variation (CV) ranging from 0.32% to 24.3%, and an average CV of 7.38%, indicating acceptable precision for PBET Pb determinations.

Lead desorption study

Desorption experiments are essential for evaluating the reversibility of metal retention and their potential for remobilization under changing soil chemical conditions (Scheidegger & Sparks, 1996). Desorption is often slower and less complete than adsorption, indicating an apparent irreversibility commonly referred to as hysteresis (Verburg & Baveye, 1994). This behavior may arise from experimental effects, such as incomplete equilibration during adsorption, as well as from true hysteresis associated with strong binding mechanisms (Scheidegger & Sparks, 1996). These processes are influenced by soil properties, including mineralogy, organic matter, and the duration of metal-soil interactions (aging) (Scheidegger & Sparks, 1996). In addition, processes such as leaching and acidification can decrease soil pH and promote metal release through proton competition and changes in ionic strength (Ibrahim et al., 2024; Verburg & Baveye, 1994). As a result, in contaminated soils, desorption controls the extent to which metals can re-enter the soil solution and become bioavailable for transport or biological uptake (Scheidegger & Sparks, 1996). Lead desorption was assessed using a modified extraction test developed by Hettiarachchi et al. (2003). Briefly, 1.00 g of air-dried soil sieved to <2 mm was suspended in 20 mL of 0.01 M $\text{Ca}(\text{NO}_3)_2$ in 50 mL polypropylene centrifuge tubes. The suspension pH was adjusted to 5.5 ± 0.2 using 0.1 M HNO_3 or 0.1 M NaOH , and the total volume was brought to 25 mL with 0.01 M $\text{Ca}(\text{NO}_3)_2$. All tubes were sealed and shake at 140 rpm (low speed) for 48 h at room temperature using a linear shaker. Solution pH was monitored after 4 h and again after 48 h of equilibration and readjusted as necessary to maintain the target pH, with the volume of acid or base added recorded. After equilibration, samples were centrifuged at 2500 rpm for 30 min, and the supernatant was filtered through 0.45 μm nylon syringe filters and stored at 4 °C until analysis. Extractions where the final pH deviated by more

than ± 0.2 units from the target pH (pH = 5.5) were discarded and repeated to ensure strict pH control and data quality. The solid residue was resuspended in 20 mL 0.01 M $\text{Ca}(\text{NO}_3)_2$ and the desorption experiment as previously described was repeated at least one more time. Lead concentrations in the extracts were quantified by Varian GTA 120 Graphite Tube Atomizer w/AA 240Z Zeeman Atomic Absorption Spectrometer (GF-AAS). The desorbed Pb (mg kg^{-1}) was divided by the total concentration (mg kg^{-1}) and expressed as percentage (%) (Eq.5.2).

Quality assurance/quality control procedures included method blanks, check standards and duplicates. The check standards showed an average recovery factor $100\% \pm 9.17$ ($\pm \text{SD}$). Likewise, the duplicate analyses, with coefficients of variation (CV) ranging from 0.10% to 23.8%, and an average CV of 10.3%, indicating acceptable precision for desorbed Pb determinations.

Equation 5.2. Desorbed Pb (%) calculation based on total Pb concentration

$$\text{Desorbed Pb (\%)} = \left(\frac{\text{Desorbed Pb mg kg}^{-1}}{\text{Total Pb mg kg}^{-1}} \right) * 100$$

Synchrotron-based bulk-XANES, μ -XRF, and μ -XANES analyses

To evaluate the effect of treatments on Pb speciation and its elemental associations, as well as to understand the mechanisms of long-term Pb immobilization, the chemical speciation of Pb and, the spatial distribution and associated elements were analyzed using X-ray absorption near edge structure, micro-X-ray fluorescence, and micro-X-ray absorption near-edge structure spectroscopy at the Advanced Photon Source (APS) of Argonne National Laboratory. Samples included two inorganic P treatments (TSP and APP), an organic P treatment (BS-LW), a Biochar treatment, a combined Biochar+APP treatment, and an untreated control. For each treatment,

composite soil samples were prepared at the 12-month sampling, combining equal amounts of soil collected from eight individual plots (Since no significant effect of cover-type was observed). The composite samples were air-dried, passed through a $<250\ \mu\text{m}$ sieve, and then gently ground to ensure a homogeneous powdered soil and minimize self-absorption during the analyses.

Bulk X-ray absorption near edge structure measurements, soil samples were mounted in Plexiglas holders. Bulk XANES spectra were acquired under ambient conditions across the Pb L₃-edge (12,005 eV) at the 5-BM-B beamline of the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT), using a Si(111) monochromator and a pair of horizontal focusing mirrors to condition and focus the incident beam.

For the synchrotron-based μ -XRF and μ -XANES analyses, powdered samples were mounted on a sample holder by spreading a thin layer of soil onto X-ray-transparent Kapton tape, then covering it with a second layer of Kapton secure the sample during analyses. Data collections were performed at the X-ray microprobe beamline (13-ID-E) of the GeoSoilEnviro Consortium for Advanced Radiation Sources (GSECARS). The beamline is equipped with a micro-focused X-ray beam generated using Kirkpatrick-Baez mirrors, providing a beam size around 1 x 1 mm, and operates over an energy range of 2.4-28 keV. A cryogenically cooled double-crystal monochromator with Si(111) and Si(311) crystals was used for continuous X-ray absorption spectroscopy scanning. Elemental mapping was conducted using 3-element silicon drift detector (SDD), allowing high-sensitivity μ -XRF mapping and the identification of elemental hotspots. Subsequently, μ -XANES spectroscopy was performed on selected regions to determine the chemical speciation of Pb and associated elements. Selected elemental hotspots (intensity > 0.6) identified from μ -XRF maps were targeted for μ -XANES analysis. Linear

combination fitting (LCF) of normalized bulk-XANES and μ -XANES spectra was performed using Athena software to quantify the relative contributions of Pb-bearing phases.

Reference compounds included pyromorphite, chloropyromorphite, hydroxypyromorphite, Pb-organic complexes (Pb bound to fulvic acid, and Pb bound to humic acid), Pb sorbed Fe, galena, anglesite, Pb-carbonate, Pb associated with kaolinite and calcite, were included. Standards contributing less than 5% were excluded from the final fits. The quality of the fits was evaluated using the lowest residual value (R-factor), with values close to unity indicating acceptable fits.

Statistical Analyses

A completely randomized block design (RCBD) with four replicates was used. One-way analyses of variances (ANOVA) and linear mixed-effects models were used, considering treatment and sampling time as fixed effects, and cover type and replicates as random effects. When significant differences were detected, post hoc comparisons were performed using Tukey's honestly significant difference (HSD) test ($P \leq 0.05$) and Dunnett's test ($P \leq 0.05$) in JMP® 19 student version (JMP Statistical Discovery LLC, 2025). In addition, multivariate statistical analyses, including principal component analysis (PCA) were conducted using selected soil properties in a JupyterLab Notebook (Python environment).

Results and Discussion

Initial physicochemical soil characterization

The initial soil characterization is shown in Table 5.1. In general, the site exhibited a slightly alkaline soil pH, total organic carbon (TOC, %) was within the normal range, and the soil texture was classified as sandy loam. The initial extractable-P concentration averaged 28.5 mg kg⁻¹, indicating moderate P availability prior to treatment application. Based on the Missouri

residential risk-based target level (RBTL = 260 mg kg⁻¹) established under MRBCA guidance, the site could be classified as moderately contaminated, with a mean total Pb concentration of 491 mg kg⁻¹ exceeding the threshold by approximately 1.9-fold on average (MRBCA, 2006). Bioaccessible and extractable Pb concentrations were within ranges commonly reported for mildly to moderately Pb-contaminated urban soils. Overall, the high coefficients of variation observed for total Pb (74.4%), bioaccessible Pb (90%), and extractable Pb (56.5%) showed the strong spatial heterogeneity commonly found in urban soils affected by mixed anthropogenic contamination sources.

Table 5.1. Initial selected soil physicochemical properties

Soil properties	Mean	Std Dev	Max	Min	CV
Total Pb (mg kg ⁻¹)	491	365	1878	192	74.4
Bioaccessible Pb (mg kg ⁻¹)	66.25	60.25	283	19	90.9
Extractable Pb (mg kg ⁻¹)*	0.14	0.07	0.24	0.07	56.5
Extractable-P (mg kg ⁻¹) *	28.5	8.78	40.6	8.84	30.8
Soil pH (1:2.5 soil: water ratio)	7.23	0.20	7.67	6.98	2.82
Cation Exchange Capacity (cmol ₍₊₎ kg ⁻¹)	25.7	0.65	-	-	-
Total organic carbon (%)	6.41	0.18	-	-	-
Total Nitrogen (%)	0.40	0.01	-	-	-
Particle size distribution	Sand		Silt	Clay	
			(%)		
	32		53	15	
Soil textural class	Sandy loam				

* Extractable Pb and P (mg kg⁻¹) determined using 0.01 M Ca(NO₃)₂

Biochar characterization

The native wood mix biochar showed a high total organic carbon content (TOC, %) of 64.2%, along with a total nitrogen content (TN, %) of 5.46%. In addition, the cation exchange capacity (CEC) was $18.7 \text{ cmol}_{(+)} \text{ kg}^{-1}$, indicating a moderate capacity for nutrient and cation retention in the soil. Regarding the analyzed elements, the total Pb concentration was negligible (0.01 mg kg^{-1}), while the total P concentration was relatively low (44.8 mg kg^{-1}).

Fourier transformed infrared spectroscopic analysis

The FTIR spectrum of the studied biochar exhibited prominent absorption bands at approximately 3400 , 1650 , and 1080 cm^{-1} , corresponding to -OH , C=O/C=C , and C-O functional groups, respectively (Figure 5.1). These bands are consistent with those reported for lignocellulosic biochar produced through thermochemical conversion, where hydroxyl, aromatic, and aliphatic groups originate from the thermal decomposition of lignin, cellulose, and hemicellulose (Saraugi et al., 2026; Tu et al., 2022; Janu et al., 2021; Uchimiya et al., 2011). The broad -OH band near 3400 cm^{-1} reflects hydrogen-bonded hydroxyl groups, commonly observed in biochar produced at moderate pyrolysis temperatures ($\sim 600^\circ\text{C}$). Similarly, the band around 1650 cm^{-1} is attributed to aromatic C=C and carbonyl (C=O) stretching vibrations, indicating the formation of lignin-derived aromatic structures during pyrolysis (Vandana et al., 2025; Tu et al., 2022; Keiluweit et al., 2010). The peak near 1080 cm^{-1} corresponds to C-O stretching in phenolic, ether, or alcohol groups, whose intensity tends to decrease with increasing pyrolysis temperature due to dehydration, decarboxylation, and the breakdown of glycosidic bonds (Muraro et al., 2026; Keiluweit et al., 2010). Overall, these spectral features, including broad bands between $3500\text{-}3000 \text{ cm}^{-1}$ associated with -OH groups and aromatic C=C vibrations in the $1600\text{-}1650 \text{ cm}^{-1}$ range, reflect the formation of a condensed aromatic structure during

carbonization following the release of volatile compounds (Keiluweit et al., 2010; Sun et al., 2011). Additional bands observed at $\sim 1700\text{ cm}^{-1}$ and $1180\text{--}1030\text{ cm}^{-1}$ further confirm the presence of carbonyl ($\text{C}=\text{O}$) and C-O functional groups, respectively, which are commonly associated with lignocellulosic biochar (Uchimiya et al., 2012, 2011). These oxygen-containing functional groups, particularly hydroxyl ($-\text{OH}$) and carboxyl ($-\text{COOH}$), play a key role in Pb stabilization by providing reactive surface sites for surface complexation and coordination with Pb^{2+} (Beesley et al., 2011; Uchimiya et al., 2011).

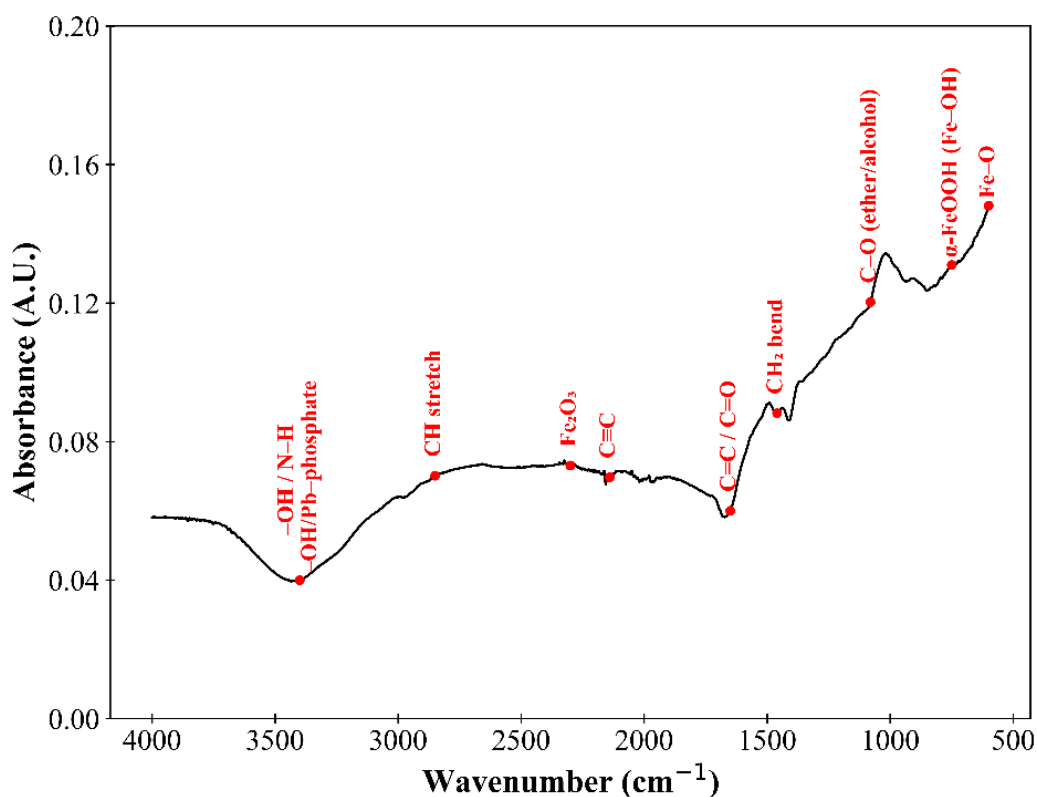


Figure 5.1. Fourier-transform infrared (FTIR) spectrum of the biochar showing the main functional groups

Microscopic analysis

Biochar pore structure (SEM)

The morphology of the biochar is presented in Figure 5.2. Scanning electron microscopy (SEM) allows the identification of significant differences in the surface morphology of biochar (Pai et al., 2026). The SEM images revealed a heterogeneous, highly porous structure, characterized by irregular macropores (A), interconnected mesopores (B), and preserved plant-derived cellular channels (C). This hierarchical porosity increases surface area and reactive sites, improving water retention and sorption processes, which may contribute to the immobilization of heavy metals and the overall improvement of soil physicochemical properties (Joseph et al., 2021; Pai et al., 2026).

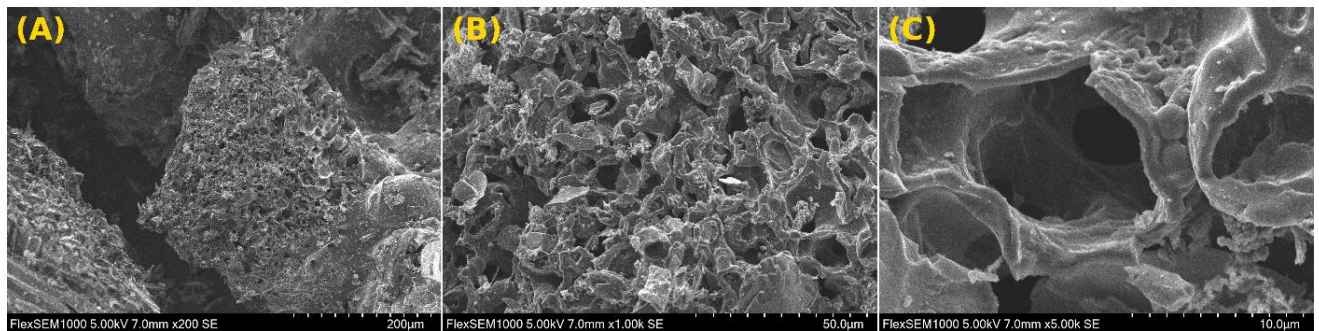


Figure 5.2. Scanning electron microscopy (SEM) micrographs illustrate the hierarchical pore structure of the biochar. (A) Low-magnification image showing overall particle morphology, (B) medium-magnification view of the interconnected porous network, and (C) high-magnification image highlighting internal pore walls and micro-scale structural features. Scale bars: (A) 200 μm, (B) 50 μm, and (C) 10 μm

Microstructure and Elemental Mapping (SEM-EDS)

Images of SEM and EDS analyses are presented in Figure 5.3. The results revealed a highly porous heterogeneous biochar structure, dominated by carbon, with localized enrichments of mineral elements (Ca, K, Si, and P). Elemental mapping showed a heterogeneous distribution

of these elements within the carbon matrix, indicating the presence of ash-derived mineral phases that may contribute to pH buffering, nutrient supply, and heavy-metal immobilization mechanisms (Cairns et al., 2022).

The elemental composition of the biochar was determined by SEM-EDS, with values reported as the mean of four analyses ($n = 4$). It is important to note that EDS is a surface-sensitive technique; the results primarily represent the elemental composition of the biochar surface and may not fully reflect its bulk composition. Therefore, interpretation should be made with caution. In this context, biochar exhibited a dominant carbon content ($86.51 \pm 3.3\%$), followed by oxygen ($11.42 \pm 2.8\%$), confirming its highly carbonaceous nature and the presence of oxygenated functional groups on its surface. Mineral elements were detected in much smaller proportions by SEM-EDS, including Si ($0.49 \pm 0.3\%$), P ($0.40 \pm 0.2\%$), K ($0.38 \pm 0.1\%$), Al ($0.16 \pm 0.1\%$), Ca ($0.15 \pm 0.1\%$), Fe ($0.14 \pm 0.1\%$), and Mg ($0.13 \pm 0.1\%$) (Figure C). These results are consistent with the elemental maps shown in Figure B, where carbon dominates the biochar matrix, while mineral elements such as Si, P, K, Al, Ca, Fe, and Mg appear heterogeneously distributed in specific zones, likely associated with ash-derived mineral phases.

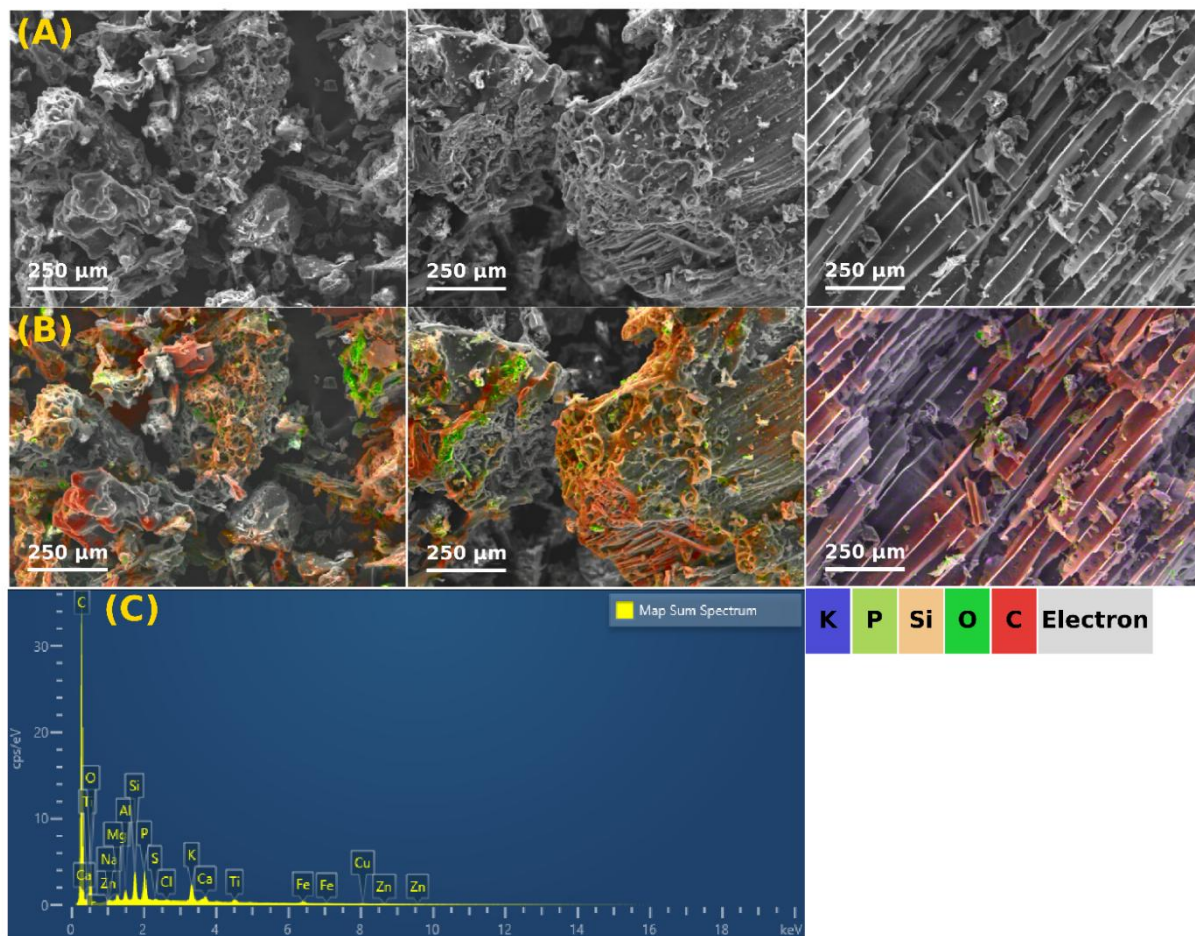


Figure 5.3. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) elemental analysis of the biochar. (A) SEM images illustrating the heterogeneous, porous, and fibrous microstructure of biochar particles. (B) Elemental mapping showing the spatial distribution of major elements (K, P, Si, O, and C) associated with the biochar surface and pore structures. (C) EDS spectrum confirming the presence of dominant mineral and carbonaceous phases within the biochar

Laboratory incubation study

Soil pH

The application of phosphorus-based amendments and biochar treatments had a significant effect on soil pH (Figure 5.4). The mixed-effects model showed highly significant effects of treatment, sampling time, and their interaction ($p < 0.0001$) on soil pH (Table 5.2).

These results indicate that soil pH varied significantly among treatments and across sampling times.

Table 5.2 Analysis of variance (ANOVA) results from the mixed-effects model evaluating the effects of treatment, time, and their interaction on soil pH

Source	Number of Parameters	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F-value	p-value
Treatment	11	11	96	42.294	<.0001
Sampling Time	3	3	96	54.001	<.0001
Treatment * Sampling Time	33	33	96	6.0146	<.0001

For example, at 1 month, biochar and BS-SJ increased soil pH by approximately 0.85 units relative to the control. By 3 months, Biochar maintained the highest pH values (~7.6), although statistical differences among some treatments were reduced. In contrast, MAP, DAP, and APP consistently exhibited the strongest acidifying effects, decreasing pH by approximately 0.85 units, likely due to nitrification of ammonium and proton release during P transformations. At 6 months, a general decline in pH was observed across most treatments, indicating progressive soil acidification (Figure 5.4). Biochar combined with MAP, DAP, and APP showed pH values approximately 0.75 units lower than the control ($p < 0.05$), whereas MAP, DAP, and APP alone remained about 0.85 units below the control ($p < 0.05$), confirming sustained acidification. Notably, BS-SJ exhibited a moderate increase in pH (0.39 units relative to the control), suggesting a delayed liming effect; however, this increase was not statistically significant. Finally, at 12 months, pH values stabilized, with the control (pH = 7.05) and BS-SJ (pH = 7.14) remaining neutral to slightly alkaline conditions. In contrast, treatments containing inorganic P sources and their mixtures with biochar remained significantly lower, ranging from 5.97 (APP) to 6.23 (Biochar+MAP), corresponding to reductions of 0.82 to 1.08 pH units

relative to the control. Among these, APP exhibited the strongest acidifying effect, followed by DAP and MAP (Figure 5.4).

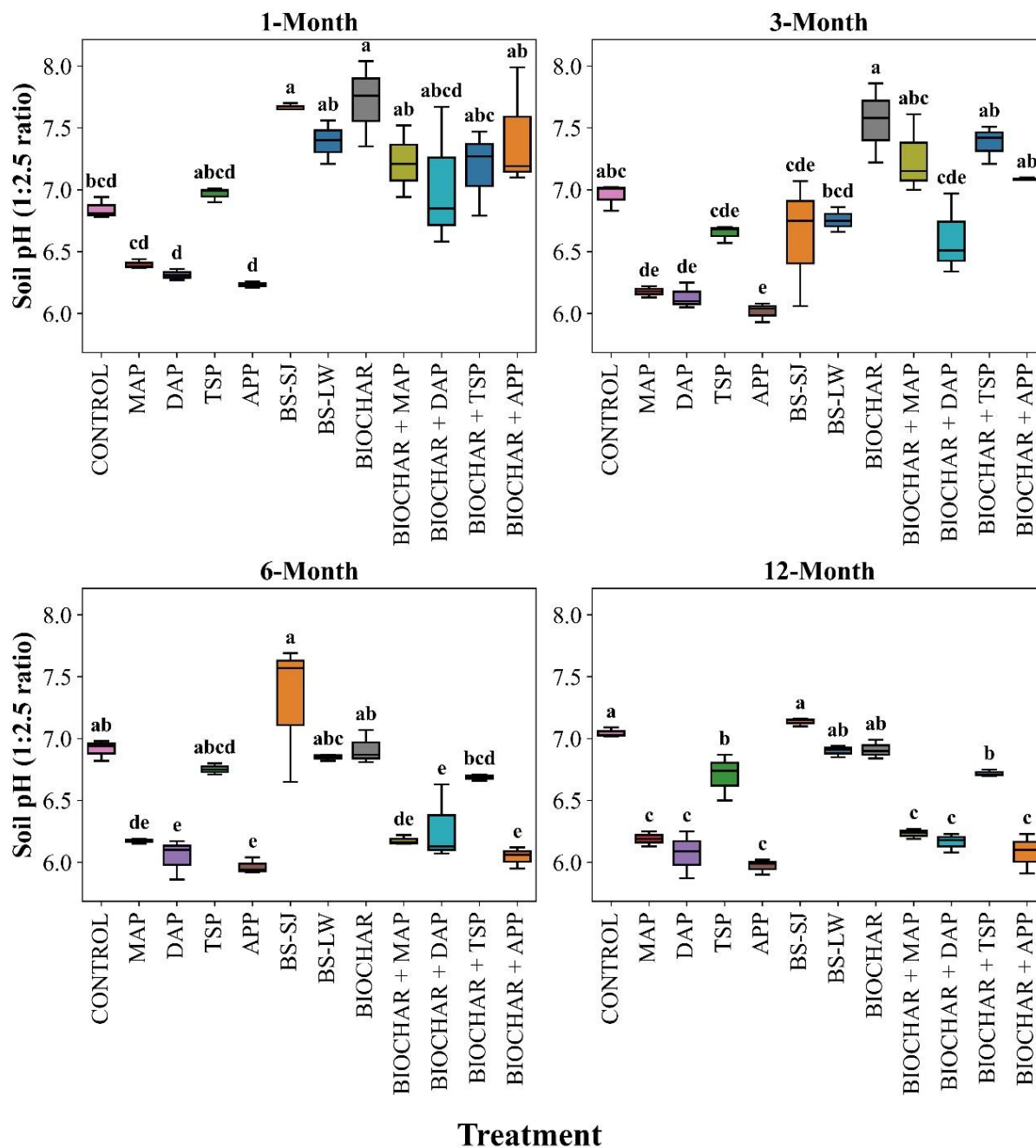


Figure 5.4. Soil pH (1:2.5 soil:water ratio) as affected by phosphorus-based amendments, biosolids, biochar, and their combinations across four incubation periods (1-, 3-, 6-, and 12-month). Boxplots represent the distribution of pH values for each treatment, showing median, interquartile range, and variability. Different letters within each site indicate statistically significant differences among treatments, as determined by Tukey's HSD test

($p < 0.05$). Control (unamended soil); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

Overall, these results demonstrate that soluble P fertilizers induce sustained soil acidification through proton-generating reactions, whereas biochar and biosolids contribute to pH buffering, with direct implications for P availability, mineral phase stability, and Pb immobilization mechanisms in contaminated soils.

In vitro Pb bioaccessibility

The application of phosphorus-based amendments and biochar treatments significantly reduce bioaccessible Pb (%) compared with the control (Figure 5.5). The mixed-effects model showed highly significant effects of treatment, time, and their interaction on soil Pb bioaccessibility ($p < 0.0001$ for all factors) (Table 5.3). These results indicate that bioaccessible Pb (%) varied consistently among treatments and sampling times.

Table 5.3 Analysis of variance (ANOVA) results from the mixed-effects model evaluating the effects of treatment, time, and their interaction on Pb bioaccessibility

Source	Number of Parameters	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F-value	p-value
Treatment	11	11	96	15.48253	<.0001
Sampling Time	3	3	96	17.08021	<.0001
Treatment * Sampling Time	33	33	96	3.58984	<.0001

For example, at the 1-month sampling, BS-SJ treatment showed a significant increase in Pb bioaccessibility, approximately 1.54-fold higher than the control (Figure 5.5). The 3-month

sampling showed that BS-LW treatment exhibited Pb bioaccessibility 1.67-fold lower than the control ($p < 0.05$). At the 6-month sampling, the inorganic P treatments (DAP, TSP, and APP) showed, on average, reductions of up to 1.48-fold relative to the control ($p < 0.05$), while organic treatment BS-LW exhibited a consistent reduction of 1.62-fold relative to the control. In addition, biochar treatments combined with MAP, DAP, and APP showed an average reduction of 1.62-fold relative to the control, whereas biochar applied alone did not show a clear reduction at this sampling time (Figure 5.5). Finally, at the 12-month sampling, a content effect of the P-based amendments and biochar treatments was observed. Treatments including, MAP, DAP, TSP, and APP, resulted in average, a 1.33-fold reduction in Pb bioaccessibility, while BS-LW exhibited a 1.22-fold reduction, both relative to the control. Finally, biochar combined with TSP, DAP, MAP, and APP consistently reduced Pb bioaccessibility by up to 1.34-fold compared with the control. In contrast, biochar applied alone showed no significant differences ($p > 0.05$). Overall, the significant reductions depended on the sampling time and the type of amendment applied. Phosphorus-based treatments and biochar, combined with inorganic P, showed high potential to reduce bioaccessible Pb (%) compared with the untreated control ($p < 0.05$). These treatments exhibited the most consistent long-term reductions in Pb bioaccessibility, becoming more effective after 6 months and stabilizing by 12 months. In contrast, biochar applied alone did not show a clear or consistent response throughout the incubation period (Figure 5.5).

The laboratory incubation study showed the most consistent reductions in bioaccessible soil Pb (%) after six months of application and remained stable up to 12 months. This suggests that the stabilization processes of bioaccessible Pb are time-dependent, as amendments require a stabilization period to progressively modify soil physicochemical conditions, ultimately controlling Pb bioaccessibility.

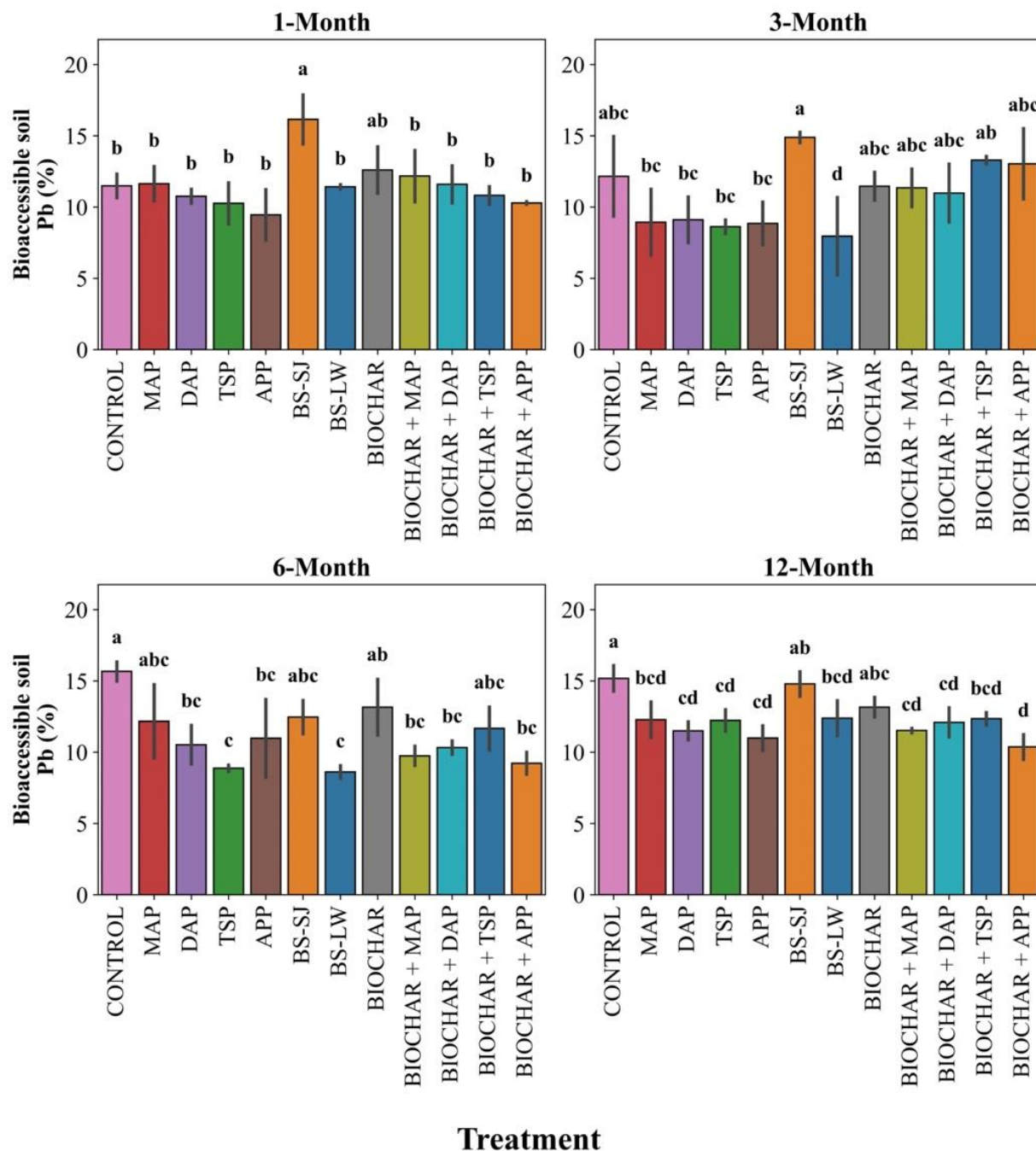


Figure 5.5. Bioaccessible soil Pb (as % of total) affected by phosphorus-based amendments, biosolids, biochar, and their combinations across four incubation periods (1,3, 6 and 12 months). Different letters on the top of the bars within each site indicate statistically significant differences among treatments, as determined by Tukey's HSD test ($p < 0.05$). Control (unamended soil); MAP (Monoammonium phosphate); DAP (Diammonium phosphate); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-SJ (Class A Biosolids, Saint Joseph), BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

These findings are consistent with other laboratory-based studies (Scheckel & Ryan, 2004; Kastury et al., 2019), which indicate that P amendments do not significantly affect Pb bioaccessibility in the short term. However, over longer incubation periods, significant reductions in Pb bioaccessibility have been consistently reported, highlighting the importance of time-dependent stabilization processes. Similar findings were reported by Hettiarachchi et al. (2001), who in an incubation study observed reductions of approximately 20% in the gastric phase and up to 60% in the intestinal phase following P amendment application. In addition, Farfel et al. (2005), using urban soils contaminated with Pb (1000 mg kg^{-1}), found that the application of biosolids resulted in reductions in Pb bioaccessibility up to 67% after one year, indicating that biosolids promote long-term Pb stabilization process.

To evaluate the effectiveness of selected treatments under field conditions and to further investigate the mechanisms responsible for Pb bioaccessibility reduction, a second experiment was designed using a representative subset of amendments. The following treatments were selected: (1) inorganic P amendments (TSP and APP), (2) an organic P amendment (BS-LW), (3) biochar, (4) the combination biochar + APP, and (5) an unamended control. This selection allows the comparison of treatments with potentially different mechanisms.

Field Study

Soil properties affected by phosphorus- and biochar-based treatments

The primary soil properties affected by the P and biochar-based amendment were soil pH, total organic carbon (TOC, %), and extractable P concentration; the main results are presented from Figure 5.7 to Figure 5.9. Among the evaluated soil properties, soil pH showed the most pronounced response to the applied treatments over time (Figures 5.6 and 5.7). The mixed-effects model showed a highly significant effect of treatment on soil pH ($p < 0.0001$) and

sampling time ($p < 0.0001$). Whereas the treatment x sampling time interaction was not significant ($p = 0.137$) (Table 5.4).

Table 5.4. Analysis of variance (ANOVA) results from the mixed-effects model evaluating the effects of treatment, time, and their interaction on soil pH

Source	Number of Parameters	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F-value	p-value
Treatment	5	5	122	34.41	<.0001
Sampling Time	2	2	122	28.65	<.0001
Treatment * Sampling Time	10	10	122	1.526	0.137

The variance component analysis showed that the residual variance accounted for approximately 69% of the total variability, followed by replicates (27%), while cover type contributed less than 3% ($p = 0.6504$) (Table 5.5). The effects of phosphorus- and -biochar-based amendments on soil pH, based on mixed-effects model analysis, are presented in Figure 5.6. The biochar treatment exhibited the highest pH (mean = 7.40) and did not differ significantly from the control (mean = 7.32). TSP showed an intermediate response (mean = 7.19), overlapping with both higher and lower statistical groups, while BS-LW (mean = 7.09) showed a significant reduction of 0.23 pH units relative to the control. In contrast, APP (mean = 6.84) and Biochar+APP (mean = 6.82) resulted in the lowest pH, which were values statistically different from the control and biochar treatments, indicating a consistent acidifying effect, with an average reduction of 0.49 pH units relative to the control (Figure 5.6).

Table 5.5. Contribution of random effects (replicates and cover type) and residual variance to total variability in soil pH based on mixed-effects model analysis

Variance Component	Variance Ratio	Estimate	Std Error	95% Lower	95% Upper	Wald test p-Value	Percentage of Total
Replicates	0.397	0.016	0.014	-0.011	0.044	0.252	27.7
Cover type	0.035	0.001	0.003	-0.004	0.007	0.613	2.45
Residual	-	0.041	0.005	0.032	0.053	-	69.8
Total	-	0.058	0.015	0.037	0.105	-	100

Overall, these results demonstrated that biochar maintained or slightly increased soil pH (+0.08 units) due to its liming effect (Beesley et al., 2011; Cao & Harris, 2010), whereas APP and the Biochar+APP treatment reduced soil pH by approximately 0.45-0.50 units throughout the 12-month period. These results suggested that APP was the primary driver of soil acidification. This effect can be attributed to the hydrolysis of ammonium polyphosphate under acidic conditions, which releases protons (H^+), coupled with subsequent nitrification of ammoniacal N, thereby contributing to acidity (McBeath et al., 2007). Similarly, Dai et al. (2025), using biochar produced via pyrolysis at 500 °C and modified with phosphoric acid (H_3PO_4), reported that the biochar pH was reduced by an average of approximately 6 units relative to the unmodified biochar (pH 10.2), and by 4.8 units relative to the unamended soil pH (pH 9.01), confirming that acid treatment is the primary driver of biochar acidification. According to Elbana et al. (2025), reductions in pH ranging from 0.11 to 0.44 units in biochar derived from palm fronds (PF) and guava branches (GB), treated with inorganic P sources (superphosphate, 13% P_2O_5), can also be attributed to chemical reactions that promote the protonation of functional groups and the formation of aliphatic and phenolic hydroxyl groups. This transformation shifts the typically alkaline nature of biochar, generally associated with its ash content and basic cations, toward a more acidic system (Elbana et al., 2025; Wang et al., 2022). In contrast, TSP and BS-LW did not differ significantly from the control. In Chapters 2

and 3 of these dissertations, similar results were reported: TSP applied at a 1:4 molar ratio (P:Pb) reduced the pH by 0.3 units relative to the control after 12 months of treatment. Similarly, the application of Biosolids Class A showed a slight alkalinizing effect, maintaining pH values similar to or slightly higher than the control.

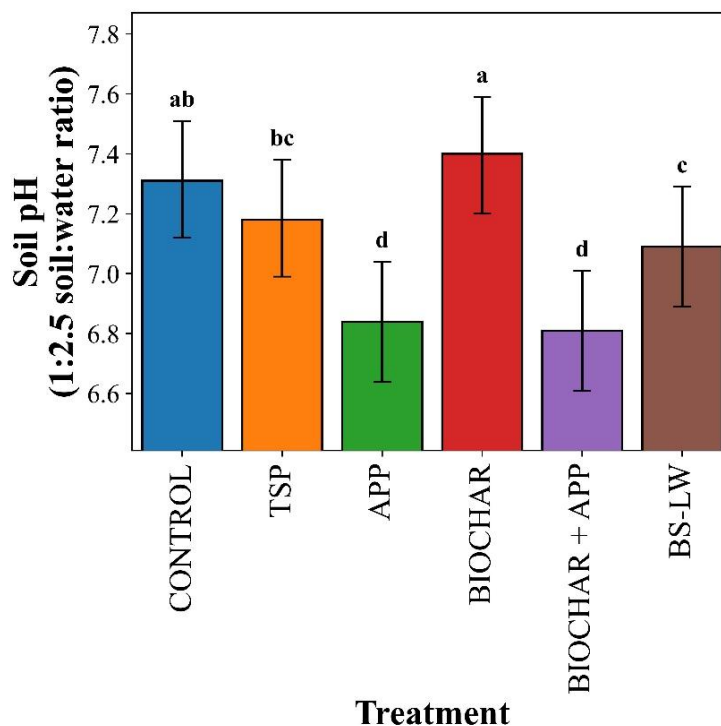


Figure 5.6. Soil pH under phosphorus- and -biochar-based treatments. Statistical comparisons were performed using a mixed-effects model; different letters above the bars indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$). Control (unamended soil); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

The effect of sampling time on soil pH, based on mixed-effects model analysis, is presented in Figure 5.7. In general, soil pH exhibited an initial decrease followed by stabilization over time. The greatest acidification was observed at the 1-month sampling, when soil pH decreased to approximately to pH 6.93. Over time, pH values recovered, reaching levels

comparable to the initial condition at 6 months (pH = 7.23) and remaining relatively stable at 12 months (pH = 7.15). This pattern suggests an initial acidification followed by stabilization of soil pH, likely driven by the buffering capacity of the soil.

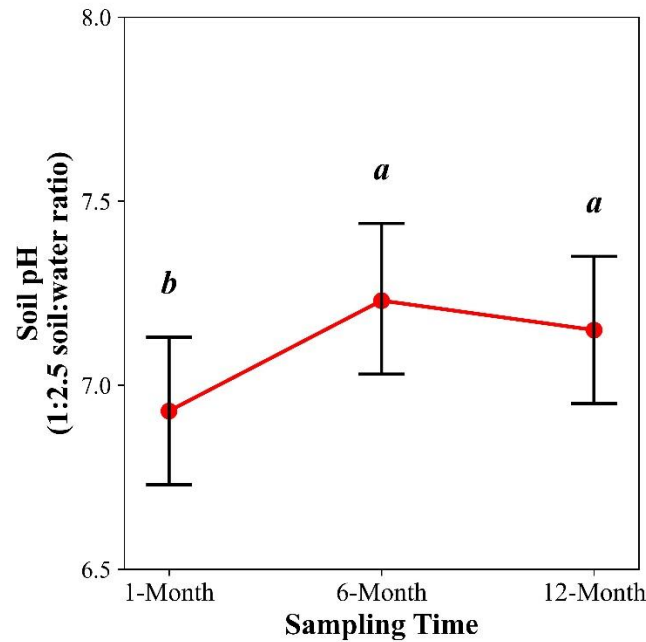


Figure 5.7. Effect of sampling time on soil pH (1:2.5 soil:water ratio) in the field experiment, based on mixed-effects model analysis. Points represent least squares means, and error bars indicate 95% confidence intervals. Different lowercase letters denote significant differences among sampling times according to Tukey's HSD test ($p < 0.05$).

To obtain meaningful results, the analysis of total organic carbon (TOC percentage), measurements were conducted at the 12-month sampling and combined samples from grass and mulch cover type (Figure 5.8). There was an increasing trend in (TOC, %) with the application of organic amendments, particularly biochar and biosolids (Figure 8). On average, biochar and BS-LW resulted in approximately 1.04-and 1.12-fold higher TOC (%) values relative to the control, while the Biochar+APP treatment showed the highest increase (1.19-fold). Despite these apparent increases, most treatments did not differ statistically from the control, likely due to soil

variability within treatments. In contrast, APP exhibited the lowest TOC (%) values and was significantly lower than Biochar+APP. Overall, these results suggest that organic amendments tend to enhance soil organic carbon, although these effects were not always statistically significant under field conditions (Figure 5.8).

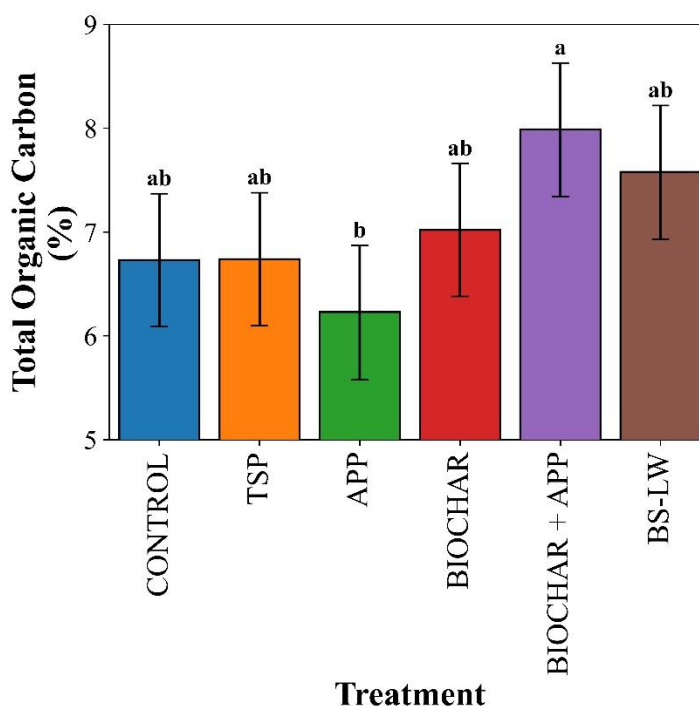


Figure 5.8. Effect of phosphorus- and -biochar-based amendments on total organic carbon (TOC, %) at 12 months sampling time. Bars represent treatment means and standard deviation (SD). Different letters above the bars denote significant differences among treatments according to Tukey's test ($p < 0.05$). Control (unamended soil); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

In general, biochar-based and organic amendments increased TOC (%), by average of approximately 1.11-fold, relative to the control at 12-month sampling. The increase suggests that both amendments could enhance TOC by the direct addition of organic matter to the soil, which may be stabilized through physical protection with the soil aggregates, sorption onto mineral surface, and the formation of more recalcitrant carbon pools. Similar results were reported by Ma

et al. (2023), who evaluated maize straw-derived biochar produced at 450 °C (< 2 mm) and applied it as a surface amendment, later incorporated into the plow layer (0-15 cm) under a winter wheat-summer maize rotation system. Found that, after four years, biochar application increased TOC by approximately 1.10-fold compared to the unamended control, confirming the potential of biochar to enhance soil carbon storage under field conditions. Likewise, Weng et al. (2022) reported that biochar application in a managed subtropical pasture increased SOC by approximately 1.43-fold relative to the control after 9.5 years, with both single and repeated applications showing significant effects. In sum, the increase in SOC following biochar application can be attributed to the direct input of stable, recalcitrant carbon that resists microbial decomposition, as well as reduced SOC mineralization (negative priming) and enhanced carbon stabilization within soil aggregates. Although a small fraction of labile carbon (3-4%) is rapidly mineralized, most biochar-derived carbon remains in the soil due to its stable structure and physical protection within aggregates (Tian et al., 2024; Ma et al., 2023). In addition, Scharenbroch et al. (2013) conducted a controlled microcosm experiment using contrasting soil types (sand, silt loam, and compacted clay), where biosolids were applied as surface amendments at agronomic rates (25 Mg ha⁻¹ yr⁻¹). The biosolids, characterized by high organic matter and nutrient content, were evaluated under greenhouse conditions over 18 months, demonstrating their potential to influence soil properties, particularly increased SOC relative to the control (NUL) across all soil types, with approximate increases of 1.1-1.4-fold in fine-textured soils and up to ~3-fold in sandy soils, reflecting both the direct input of organic matter and the stronger relative response in soils with low baseline carbon content. Comparable results were reported by Haney et al. (2015), who conducted a long-term field study in Texas to evaluate the effects of anaerobically digested biosolids applied to silt loam soils under non-till forage systems.

Biosolids were surface applied at rates ranging from 20 to 60 Mg ha⁻¹ yr⁻¹ over periods of up to 20 years. Results showed that biosolid application significantly increased SOC stocks, with responses positively correlated to both application rate and duration. After 8 years of continuous applications, SOC increased by approximately 32-92% in surface soils compared to the unamended control. Moreover, long-term applications (20 years at 20 Mg ha⁻¹ yr⁻¹) resulted in an additional 36% increase in SOC relative to shorter-term treatments, highlighting the strong potential of biosolids to promote sustained carbon accumulation in soils. Overall, biosolid application increased SOC across soils, primarily due to the direct input of organic matter and its relatively lower C/N ratio compared to other amendments. These results indicate that biosolids not only contribute to immediate increases in SOC but also promote long-term carbon accumulation, with effects extending beyond the surface layer and persisting over time (Haney et al., 2015; Scharenbroch et al., 2013).

The application of P-based and biochar-based treatments significantly increased extractable P compared with the control (Figure 5.9). The mixed-effects model showed a highly significant effect of treatment on extractable P concentrations ($p < 0.0001$), whereas sampling time ($p = 0.6596$) or the treatment x sampling time interaction ($p = 0.0804$) was not significant (Table 5.6). The variance component analysis showed that the residual variance accounted for the largest proportion of the total variability (79.1%), followed by cover type (12.7%) and replicates (8.2%). Both random effects were non-significant ($p > 0.33$), indicating that spatial variability among replicates and differences in soil cover had a relatively minor influence compared with the treatment effect (Table 5.7).

Table 5.6. Contribution of random effects (replicates and cover type) and residual variance to total variability in extractable P based on mixed-effects model analysis

Variance Component	Variance Ratio	Estimate	Std Error	95% Lower	95% Upper	Wald test p-Value	Percentage of Total
Replicates	0.103	372.690	385.69	-383.77	1128.1	0.335	8.18
Cover type	0.161	577.451	887.33	-1161.6	2316.5	0.515	12.7
Residual		3597.78	460.64	2841.6	4703.4		79.1
Total		4547.42	1063.38	3016.1	7637.2		100

Table 5.7. Analysis of variance (ANOVA) results from the mixed-effects model evaluating the effects of treatment, time, and their interaction on extractable P

Source	Number of Parameters	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F-value	p-value
Treatment	5	5	122	48.554	<.0001
Sampling Time	2	2	122	0.4174	0.6569
Treatment * Sampling Time	10	10	122	1.7342	0.0804

The extractable P concentration showed the following decreasing order: Biochar+APP > TSP > APP > Biochar > BS-LW >> Control (Figure 5.9). The Biochar+APP treatment showed values 4.14-fold higher than the control, while the inorganic treatments (TSP and APP) showed, on average, concentrations 3.63-fold significantly higher than the control. However, the biochar and BS-LW treatments did not show significant increases in extractable P ($p > 0.05$) (Figure 5.9). In general, the Biochar+APP combination exhibited the highest extractable P concentrations, highlighting the complementary behavior between APP and biochar: APP serves as a source of readily extractable P, while biochar may act as a carrier or matrix that favors its retention and reduces its fixation in the soil. The increase in extractable P observed in the Biochar+APP treatment can be explained by the combined effect of soil acidification and reduced P fixation.

The hydrolysis of ammonium polyphosphate releases protons, thereby decreasing soil pH and enhancing P solubility (Wang et al., 2022; McBeath et al., 2007). Additionally, biochar provides reactive surfaces that improve P retention and limit losses, thereby increasing extractable P concentrations (Glaser et al., 2002).

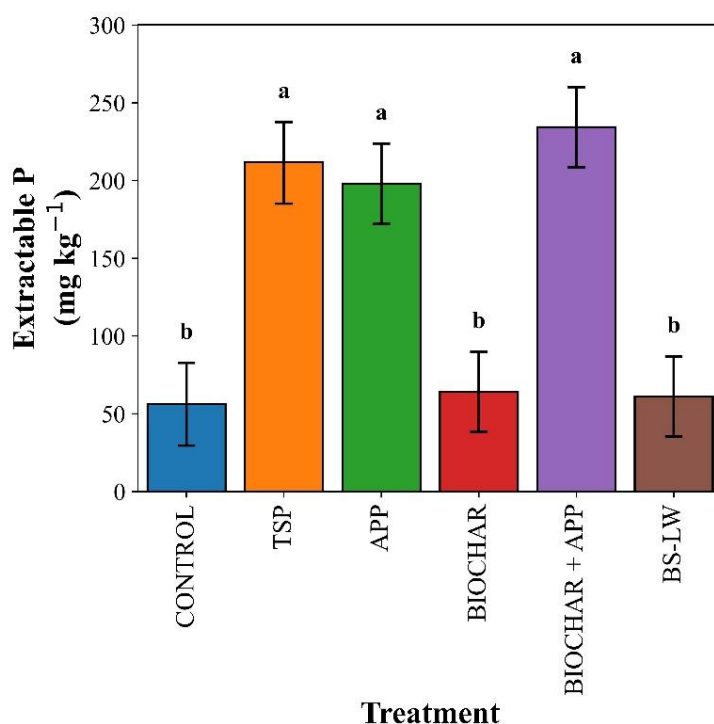


Figure 5.9. Extractable P concentration under phosphorus- and -biochar-based treatments. Statistical comparisons were performed using a mixed-effects model; different letters above the bars indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$). Control (unamended soil); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

Similar responses have been reported by Arif et al. (2016), where in a maize-wheat field experiment conducted on an alkaline silty clay loam soil, biochar (10 t ha⁻¹) combined with organic and inorganic P sources (100 kg P ha⁻¹) increased soil available P by approximately 1.1-1.4-fold compared to treatments without biochar, highlighting its role in enhancing P retention and reducing fixation under field conditions. In addition, the inorganic P amendments, increase in available P following fertilizer application is primarily driven by the rapid dissolution of

inorganic P sources, which release orthophosphate ions into the soil solution and increase the labile P pool (Wang et al., 2015; Malik et al., 2012)

In vitro Pb bioaccessibility

The treatment application showed a consistent trend toward reducing bioaccessible soil Pb (%) compared to the untreated control. However, the statistical significance of these reductions depended on the type of treatment applied (Figure 5.10). The mixed-effects model showed a significant effect of treatment on bioaccessible Pb (%) ($p < 0.0001$), whereas sampling time ($p = 0.2125$) or the treatment x sampling time interaction ($p = 0.6165$) was not significant (Table 5.8). Variance component analysis indicated that most of the variability was explained by the residual term (97.7%), followed by replicates (2.33%), while cover type contributed negligibly. These results confirm that treatment was the primary driver of variation in bioaccessible soil Pb (%) (Table 5.9).

Table 5.8. Analysis of variance (ANOVA) results from the mixed-effects model evaluating the effects of treatment, time, and their interaction on bioaccessible soil Pb (%)

Source	Number of Parameters	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F-value	p-value
Treatment	5	5	122	3.633	<0.0001
Sampling Time	2	2	122	15.91	0.2125
Treatment * Sampling Time	10	10	122	2.217	0.6165

Table 5.9. Contribution of random effects (replicates and cover type) and residual variance to total variability on bioaccessible soil Pb (%) based on mixed-effects model analysis

Variance Component	Variance Ratio	Estimate	Std Error	95% Lower	95% Upper	Wald test p-Value	Percentage of Total
Replicates	0.0238	0.1335	0.2363	-0.327	0.598	0.566	2.33
Cover type	-0.0043	-0.0245	0.0775	-0.176	0.127	0.751	0.00
Residual	-	5.6813	0.7215	4.495	7.410	-	97.7
Total	-	5.8169	0.7554	4.579	4.579	-	100

Consistent with these findings, TSP and APP produced the greatest reductions in bioaccessible soil Pb, averaging 1.22-fold lower than the control. In contrast, BS-LW, biochar, and Biochar+APP resulted in decreases from 1.05, 1.08 to 1.19-fold lower than the control, respectively. Despite these apparent differences, the reductions observed with BS-LW, biochar, and Biochar+APP were not statistically significant compared with the control ($p > 0.05$; Figure 5.10). The results of the incubation experiment demonstrated that inorganic P sources reduced bioaccessible soil Pb by up to 22.1%, while organic sources (BS-LW) by up to 12.4%, and biochar combined with inorganic sources reduced it by up to 23.1%. However, under real field conditions, inorganic P treatments (TSP and APP) were the most effective in reducing soil bioaccessible Pb (%), showing high potential for reducing bioaccessible Pb, with an average reduction of approximately 20% compared with the control. Similar results were reported by Hettiarachchi & Pierzynski. (2004) and Scheckel & Ryan. (2004) who, using inorganic P treatments such as TSP, APP, and MAP, observed reductions of approximately 30.4% in bioaccessible soil Pb compared with the control. Likewise, Alankarage et al. (2024) and Gu et al. (2020) reported reductions in Pb bioavailability of approximately 10-18% when applying phosphate amendments such as TSP, struvite, and phosphate rock (PR), compared with untreated soils. In general, these results support the effectiveness of P amendments in reducing Pb

bioaccessibility, while also highlighting that most available evidence is derived from controlled laboratory studies, with limited validation under field conditions.

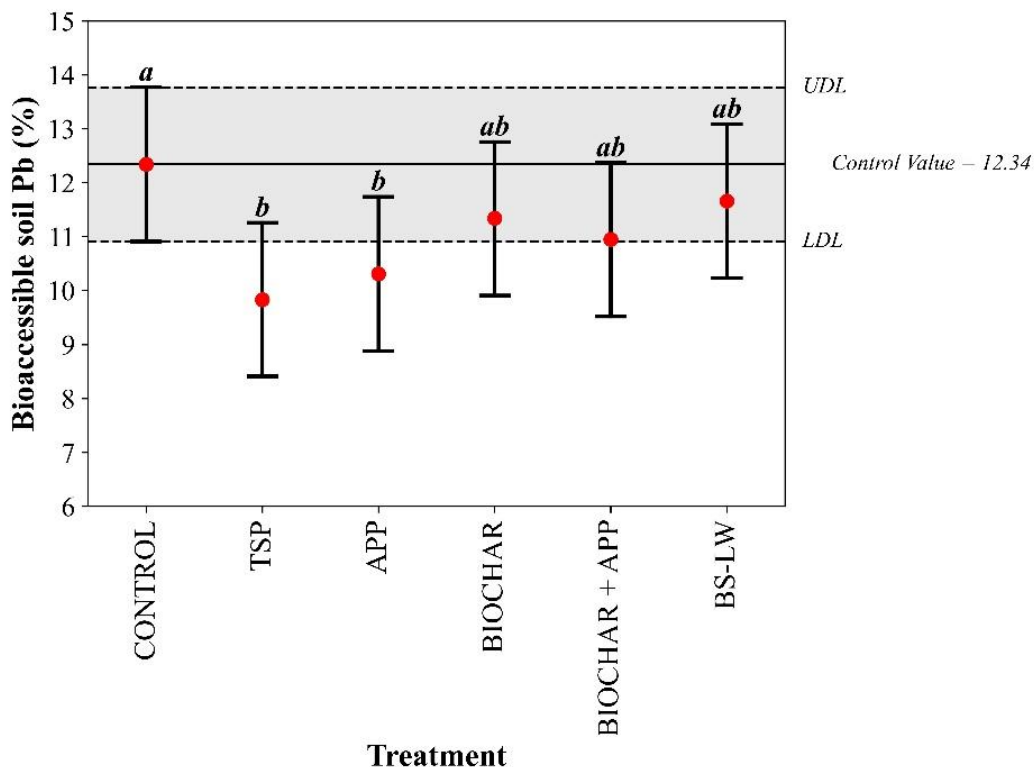


Figure 5.10. Bioaccessible soil Pb (as % of total) affected by phosphorus- and -biochar-based treatments. Points represent treatment means, and bars indicate the standard deviation. The shaded area represents the desorption range of the control soil based on Dunnett's test. Statistical comparisons were performed using a mixed-effects model; different letters above the points indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$). Control (unamended soil); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

Lead desorption

The results showed that P-based and biochar treatments reduced a considerable proportion of desorbable Pb in the amended soils (Figure 5.11). The mixed-effects model showed a significant effect of treatment ($p < 0.0001$); sampling time and the treatment x time interaction were not significant ($p > 0.05$) (Table 15.0). Variance component analysis indicated

that most of the variability was explained by the residual term (69.8%), followed by replicates (28.1%), while cover type contributed minimally (2.1%) (Table 5.11). These results confirm that treatment was the primary driver of variation in desorbed Pb. The control showed the highest desorbable Pb%, which differed significantly from most treatments. Compared to the control, desorbable Pb decreased in TSP (1.53-fold), followed by biochar (1.67-fold), Biochar+APP (1.87-fold), and BS-LW (1.91-fold). However, APP showed a 1.39-fold reduction relative to the control; no statistical differences were observed (Figure 5.11).

Table 5.10. Analysis of variance (ANOVA) results from the mixed-effects model evaluating the effects of treatment, time, and their interaction on desorbable Pb (%).

Source	Number of Parameters	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F-value	p-value
Treatment	5	5	124	5.121	<0.0003
Sampling Time	2	2	124	1.424	0.2424
Treatment * Sampling Time	10	10	124	0.944	0.4951

Table 5.11. Contribution of random effects (replicates and cover type) and residual variance to total variability desorbable Pb (%) based on mixed-effects model analysis.

Variance Component	Variance Ratio	Estimate	Std Error	95% Lower	95% Upper	Wald test p-Value	Percentage of Total
Replicates	0.00015	1.93e-5	-2e-8	-0.327	0.00002	0.984	2.33
Cover type	0.01899	2.00e-7	-9e-5	-0.176	0.00013	0.6832	0.00
Residual	-	1.20e-3	10e-4	4.495	0.0016	-	97.7
Total	-	1.27e-3	10e-4	4.579	0.0017	-	100

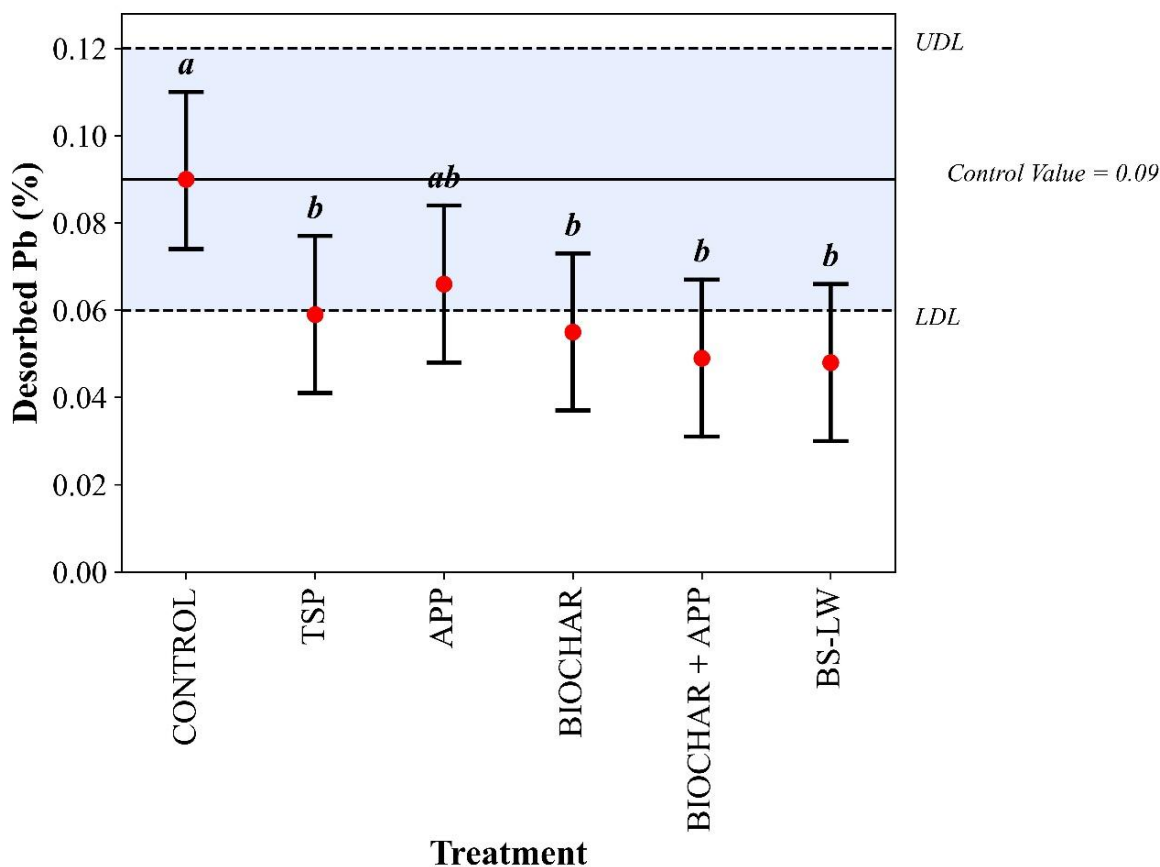


Figure 5.11. Desorbed Pb (as % of total) affected by phosphorus- and -biochar-based treatments. Points represent treatment means, and bars indicate the standard deviation. The shaded area represents the desorption range of the control soil based on Dunnett's test. Statistical comparisons were performed using a mixed-effects model; different letters above the points indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$). Control (unamended soil); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

Phosphorus-based treatments and biochar significantly reduced desorbed Pb in the amended soils. The largest reductions were observed in BS-LW (1.91-fold) and Biochar+APP (1.87-fold), followed by biochar (1.67-fold), and TSP (1.53-fold) relative to the control. Similar results were reported by Gomes et al. (2022), who evaluated Pb desorption in soils amended with phosphate, lime, biochar, and biosolids using a stirred-flow reactor system coupled with Mehlich-III extraction to assess Pb desorption kinetics under controlled laboratory conditions. In that study, inorganic amendments, particularly phosphate, were the most effective in reducing Pb

desorption, decreasing the desorbed fraction to approximately 5-8%, whereas organic amendments, such as biochar and biosolids, showed a lower capacity to reduce Pb desorption kinetics. Similarly, Cao & Harris (2010) conducted a sorption experiment to evaluate the capacity of biochar to retain Pb in aqueous solution. When 0.25 g of biochar derived from dried manure solids, pyrolyzed at 500 °C, was mixed with 50 mL of a Pb solution at 200 mg L⁻¹ prepared in 0.01 M NaNO₃ as a background solution, approximately 93% of the Pb was removed from the solution.

Multivariate evaluation of Pb stabilization mechanisms based on wet-chemical data

The PCA analysis was conducted using data from the 12-month sampling period. Samples were combined by cover type and differentiated by treatment. In addition, the PCA was performed using only variables related to Pb mobility and stabilization to evaluate treatment-induced effects while avoiding bias from initial differences in total Pb concentrations (Figure 5.12). Overall, the PCA revealed clear separation patterns among treatments, indicating distinct chemical responses. For example, the APP treatment was positioned separately in the upper left quadrant of the PCA space and was characterized by high extractable P (189 mg kg⁻¹), a slightly lower soil pH (6.9), reduced Pb desorption (0.06%), and the lowest Pb bioaccessibility (10.3%). The application of APP primarily increased extractable P while slightly acidifying the soil. Under these conditions, APP was mainly associated with precipitation-driven mechanisms involving Pb-phosphate species. Similarly, TSP treatment occupied an intermediate position closer to the origin, suggesting a moderate chemical response relative to APP. Although P addition occurred, the shift in multivariate space was less pronounced, indicating a comparatively weaker precipitation effect. In contrast, the control and biochar treatments were located on the positive side of PC1 and appeared relatively close to each other, indicating similar

geochemical behavior. Both treatments showed comparable extractable P concentrations (68.9 and 81.4 mg kg⁻¹, respectively) and near-neutral soil pH (~7.3). Under these conditions, biochar applied alone produced only minor changes in Pb mitigation pathways, suggesting that dominant processes were related to surface complexation rather than to Pb-phosphate precipitation. The BS-LW treatment was displaced toward negative PC2 values, indicating a differentiated response likely associated with TOC (%) and enhanced potential for organic surface complexation. Finally, the Biochar+APP treatment was in the lower left quadrant, separated from both APP and biochar alone, suggesting a combined chemical influence. This positioning suggests a synergistic mechanism in which Pb-P precipitation and organic complexation may occur simultaneously. Consistently, this treatment showed low desorbed Pb (0.03%) and reduced bioaccessible Pb (12.5%), together with high extractable P (192 mg kg⁻¹) and elevated TOC (7.9%). These results indicate a complementary stabilization effect when combined with APP (Figure 5.12). In general, the effects of the treatments on soil physicochemical properties suggests the presence of different mechanisms involved in the reduction of Pb bioaccessibility and Pb desorption. The PCA analysis grouped treatments based on similarities in soil chemical properties and Pb fractions, allowing identification of distinct stabilization pathways. Treatments with similar geochemical responses clustered together, while those with different mechanisms were clearly separated in the multivariate space. In this context, APP and TSP treatments formed an independent cluster, characterized by high extractable P concentrations, a decrease in soil pH, and the lowest fractions of bioaccessible and desorbed Pb, indicating a mechanism dominated by precipitation.

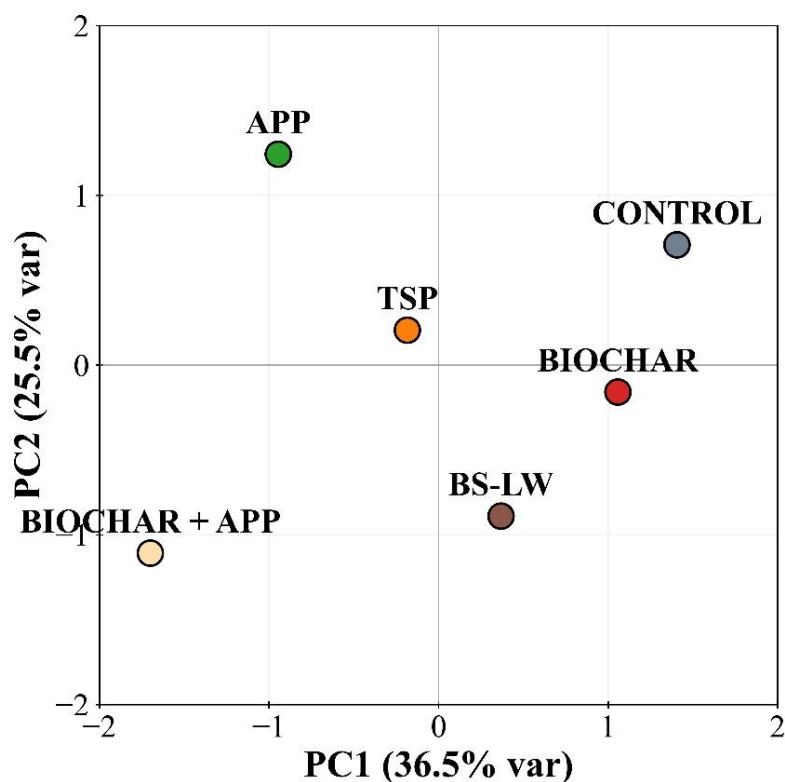


Figure 5.12. Principal component analysis (PCA) clustering of treatment means showing the distribution of soil amendments based on the evaluated soil chemical properties. Treatments located in similar regions of the PCA space indicate similar dominant chemical mechanisms as reflected by the wet-chemical data. Control (unamended soil); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

The slight soil acidification likely increased Pb^{2+} concentrations in the soil solution, enhancing its reaction with available P and promoting the formation of stable Pb-phosphate minerals. These results are consistent with previous studies showing that under low pH and high P availability, Pb is transformed into stable Pb-phosphate minerals (pyromorphite-group). Cao et al. (2008) and Scheckel & Ryan. (2004) reported that inorganic phosphate sources effectively promote pyromorphite formation, with their effectiveness increasing as P solubility increases and soil pH decreases. Under these conditions, unstable Pb phases dissolve and are progressively transformed into chloropyromorphite, thereby reducing Pb solubility. Biochar+APP treatment

formed a distinct cluster, characterized by high extractable P concentrations, a decrease in soil pH, high SOC (%) and reduces in bioaccessible and desorbed Pb (%), indicating a combined mechanism of precipitation and organic complexation. Under these conditions, biochar likely reduced P fixation and facilitated its interaction with Pb, promoting the formation of stable Pb-phosphate phases while simultaneously contributing to Pb stabilization through surface complexation. These results are supported by previous studies. For example, Luo et al. (2022) reported that biochar modified with TSP and hydroxyapatite reduced bioaccessible Pb by 16.3% to 92.3% after a 90-day incubation period. Likewise, (Muraro et al. (2026) showed that P-loaded biochar, produced by immersing raw and activated biochar in a saturated KH_2PO_4 solution, reduced Pb bioavailability by more than 50% during a 45-day incubation study. These findings further support the role of biochar as a carrier and reactive surface for phosphorus, enhancing Pb immobilization through combined precipitation and sorption mechanisms. Biochar clustered with the control treatment, suggesting minimal changes in Pb stabilization pathways, with processes primarily governed by surface complexation mechanisms. Similar results were reported by Alankarage et al. (2024), who conducted a study under controlled laboratory conditions using a Pb-contaminated urban soil collected from a public park near a Pb-Zn smelter in Port Pirie, Australia, and evaluated using both in vitro and in vivo approaches. Their findings indicated that biochar showed limited effectiveness in reducing Pb bioaccessibility and bioavailability in contaminated soils, with in vitro reductions ranging from approximately 10-13%. These results suggest that, although biochar can induce minor changes in Pb behavior, it is insufficient to promote substantial Pb stabilization when applied alone. Consequently, under field conditions, biochar alone may not provide significant reductions in Pb bioaccessibility.

Synchrotron-based analyses

Soil Pb speciation (bulk-XANES)

The XANES analysis combined with linear combination fitting (LCF) showed that the unamended control soil was primarily dominated by Pb-sorbed-Fe (70%), accompanied by a smaller fraction associated with Pb bound to fulvic acid-like species (29%), reflecting the natural behavior of Pb in untreated urban contaminated soils. In contrast, inorganic P treatments (TSP and APP) promoted the formation of pyromorphite-like species, ranging from 29 to 34%. While BS-LW, biochar, and Biochar+APP treatments, Pb was highly associated with stable organic species (Pb bound to humic acid-like), accounting for 30%, 36%, and 56% of total Pb speciation, respectively. The overall speciation of soil Pb (bulk XANES) confirmed that Pb stabilization in inorganic P treatments (TSP and APP) was predominantly controlled by precipitation mechanisms, as evidenced by the significant contribution of pyromorphite-like species (29-34%). Consistent with PCA clustering, treatments that decreased soil pH while increasing P availability favored precipitation-driven stabilization, as these conditions enhanced Pb^{2+} solubility and promoted the formation of stable Pb-phosphate phases. This mechanism is well supported in the literature, where phosphate amendments could create favorable conditions for Pb immobilization through increased P availability and slight acidification. Under such conditions, primary Pb minerals (galena, anglesite, Pb carbonate species) dissolve and are progressively transformed into stable chloropyromorphite, resulting in a significant reduction in Pb solubility in contaminated soils (Karna et al., 2018; Cao et al., 2008, 2002). Similar mechanisms were reported by Baker et al. (2014), who demonstrated that inorganic P sources, such as APP and TSP, favor the formation of stable Pb-phosphate minerals over time. In their study, pyromorphite contributions reached 22% and 19% for APP and TSP, respectively, confirming the effectiveness

of soluble P sources in promoting precipitation-driven Pb stabilization. The authors also report that, these processes are primarily controlled by P availability and soil pH, which regulate Pb^{2+} solubility and the extent of precipitation reactions. However, under typical soil conditions, limitations in P or Pb availability can restrict these reactions, leading to the initial formation of metastable phases such as plumbogummite. Over time, and with increasing P availability, these phases can progressively transform into more stable pyromorphite. Overall, these findings demonstrate that Pb stabilization is a dynamic and time-dependent process governed by local geochemical conditions. In contrast, BS-LW, biochar, and Biochar+APP treatments were characterized by a predominance of Pb associated with humic acid-like species (30-56%), indicating that Pb stabilization was primarily governed by organic complexation and surface sorption rather than precipitation of Pb-phosphate minerals. These amendments also contributed to increased inputs of stable organic matter, enhancing soil carbon stocks and promoting long-term carbon persistence. The accumulation of recalcitrant carbon favors the formation of organo-mineral associations and provides reactive binding sites for Pb, reinforcing stabilization through organic-driven mechanisms (Haney et al., 2015; Scharenbroch et al., 2013). Similar results were reported by Gomes et al. (2022), who showed that biosolid-amended soils contained a high proportion of Pb associated with organic species (~48%). Likewise, results from Chapter 3 of this dissertation indicated that biosolids promoted Pb association with stable organic matter, particularly Pb bound to humic acid-like species, ranging from 15 to 44%. These findings support that Pb stabilization in biosolid-treated soils is primarily driven by organic complexation mechanisms.

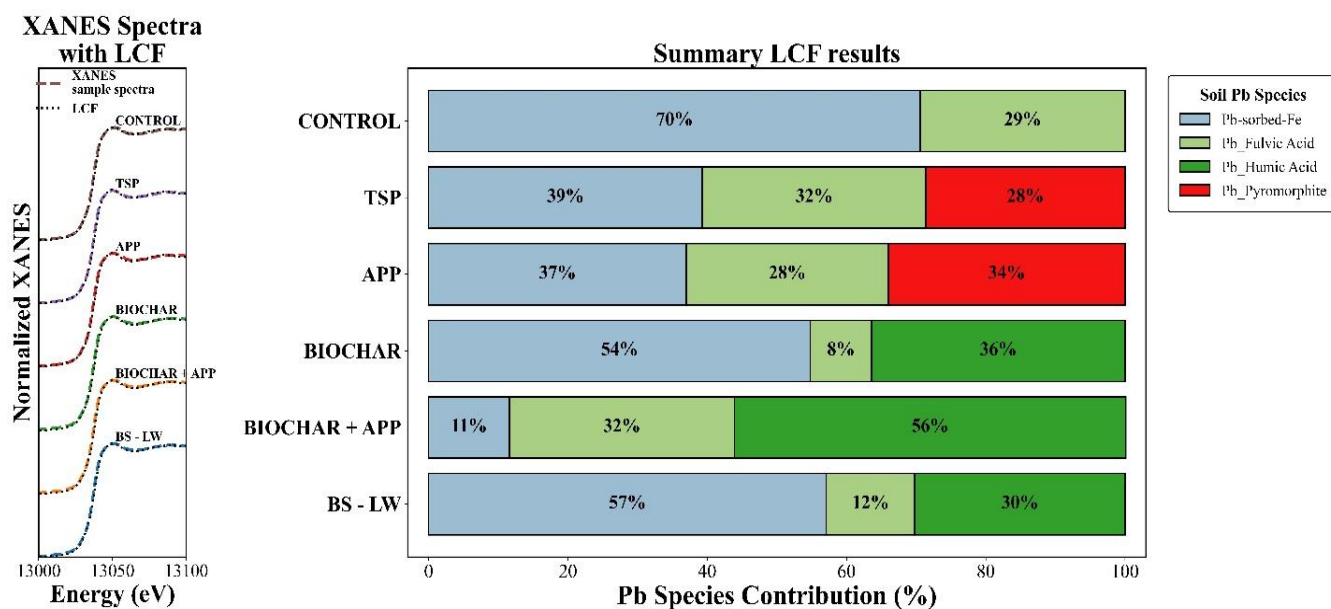


Figure 5.13. Normalized Pb L₃-edge XANES spectra with linear combination fitting (LCF) results (left) and relative contribution (%) of Pb species derived from LCF analysis (right) as affected by phosphorus-based and Biochar treatments at 12 months sampling time. Stacked bars represent the proportional contribution of individual Pb species for each treatment. Control (unamended soil); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

Biochar amendments reduce Pb bioaccessibility primarily through adsorption and surface complexation, which are strongly influenced by both soil properties (e.g., pH, organic carbon content, and cation exchange capacity) and biochar characteristics. Biochar enriched in oxygen-containing functional groups, such as carboxyl, hydroxyl, and carbonyl groups, exhibits a greater affinity for metal binding (Uchimiya et al., 2011). Consistent with this mechanism, Alankarage et al. (2024) reported that Pb speciation in biochar-amended soils was dominated by Pb associated with Fe oxides (ferrihydrite; 50-55%), followed by hydroxyapatite-like phases (30-35%), with minor contributions from organic or citrate-bound Pb (10-15%), indicating that Pb retention was primarily governed by surface sorption rather than precipitation processes.

Micro-X-ray fluorescence and micro-XANES soil Pb speciation

Micro-XRF elemental maps were acquired in triplicate for each treatment to enhance spatial representativeness. From each map, six Pb hotspots were selected based on fluorescence intensity values greater than 0.6 (normalized units) for μ -XANES analysis. To verify that the selected points corresponded to Pb, μ -XRF spectra were analyzed by identifying the characteristic Pb lines $L\alpha_1$ (10.551 keV), $L\beta_2$ (12.614 keV), $L\beta_3$ (12.795 keV), and $L\beta_4$ (12.307 keV) (Figure 5.14).

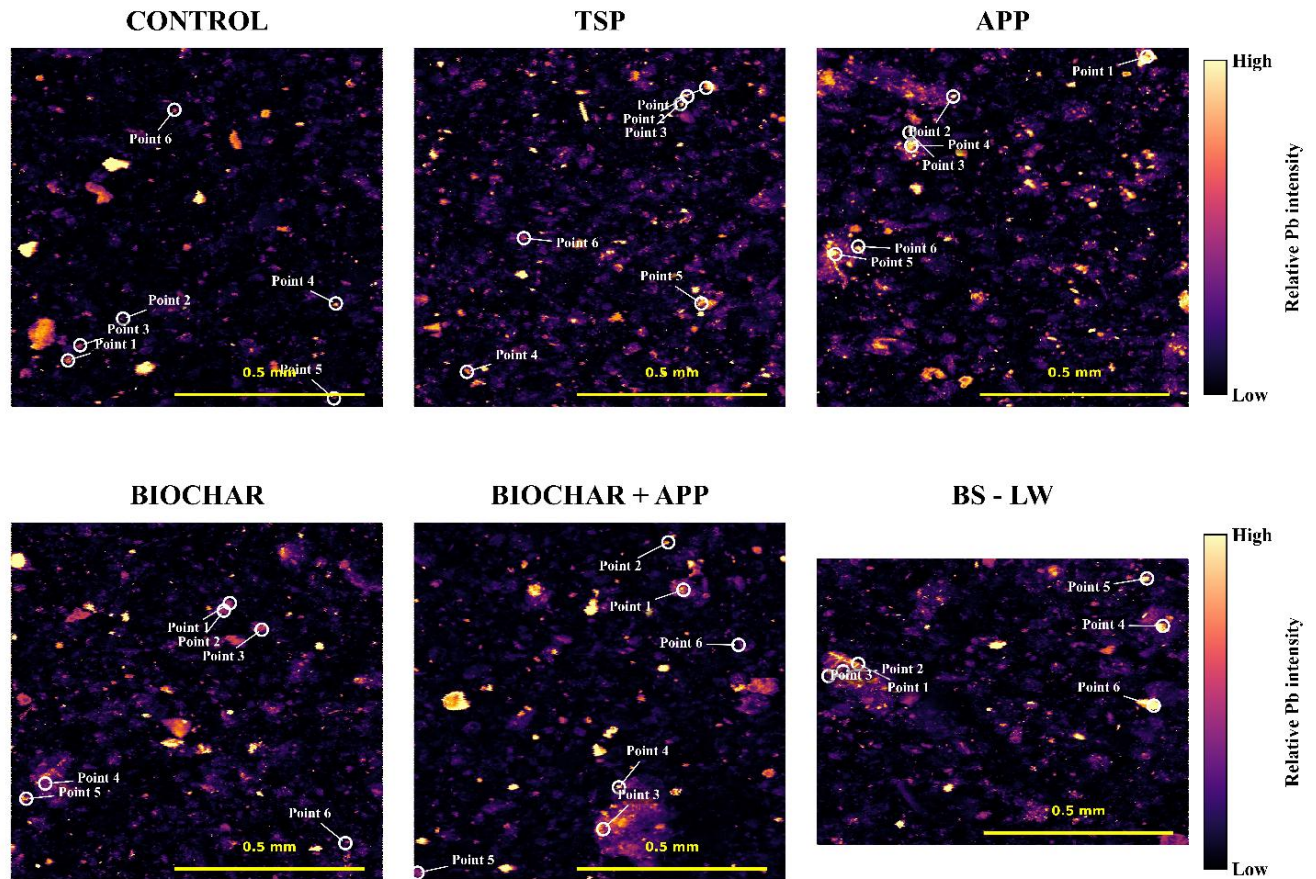


Figure 5.14. Micro-X-ray fluorescence maps showing the spatial distribution of Pb in composited field soil samples collected 12 months after treatment application. The color scale (magma) ranges from dark (purple-black) indicating low fluorescence intensity to bright (yellow-white) indicating high Pb concentrations. Shading is relative within each map. Selected points indicate locations where μ -XANES analyses were performed to determine Pb speciation. Control (unamended soil); TSP (Triple super phosphate); APP (Ammonium polyphosphate); BS-LW (Class A Biosolids, Lawrence); Biochar (Wood mix-biochar).

For micro-X-ray absorption near edge analyses, each hotspot was scanned four times to ensure spectral quality. However, for linear combination fitting (LCF), only hotspots exhibiting high spectra quality were selected, μ -XANES, results are presented in Table 5.12. In general, the LCF results showed treatment-dependent differences in Pb speciation. For example, in the unamended Control, Pb was primarily associated with Pb-sorbed-Fe phases, indicating the predominance of surface sorption mechanisms limited mineral transformation. In contrast, in the inorganic-P treatments, chloropyromorphite-like solids were the dominant Pb species in most of the hotspots analyzed, accounting for 89.8% and 81.5% in TSP and APP, respectively. On the other hand, Biochar and Biochar+APP treatments, soil Pb was associated with both sorbed-Fe-like species and chloropyromorphite-like phases. On average, Pb associated with Fe-like phases represented 20% and 23%, respectively. The chloropyromorphite-like species reached up to 77% and 75% for the respective treatments. Additionally, the Biochar+APP treatment showed Pb associated with stable organic matter species (Pb bound to humic acids-like) reached up to 11% in one of the samples. Finally, the BS-LW treatment showed the highest percentage of Pb associated with Fe-sorbed-like species among the treatments (~42%). Additionally, the chloropyromorphite-like fraction was approximately 47%.

The Linear combination fitting from μ -XANES results showed that inorganic P treatments (TSP and APP) were dominated by chloropyromorphite-like species, confirming that inorganic P sources stabilized Pb through Pb-phosphate precipitation. This is not surprising, as we focused on Pb hot spots specifically for this analysis. This mechanism is strongly governed by both Pb and P availability and soil pH, as higher P concentrations combined with slight acidification enhance Pb^{2+} solubility and promote the formation of stable Pb-phosphate minerals (Karna et al., 2018; Baker et al., 2014; Scheckel & Ryan, 2004; Cao et al., 2002, 2008). In

contrast, micro-scale results revealed that biochar and Biochar+APP treatments showed a strong contribution of chloropyromorphite-like species, reaching up to 75% and 77%, respectively, while a smaller fraction was associated with Pb bound to humic acid-like. These results suggest that, although biochar is typically associated with surface-driven mechanisms (bulk XANES), it can also facilitate Pb-phosphate precipitation under favorable conditions in selected areas. This behavior may be attributed to the physicochemical properties of biochar, including its high surface area, porous structure, and the abundance of oxygen-containing functional groups ($-OH$, $C=O/C=C$, and $C-O$), which provide reactive sites that enhance Pb retention and may promote localized Pb-P interactions (Uchimiya et al., 2011). Beesley et al. (2011) reported that biochar produced at temperatures above 500 °C exhibits increased aromaticity, surface area, and alkalinity, thereby enhancing metal retention through sorption and, to a lesser extent, precipitation. However, the addition of the inorganic P source did not substantially modify the dominant Pb stabilization mechanism in the Biochar+APP treatment. In this context, the contribution of chloropyromorphite-like species was comparable between two treatments, at approximately 77% in Biochar+APP and 75% in biochar alone, indicating that Pb stabilization was primarily controlled by biochar, with no significant additional effect of inorganic P under the evaluated conditions. Finally, the BS-LW treatment was characterized by Pb bound to Fe and a chloropyromorphite-like species, suggesting stabilization through a combination of sorption onto Fe phases and Pb-phosphate formation. Similar results were reported by Wekumbura et al. (2023), who found that Class A Fe-rich biosolids were more effective than other treatments in reducing bioaccessible Pb while maintaining the natural soil pH. Sequential extraction and synchrotron-based analyses revealed a higher proportion of chemically stable Pb species in biosolids-treated soils, including Pb precipitated as pyromorphite (30-40%). Overall, the results

demonstrate that phosphorus-based amendments and biochar-based treatments promoted contrasting Pb stabilization mechanisms, dominated by Pb–phosphate precipitation and organic complexation and sorption, respectively. In some cases, the combination of amendments showed complementary or synergistic effects that enhanced Pb stabilization. However, inorganic P sources remained more effective in the long term for reducing Pb bioaccessibility, mainly due to the formation of highly stable pyromorphite-group minerals.

Table 5.12. Linear combination fitting (LCF) results from micro-X-ray absorption near edge (μ -XANES) analyses showing the percentage contribution of Pb species at selected hotspots across treatments. A dash (-) indicates that no point spectrum.

Treatment	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Pb species
Percentage of contribution (%)							
CONTROL	100	-	100	100	100	-	Pb_sorbed_Fe
TSP	13.5	17	0	-	-	-	Pb_sorbed_Fe
	86.5	83	100	-	-	-	Pb_Chloropyromorphite
APP	22.2	13.7	18.9	14.5	22.8	-	Pb_sorbed_Fe
	77.8	86.3	81.1	85.5	77.2	-	Pb_Chloropyromorphite
BIOCHAR	42.5	9.5	16.3	-	-	-	Pb_sorbed_Fe
	57.5	90.5	83.7	-	-	-	Pb_Chloropyromorphite
BIOCHAR + APP	59.8	-	0	2.9	-	-	Pb_sorbed_Fe
	0	-	11	0	-	-	Pb_Humic Acid
	40.2	-	89	97.1	-	-	Pb_Chloropyromorphite
BS-LW	48.6	46.4	27.3	-	55	33.2	Pb_sorbed_Fe
	51.4	53.6	72.7	-	45	66.8	Pb_Chloropyromorphite

Conclusions

The results of this study demonstrate that phosphorus-based amendments and biochar significantly alter soil chemistry and promote distinct mechanisms of Pb stabilization in contaminated urban soils. Phosphorus- and biochar-based treatments modified key soil properties

related to Pb mobility and bioaccessibility, particularly soil pH, total organic carbon (TOC), and extractable P. These chemical modifications in soil appeared to be directly related to the mechanisms associated with Pb stabilization. Inorganic P treatments favored the precipitation of pyromorphite-like minerals, driven by changes in P availability and soil pH, thereby reducing Pb bioaccessibility and desorption. In contrast, biochar and biosolids primarily promoted organic complexation and surface sorption, associated with stable organic carbon and their reactive sites that retain Pb. The integration of wet-chemical data, multivariate analysis, and synchrotron-based spectroscopy confirmed that changes in soil properties were directly associated with transformations in Pb speciation. Inorganic phosphorus sources remain the most effective for reducing Pb bioaccessibility over the long term due to the formation of stable pyromorphite-like solid species that control Pb solubility in contaminated soils. However, these findings highlight that Pb stabilization depends on geochemical conditions and therefore requires further validation. Additional research is needed to better understand Pb-Fe associations in biosolids, as well as to further investigate Fe-modified biochar, since Fe oxides and hydroxides may enhance Pb retention through specific sorption, coprecipitation, or other stabilization processes. Furthermore, it is essential to evaluate the persistence of these mechanisms over longer periods, under different levels of contamination and across contrasting soil conditions that may alter P availability, organic carbon dynamics, and the stability of the mineral phases formed. In sum, these future research efforts will help optimize mechanism-based remediation strategies and advance more robust and transferable approaches to reduce the bioaccessible risk of Pb urban contaminated soil.

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Chapter 6 - Predictability of soil physical and chemical properties using soil sensor data: A case study using Veris P4000 soil sensor

Abstract

Soil sensors are practical tools widely used to characterize chemical and physical soil properties in situ. This study aimed to: (1) evaluate the relationships between data obtained from multiple soil sensors and selected physicochemical properties at two depths, and (2) assess how permanent soil properties influence sensor signal intensity and predictive performance across two contrasting agricultural fields in the Midwestern United States. A total of 616 soil core and probe samples were collected alongside in-situ measurements from the Veris P4000 sensor system, which integrates apparent electrical conductivity (EC_a), red visible spectrum (VIS), near-infrared (NIR) reflectance, and Force modules in two wheat-soybean rotation fields in Kansas prior to planting. Among the four sensors, NIR consistently showed the highest predictive ability for soil organic carbon (SOC) and exchangeable Phosphorus (Exch-P) ($R^2 > 0.7$), while VIS performed well for Total Nitrogen (%), Exch-P, clay, and Exch-Mg, particularly at the Colby site. The EC_a sensor showed strong associations with pH, Exch-Mg, Exch-K, and clay, reflecting its sensitivity to ionic composition and soil texture. In contrast, the Force sensor exhibited limited predictive capacity for chemical properties, suggesting its greater suitability for evaluating mechanical resistance. Finally, sensor responses varied notably between sites. At Ashland Bottoms, chemical properties such as pH and SOC were the main drivers, while at Colby, texture, compaction, and moisture played a greater role. These site-specific differences highlight the importance of considering local soil conditions when interpreting sensor data.

Overall, this study demonstrates that proximal soil sensors, when combined with appropriate preprocessing and modeling, can effectively estimate key soil properties and support site-specific soil management strategies.

Introduction

Soil sampling is key to generating field-specific information that enables agronomic decisions high precision. In this sense, conducting proper sampling ensures that soil test results are accurate, representative, and reliable (Marcaida et al., 2025). Historically, most soil properties have been determined through traditional wet chemical analyses (laboratory tests) of collected soil samples. However, the number of soil samples collected in the field is often limited compared to the total field area (Kuang et al., 2012). Soil properties can also vary significantly between sampling points within a field. In this context, when the spatial variability of the soil is unknown, point sampling is recommended using a regular grid (2.5 acres per cell) over the field (Virk et al., 2024). Samples are usually collected at specific GPS-referenced locations, and 8 to 10 soil cores are combined. After laboratory analysis, the data are interpolated to generate continuous maps that realistically represent the spatial distribution of soil properties (Marcaida et al., 2025). In contrast, precision agriculture leverages rapidly evolving information and electronic technologies to enhance soil management and agronomy practices. Soil sensors are practical tools that are increasingly used for in-situ characterization of several chemical and physical soil properties (Ji et al., 2019). In general, sensors generate extensive datasets across entire fields, offering a significant advantage over traditional soil sampling and laboratory analysis methods. For example, by capturing spatial variability within fields, soil sensors facilitate the in-situ collection of large volumes of high-resolution data on the dynamic interactions between physical and chemical soil properties. This enhanced information supports more precise and efficient site-specific soil management and agronomic decision-making (Kuang et al., 2012; Sudduth et al., 2005). Soil sensors showed several advantages in predicting key physical and chemical properties, such as exchangeable nutrients, pH, soil organic carbon (SOC),

total nitrogen (TN), soil mineral fraction (sand, silt, and clay %), cation exchange capacity (CEC), and soil moisture content (Pan et al., 2014). In this context, determining soil pH using soil sensors is a valuable tool. Soil pH plays a key role in numerous soil processes, directly influencing the recycling and availability of nutrients for crops, as well as the mobility and distribution of potentially toxic compounds within the soil profile (Neina, 2019). Likewise, accurate real-time data on the availability of major soil nutrients allows for more efficient fertilization and helps prevent overapplication. For instance, it has been shown that many farmers tend to underestimate actual soil phosphorus (P) test levels and continue applying it unnecessarily, even when their soils already contain adequate or excessive concentrations (Kile et al., 2025). For example, in a study conducted by Ji et al. (2019), where using the visible and near-infrared reflectance spectrometer (Spectra) demonstrated a high predictive capability for key variables such as soil organic matter (SOM), as well as exchangeable calcium (Ca), magnesium (Mg), and aluminum (Al), achieving coefficients of determination $R^2 > 0.85$ in all cases. Similarly, the gamma-ray spectroscopy (Gamma-ray) sensor showed comparable performance in predicting SOM and available Mg ($R^2 > 0.80$). The DUALEM sensor, based on electromagnetic measurements of apparent soil conductivity, also produced good results for estimating available Ca, Mg, K, and SOC, highlighting its sensitivity to exchangeable cation concentrations ($R^2 > 0.79$). Additionally, the proximal electrical conductivity sensor (ECP) proved useful for predicting Ca, Mg, and Al, although with lower accuracy than Spectra ($R^2 > 0.80$). Similarly, a study conducted by Pinheiro et al. (2017) in the central region of the Brazilian Amazon, using laboratory-based VIS-NIR spectral measurements, found that VIS-NIR spectroscopy is an effective technique that allowed for accurate prediction of clay content (%) and soil organic carbon (SOC), achieving coefficients of determination greater than 0.70 ($R^2 >$

0.70). These results highlight the potential of VIS-NIR spectroscopy as a fast, cost-effective, and reliable tool for characterizing soil properties in complex tropical environments. However, a study conducted by Heiniger et al. (2003) across 16 agricultural fields showed that apparent soil electrical conductivity (EC_a) did not accurately predict nutrient levels such as P, K, Ca, Mg, Zn, and Cu ($R^2 < 0.50$). However, when these nutrients were closely associated with properties that strongly influence EC_a (e.g., texture, CEC, moisture content, and dissolved salts), moderate-to-strong correlations were observed (R^2 values between 0.51 and 0.75). Additionally, subdividing fields by similar soil texture improved prediction accuracy (Heiniger et al., 2003). In a similar approach, Corwin and Lesch (2005) demonstrated that stratifying agricultural fields into homogeneous texture zones, defined through a combination of EC_a measurements, topographic attributes, and soil sampling, significantly improved the predictive performance of spatial models. By reducing within-zone variability, this method enhanced calibration accuracy, minimized the influence of local anomalies, and allowed for more reliable interpolation across management units. However, the use of sensors to estimate soil physicochemical properties is not without limitations. In this context, several studies have shown that soil texture, pH, cation exchange capacity (CEC), salt concentration, and moisture content can significantly affect the signals recorded by technologies such as apparent electrical conductivity (EC_a), visible (VIS) and near-infrared spectroscopy (NIR), among other proximal sensing tools (Corwin and Lesch, 2005). Despite advances in interpreting these signals, there remains a critical need to better understand how different soil properties influence sensor responses under real-world field conditions, especially in agricultural systems characterized by high physicochemical variability (Corwin and Lesch, 2005). This research study aims were: (i) to estimate the relationships between data obtained from multiple soil sensors and a set of selected physicochemical

properties, considering two depths; and (ii) to analyze how permanent soil properties influence the intensity of sensor signals and their predictive capacity, aiming to identify the factors that enhance or limit sensor performance in two contrasting experimental fields.

Materials and Methods

Location and sampling strategy

The research was carried out in two NSF EPSCoR project research fields located in two Kansas State University Agricultural Experimental Stations, historically managed in a wheat-soybean rotation in the Midwestern United States. The research fields were in Ashland Bottoms (-96.61551; 39.125607) and Colby (-101.06814; 39.3874567), Kansas. Prior to sampling wheat, multiple measurements were obtained using a mounted soil sampler Veris P4000-Soil Sensing System (Veris Technologies, Salina, Kansas, U.S). The Veris P4000 probe was equipped with 4 sensors including Apparent Electrical Conductivity (EC_a), Red Visible Spectrum (VIS: ranged from 557 to 1269 nm), Near Infrared Reflectance (NIR: ranged from 1323 to 2652 nm) and Force (Force) sensor. At the same time, using a soil core sampler, soil sub-samples were collected at 1 meter (m) depth. In total, around 368 soil sub-samples were collected in 2019 and 2020 in Ashland Bottoms, and around 248 soil samples were collected in 2019 and 2020 in Colby. All sampling locations were georeferenced, corrected, and validated using a precision GPS system. Soil cores were transported in ice boxes and kept in a freezer until processing. Soil sub-samples were separated into two depths surface (0 to 20 cm) and subsurface (20 to 60 cm). Measurements needed for determining approximate soil bulk density and gravimetric water content were taken. The soil sub-samples were air-dried, ground and sieved through stainless mesh (< 2 mm). Finally, soil sub samples were placed in a plastic Ziplock bag, labeled, mixed thoroughly, and stored before chemical analysis.

Spectral processing

Field measurements collected by the Veris P4000 (EC_a , VIS, NIR, and Force sensor) were combined and averaged by sampling point and depth, generating a single representative value for the 0-20 cm and 20-60 cm soil depth intervals. This approach allowed for point-to-point comparisons with soil samples collected at corresponding locations and depths. To reduce noise and enhance signal interpretation, the averaged sensor signals were preprocessed using a Savitzky-Golay filter (Savitzky & Golay, 1951). This filter was applied to selected sensor data (EC_a , VIS, NIR, and Force), fitting a third-degree polynomial (polyorder = 3) over a moving window of 11 observations (window length = 11) (Figure 6.1). The filter was applied only when the number of data points met or exceeded the required window length. The resulting smoothed values were stored in new columns within the same data frame, labeled as EC_{as} , VIS_s , NIR_s , and $Force_s$.

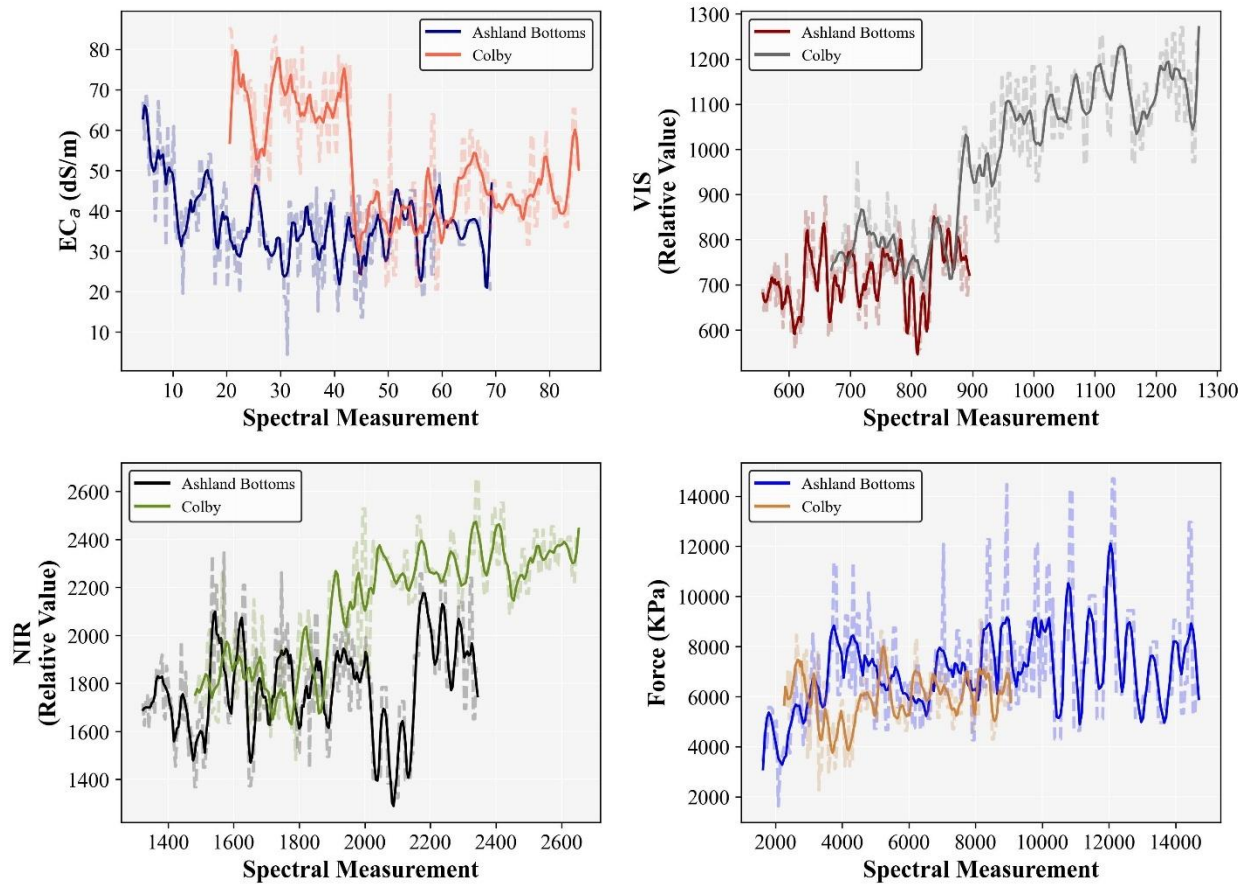


Figure 6.1 Sensor spectral data by site and transformation. Dashed lines indicate raw spectral data and solid lines correspond to the smoothed sensor signals obtained using the Savitzky-Golay transformation

Soil physical and chemical properties

Soil pH was measured with Milli-Q water ($\sim 18 \text{ M } \Omega \text{ H}_2\text{O}$) (1:5 soil/water ratio) using a pH/conductivity meter (Thermo scientific orion glass electrode, Beverly, MA), previously calibrated in buffer solutions (Thomas, 1996). Cation exchange capacity ($\text{CEC cmol}_{(+)}/\text{kg}$) was obtained by displacement method (Peter et al., 2015). Soil organic carbon (SOC %) and total nitrogen (TN %) were determined by dry combustion method using a LECO TruSpec CN combustion analyzer. Soil texture was measured using the hydrometer method (Sikora & Moore-Kucera, 2014). Additionally, Nitrate (NO_3^-) and Ammonia (NH_4^+) were determinate by 1 M KCl

extraction, using continue flow injection system (QuickChem 8500 series 2, LACHAT). Exchangeable potassium (Exch-K), calcium (Exch-Ca), magnesium (Exch-Mg) and sodium (Exch-Na) were measured using ammonium acetate ($\text{NH}_4^+\text{-CH}_3\text{COO}$) extraction. Similarly, exchangeable phosphorus (Exch-P) was assessed by Mehlich III extraction (Peter et al., 2015). Element exchangeable concentrations (except Exch-P) were quantified by a coupled plasma optical emission spectrometer (ICP-OES, Varian 720-ES). In addition, exchangeable phosphorus was quantified using a spectrophotometer (Beckman Coulter, DU 800 spectrophotometer). Finally, soil texture was determined by the hydrometer procedure, approximate bulk density (BD) was calculated using the cylindric method and soil moisture was determined using gravimetric analysis (GWC %) after drying soils at 105°C for overnight.

Statistical analysis

All statistical analyses were conducted using JMP Student version 19 and JupyterLab Notebook. A multivariate correlation analysis was performed to identify the selected soil physicochemical properties that were most strongly correlated with the Veris P4000 transformed soil sensor data. In addition, linear regression models were developed using selected soil variables and transformed sensor outputs. Principal components analyses (PCA) were conducted to identify the dominant physicochemical properties associated with sensor responses at each site. Normality was assessed using the Shapiro-Wilk test ($p > 0.05$), and homogeneity of variance was evaluated using Levene's test ($p < 0.05$).

Model validation

The model validation was carried out through Monte Carlo simulation using an automated algorithm performed using a Python script in JupyterLab Notebook developed by the authors. Briefly, linear regression models were fitted to predict soil physicochemical properties

using sensor-derived variables (EC_{as} , VIS_s , and NIR_s) as predictors. Where, validation was performed using a simulation with 1000 iterations. In each iteration, the original dataset was randomly shuffled and split into two subsets 60% for calibration and 40% for validation. The fitted model was trained on the calibration subset and subsequently evaluated on both the calibration and validation sets. Subsequently, model performance was assessed using Root Sum of Squared Errors (RSSE) and the Mean Absolute Error (MAE). Finally, the RSSE and MAE values obtained in each interaction were recorded and statistically analyzed, providing key insights into the predictive performance of each model across different sites, soil depths, and physicochemical properties.

Results and Discussion

Physicochemical soil properties

The soil physicochemical properties, including mean values and variability (standard deviation), are presented in Table 6.1. Soil characterization was based on data combined across years and differentiated by depth (surface, 0-20 cm and subsurface, 20-60 cm) for both research sites. In general, at Ashland Bottoms, the gravimetric water content (GWC %) at the surface was 1.1-times higher than subsurface. This consistent moisture retention is likely associated with a finer soil texture. For example, the clay content at the surface was 1.05 times higher than subsurface, while the silt fraction remained similar across depths (~59.2% on average). In contrast, sand content (%) at the surface was 1.08-times lower than the subsurface. In addition, soil bulk density (BD) at the surface was 10.3% higher than the subsurface, which may reflect better aggregation and lower compaction in deeper layers. Regarding nutrients, the concentrations varied in depth. For instance, exchangeable phosphorus (Exch-P) and exchangeable potassium (Exch-K) were 1.6 and 1.47-times higher, respectively, at the surface

compared to the subsurface. In contrast, exchangeable calcium (Exch-Ca) remained consistent across depths, averaging $\sim 3234 \text{ mg kg}^{-1}$. However, exchangeable magnesium (Exch-Mg) and sodium (Exch-Na) showed an opposite trend, with subsurface concentrations being 1.40 -and - 1.27-times higher, respectively, than the surface. The concentration of nitrate (NO_3^-) at the surface was significantly higher, being approximately 2.37 times greater than subsurface. In contrast, the concentration of ammonium (NH_4^+) remained relatively constant throughout the soil profile, with an average value $\sim 2.84 \text{ mg kg}^{-1}$. The cation exchange capacity (CEC) remained relatively stable across depths ($\sim 19.3 \text{ cmol}_{(+)} \text{ kg}^{-1}$ on average), while soil pH ranged from 7.65 to 7.53, remaining within a neutral to slightly alkaline at surface and subsurface, respectively. Finally, soil organic carbon (SOC %) and total nitrogen (TN %) at the surface were 1.26 -and - 1.3-times higher, respectively, than the subsurface.

In contrast, in Colby, at Colby, the gravimetric water content (GWC %) at the surface was 1.04 times lower than at the subsurface, suggesting that the distribution of particle-size fractions (texture) may influence moisture retention throughout the soil profile. For instance, clay content at the surface was 1.09 times higher than in the subsurface, while sand content (%) was also slightly higher at the surface (1.04 times). However, the silt fraction (%) was 1.05 times greater in the subsurface, which may enhance moisture retention in deeper layers. In addition, soil bulk density remained similar across depths ($\sim 1.51 \text{ g/cm}^3$ on average), further supporting the potential for greater subsurface water retention. In addition, the concentrations of exchangeable sodium (Exch-Na), calcium (Exch-Ca), and magnesium (Exch-Mg) were 2.09-, 1.05-, and- 1.09-times higher, respectively, in the subsurface compared to the surface. This accumulation aligns with elevated pH values, which ranged from slightly to moderately alkaline in both the surface and subsurface layers. Similarly, cation exchange capacity was 1.02-times higher in the

subsurface, likely due to the increased presence of exchangeable base cations. In contrast, the concentrations of Exch-P and Exch-K were significantly higher at the surface, being 1.81 and 1.60 times greater, respectively, than in the subsurface. Likewise, the concentrations NO_3^- and NH_4^+ were significantly higher at the surface, being approximately 1.38 and 1.12 times greater, respectively, than in the subsurface. Finally, the soil organic carbon and total nitrogen contents also varied with depth, with values 1.46 and 1.44 times higher, respectively, at the surface than in the subsurface.

Spectral variability across sites and sensors

In general, the signal from the EC_a sensor was considerably higher in Colby compared to Ashland Bottoms (Table 6.2). For instance, the minimum signal was 4.62 times greater, and the maximum signal was 1.47 times higher in Colby. This behavior suggests greater apparent soil conductivity at the Colby site, likely influenced by differences in soil texture, soluble salt content, or moisture conditions. Similarly, the VIS sensor also showed higher values in Colby, with the minimum and maximum signals 1.19 and 2.23 times greater than those in Ashland Bottoms, respectively. The near infrared reflectance sensor signals showed values more comparable between sites, although slightly higher in Colby, where the minimum and maximum signals were 1.12 and 1.18 times greater, respectively. This pattern suggests higher optical reflectance at the Colby, possibly associated with lower organic carbon content or differences in soil color and mineral composition.

Table 6.1. Summary of selected physicochemical soil properties in two EPSCoR research sites

Soil parameters	Ashland Bottoms				Colby			
	<i>Surface</i>		<i>Subsurface</i>		<i>Surface</i>		<i>Subsurface</i>	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GWC	26.1	5.68	23.6	6.03	24.3	5.43	25.4	4.87
BD	1.5	0.15	1.36	0.18	1.5	0.3	1.51	0.31
NO ₃ ⁻	4.96	2.36	2.09	1	95.3	111	68.8	102
NH ₄ ⁺	2.85	1.39	2.84	1.17	2.31	1.22	2.05	1.15
Exch-P	40.3	23.1	25	20.1	32.2	14.3	17.6	6.74
Exch-K	294	60.4	200	42.4	659	89.9	410	94.1
Exch-Ca	3232	478	3237	361	4259	800	4473	767
Exch-Mg	158	44.5	222	46.3	429	86	470	39.5
Exch-Na	8.91	3.47	11.4	4.01	17.3	11.3	36.3	17.9
CEC	19.1	2.26	19.5	1.65	26.8	3.13	27.5	3.66
pH	7.65	0.3	7.53	0.34	8.02	0.2	8.17	0.24
TN	0.13	0.03	0.1	0.04	0.13	0.02	0.09	0.02
SOC	1.16	0.12	0.92	0.48	0.88	0.16	0.6	0.19
Sand	15	3.81	16.2	3.76	23.6	7.11	22.5	6.28
Silt	59.2	2.65	59.2	3.6	53.5	5.58	56.6	4.19
Clay	25.8	3.46	24.5	2.97	22.9	4.91	20.9	7.34

GWC: Gravimetric Water Content expressed; **BD:** Bulk Density expressed in g/cm³; **NO₃⁻:** Nitrates; **NH₄⁺:** Ammonia expressed in mg/kg; Exchangeable P, K, Ca, Mg, Na element concentration expressed in mg/kg; **CEC:** Cation Exchange Capacity expressed in cmol₍₊₎ kg⁻¹; Soil pH (H₂O); **SOC:** Soil organic Carbon expressed in %; **TN:** Total Nitrogen expressed in %.

In contrast, the Force sensor showed an opposite trend. The minimum signal in Colby was 1.39 times higher than that in Ashland Bottoms. However, in Ashland Bottoms, the maximum signal was 1.90 times greater than in Colby. This higher penetration resistance observed in Ashland Bottoms may be related to structural differences in the soil, such as increased compaction or bulk density in the subsurface. Overall, the variations observed in signal

magnitude and range reflected how different sensors capture distinct dimensions of soil variability. For example, Force sensors respond more strongly to physical properties such as compaction and resistance, and the EC_{as} , VIS_s , and NIR_s sensors are more sensitive to chemical and optical characteristics. Finally, the observed differences between sites highlight the extent to which spectral response is influenced by local soil conditions.

Table 6.2. Spectral range of raw sensor signals by site and sensor type

Site	Sensor	Min	Max	Standard deviation
Ashland Bottoms	EC	4.45	69.3	10.6
	VIS	558	895	78.5
	NIR	1323	2344	229
	Force	1626	14695	2326
Colby	EC	20.6	85.5	15.6
	VIS	669	1270	175
	NIR	1483	2652	270
	Force	2261	9057	1224

Multiple correlation analyses

A multiple Pearson correlation analysis was conducted to evaluate the relationships between selected soil physicochemical properties and the transformed sensor data from the Veris P4000 system. Data from different years were merged for both Ashland Bottoms and Colby, and the analysis was distinguished by site and depth. Only variables with moderate to strong correlation coefficients ($r \geq \pm 0.50$) were analyzed, as shown in figures 6.2 to 6.7. For example, at the surface in Ashland Bottoms, EC_{as} showed strong positive correlations with Exch-Ca, pH, CEC, Exch-P, and clay (%), and negative correlations with GWC. This suggests that EC_{as} is sensitive to ionic retention, and moisture dynamics in these finer-textured soils (Figure 6.2). Similarly, at the subsurface in Ashland Bottoms, EC_{as} was positively correlated with pH, Exch-

Ca, Exch-K, Exch-P, and negatively with GWC (%), reinforcing its utility for characterizing deeper nutrient pools (Figure 2). At the surface in Colby, EC_{as} was positively correlated with Exch-Na, Exch-Mg, clay (%), and bulk density, and negative correlations with Exch-Ca, CEC, and sand (%). These associations highlight the influence of textural heterogeneity and higher salinity on EC_{as} at the surface. Finally, at the subsurface in Colby, positive strong correlations were found between EC_{as} and Exch-Na, Exch-K, Total N (%), SOC (%), and clay (%). Similarly, for the surface layer, EC_{as} showed a negative correlation with Exch-Ca, CEC, and sand (%). These results suggest that EC_{as} captures key variations in chemical fertility and soil texture (Figure 6.2).

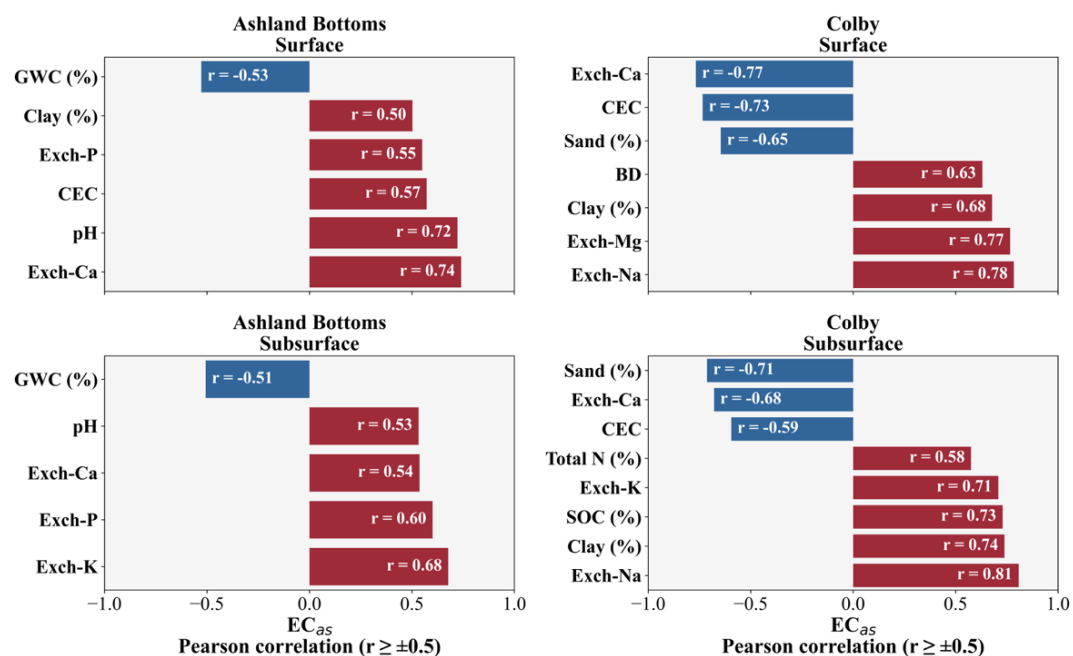


Figure 6.2. Pearson correlation matrix between apparent electrical conductivity (EC_{as}) soil sensor and significant soil variables at surface and subsurface in Ashland Bottoms and Colby sites.

The Pearson correlation analysis between the Red Visible Spectrum sensor (VIS_s) and key soil physicochemical properties in Asland Bottoms and Colby sites at both depths is shown in Figure 6.3. In general, at Ashland Bottoms-surface, VIS_s exhibited strong negative correlations with Exch-Ca, Exch-P, Exch-K, Total N (%) and SOC (%). These results indicate that soils with higher fertility and organic C content tend to reflect less visible light and retain more moisture. Similar negative correlations were observed at the subsurface between VIS_s and Exch-P, Exch-K, Total N (%), SOC (%), and CEC, reinforcing the sensor sensitivity to nutrient-rich, organic-matter-dense soils even at greater soil profile depths. In contrast, the surface soils in Colby, VIS_s were positively correlated with Exch-Ca, CEC, and sand (%), while showing negative correlations with Exch-K, Exch-Mg, Exch-Na, SOC (%), clay (%), and bulk density. These opposing relationships highlight the influence of soil texture and mineral composition on visible reflectance at this site. Similarly, at the subsurface in Colby, a strong negative correlation was observed between VIS_s and clay (%), SOC (%), Exch-K, Exch-Na, Total N (%), and Exch-Mg, while positive correlations were found with Exch-Ca, silt (%), and sand (%). This contrast between fine and coarse textured soil fractions showed how VIS_s is primarily influenced by soil texture and organic matter content.

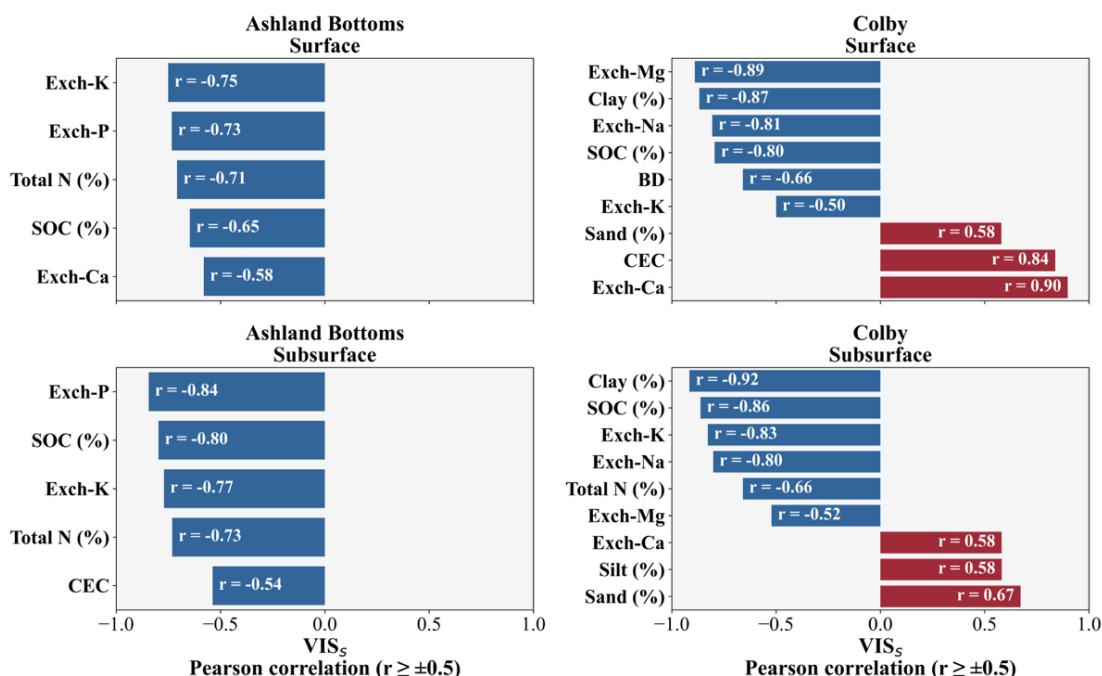


Figure 6.3. Pearson correlation matrix between Red Visible Spectrum (VIS_s) soil sensor and significant soil variables at the surface and subsurface in Ashland Bottoms and Colby sites.

The Pearson correlation analysis between the near-infrared reflectance sensor and soil properties revealed strong, site and depth-specific relationships, as shown in Figure. 6.4. At Ashland Bottoms surface, NIR_s was negatively correlated with Total N (%), Exch-P, Exch-K, Exch-Ca, and SOC (%). A similar trend was observed at the subsurface layer, where NIR_s showed negative correlations with Total N (%), Exch-P, Exch-K, SOC (%), and CEC. Interestingly, a positive correlation was found with bulk density, suggesting that denser subsurface layers tend to reflect more NIR_s radiation, possibly due to lower moisture retention and reduced organic matter content. These findings confirm the sensitivity of NIR_s to organic matter and nutrient-associated properties, particularly in finer textured, carbon-rich soils where NIR_s reflectance tends to decrease. At Colby surface, NIR_s was positively correlated with Exch-Ca, CEC, and sand (%), while showing negative correlations with SOC (%), BD, clay (%), Exch-Na, and Exch-Mg. Finally, at Colby subsurface, NIR_s exhibited strong negative correlations with

clay (%), SOC (%), Exch-K, Exch-Na, Exch-Mg, and Total N (%), along with positive correlations with Exch-Ca, CEC, silt (%), sand (%). These associations demonstrate that NIR_s effectively captures the interactive effects of soil texture, nutrient status, salinity, and organic matter across depths in the Colby site.

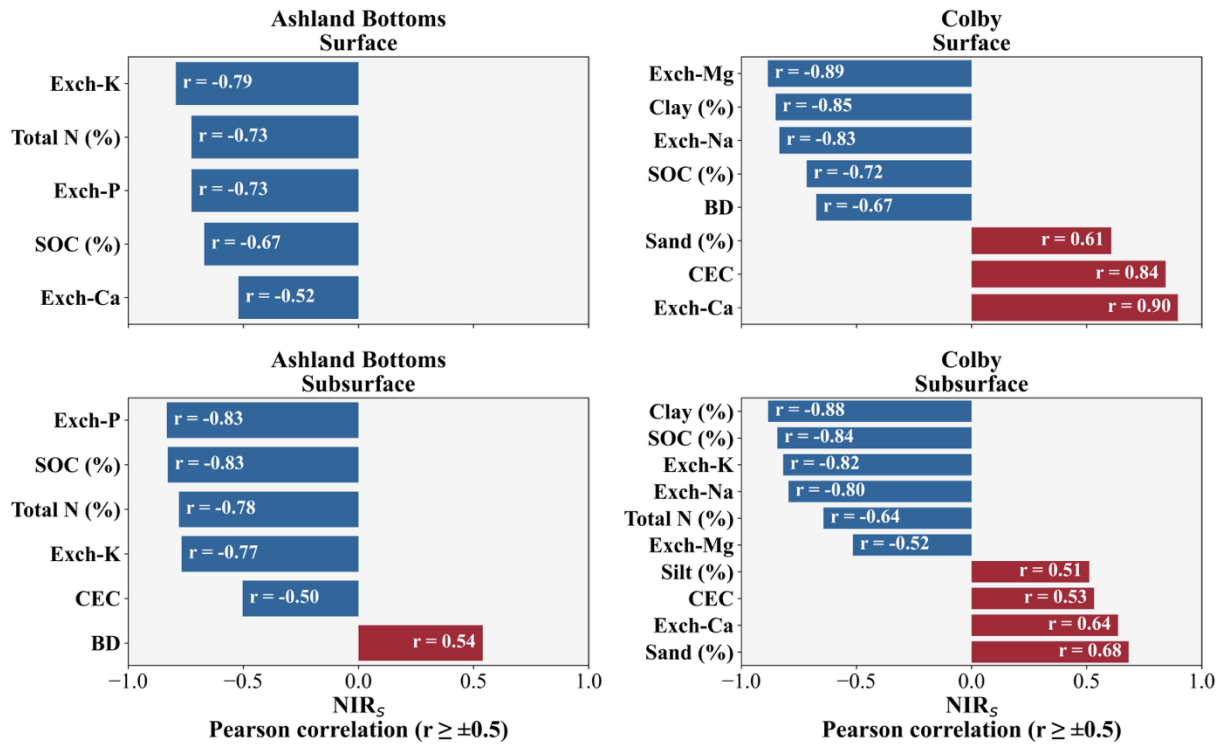


Figure 6.4. Pearson correlation matrix between Near Infrared (NIR_s) soil sensor and significant soil variables at surface and subsurface in Ashland Bottoms and Colby sites.

The correlation analysis for the Force_s sensor revealed sensor was negatively correlated with clay content (%) at both surface and subsurface depths at Ashland Bottoms (Figure 6.5). Finally, NO₃⁻ and NH₄⁺ concentrations were not significantly correlated with any of the proximal sensor signals across all sites and depths. This lack of association suggests that predicting these highly dynamic, mobile nitrogen forms may require additional covariates (e.g., microbial activity indicators) or more complex modeling techniques beyond Pearson correlations.

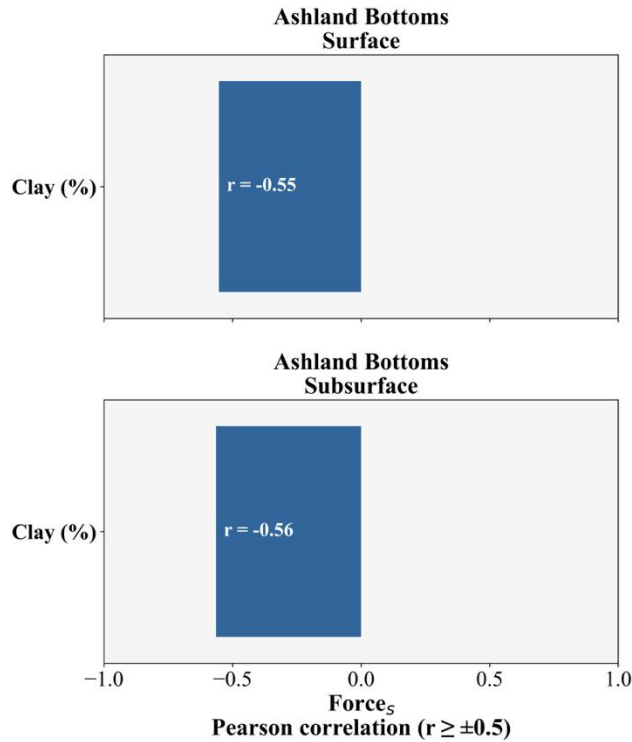


Figure 6.5. Pearson correlation matrix between Force_s soil sensor and significant soil variables at surface and subsurface in Ashland Bottoms site.

Predictions of soil chemical properties

A linear regression analysis was conducted to evaluate the predictive accuracy between selected soil physicochemical properties and transformed sensor data obtained from the Veris P4000 system. In general, the linear regression analyses conducted in Ashland Bottoms and Colby did not follow the same trends observed in the Pearson correlation analyses at either depth. For instance, in Ashland Bottoms, at the surface layer, EC_{as} (ranged from 4 to 69 ds/m) showed a statistically significant and positive linear relationship with soil pH ($p < 0.05$), with a relatively low prediction error ($RMSE = 0.22$), suggesting a moderate predictive strength (Figure 6.6). Similar results were reported by Pei et al. (2019), in which the Veris P4000 system predicted soil pH with high accuracy using EC_a , especially when applying a 30-point Gaussian

smoothing window (Savitzky-Golay data transformation), achieving $R^2 > 0.60$. This association underscores the indirect role of properties such as mineralogy, moisture, and SOC% in EC_{as} spectral response.

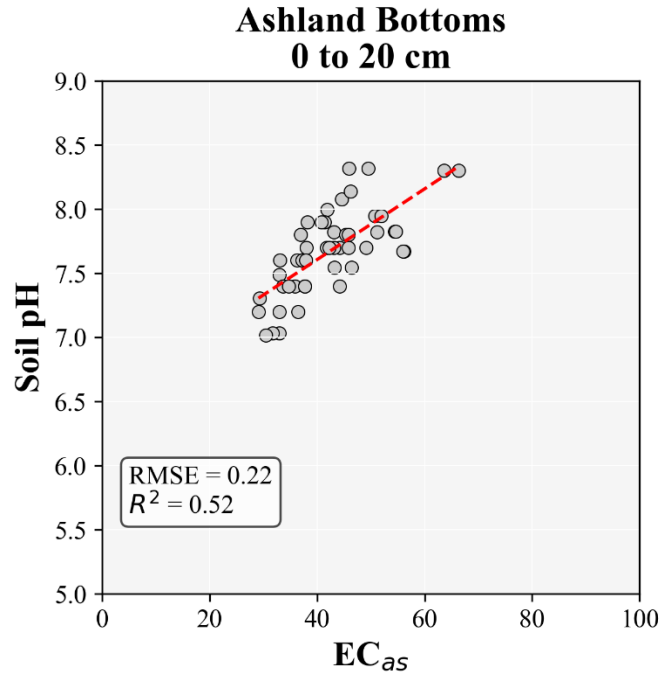


Figure 6.6. Linear regression between Apparent Electrical Conductivity (EC_{as}) soil sensor and significant soil variables in Ashland Bottoms site.

In Colby, the linear regression models involving the EC_{as} sensor (ranged from 20 to 86 ds/m) showed several significant relationships with soil properties, particularly in the subsurface layer (Figure 6.7). At the surface depth, EC_{as} was positively and significantly correlated with Exch-Mg, with a strong coefficient of determination ($R^2 = 0.69$) and a moderate RMSE ~ 46.67 mg kg⁻¹. This suggests a consistent and meaningful relationship between soil conductivity and Mg accumulation in the surface soil layer. In the subsurface, EC_{as} exhibited statistically significant relationships with both Exch-K and clay content (%). The model for Exch-K yielded an $R^2 = 0.50$ and RMSE ~ 64.81 mg kg⁻¹, while the relationship with clay (%) showed a slightly stronger fit ($R^2 = 0.55$, RMSE = 4.95%). In all three models, the residuals meet key assumptions

of normality and homoscedasticity, indicating that the regression results are robust and interpretable.

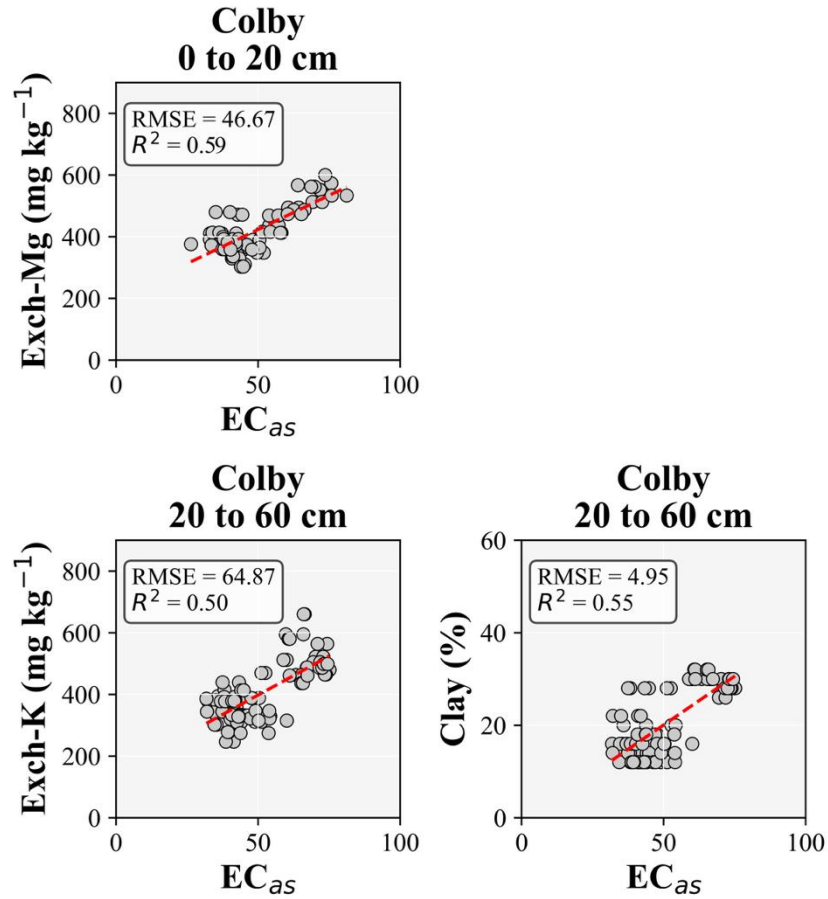


Figure 6.7. Linear regression between Apparent Electrical Conductivity (EC_{as}) soil sensor and significant soil variables in Colby site.

These results are consistent with Heiniger et al. (2003), who reported that the EC_a signal can be limited in sandy soils with low soil moisture and nutrient retention. Mazur et al. (2022) demonstrated in agricultural fields in Poland that EC_a was strongly correlated with available K ($r = 0.80$) and moderately with Mg ($r = 0.48$), validating its use as a proximal tool for characterizing the spatial variability of nutrients. The site-specific differences are consistent with the conceptual model described by Friedman (2005). In saturated soils (two-phase systems), the

EC_a is expressed as $\sigma a(\sigma_w, n)$, where $\sigma a (EC_a)$ is a function of σ_w (electrical conductivity of the soil solution) and n (porosity-texture), which becomes the dominant factor and probably can mask secondary effects of particle geometry, orientation, and interfacial attributes.

In Ashland Bottoms, the Red visible spectrum sensor ranged from 557 to 895 nm showed strong and statistically significant relationships with multiple soil properties across both depths (6.8). At the surface layer, VIS_s was negatively correlated with Total N (%) and Exch-P. The model for Total N (%) exhibited a coefficient of determination of $R^2 = 0.50$ and a very low prediction error (RMSE = 0.02%), indicating a reliable inverse trend between reflectance in the VIS_s range and nitrogen content. Similarly, the regression between VIS_s and Exch-P at this depth yielded $R^2 = 0.64$ and RMSE $\sim 17.47 \text{ mg kg}^{-1}$, reinforcing the negative association between VIS_s reflectance and labile phosphorus pools. Similarly, at subsurface layer, VIS_s also maintained a strong negative relationship with Exch-P, with $R^2 = 0.71$ and RMSE $\sim 12.96 \text{ mg kg}^{-1}$, the strongest fit among all VIS_s -based models (Figure 6.8).

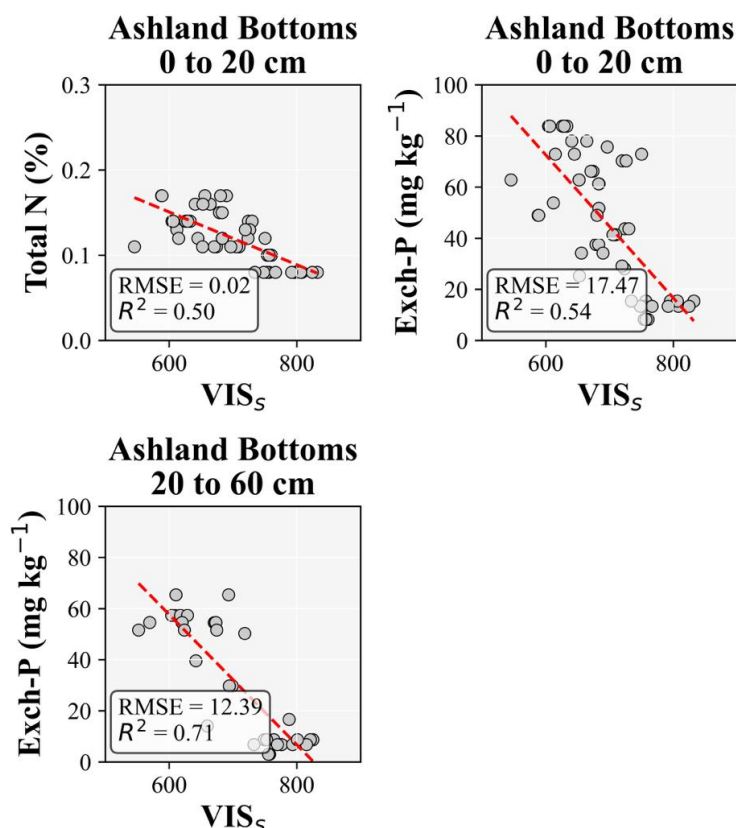


Figure 6.8. Linear regression between Visible Spectrum (VIS_s) soil sensor and significant soil variables in Ashland Bottoms site.

In Colby, the visible spectrum sensor ranged from 669 to 1269 nm showed strong and consistent negative relationships with both Exch-Mg and clay content (%), especially in the surface layer. At the 0-20 cm depth, VIS_s was significantly and inversely correlated with Exch-Mg, yielding an $R^2 = 0.79$ and RMSE $\sim 33.15 \text{ mg kg}^{-1}$, indicating a robust model with low prediction error. Likewise, in the surface, VIS_s was negatively correlated with clay (%), with an $R^2 = 0.75$ and RMSE $\sim 2.21\%$, indicating that finer-textured soils (higher clay content) tend to exhibit lower VIS_s reflectance. On the other hand, at the subsurface, VIS_s maintained a similar inverse relationship with clay (%), though with a slightly lower $R^2 = 0.64$ and RMSE $\sim 2.95\%$,

suggesting that VIS_s reflectance remains a good predictor of clay content even at depth (Figure 6.9).

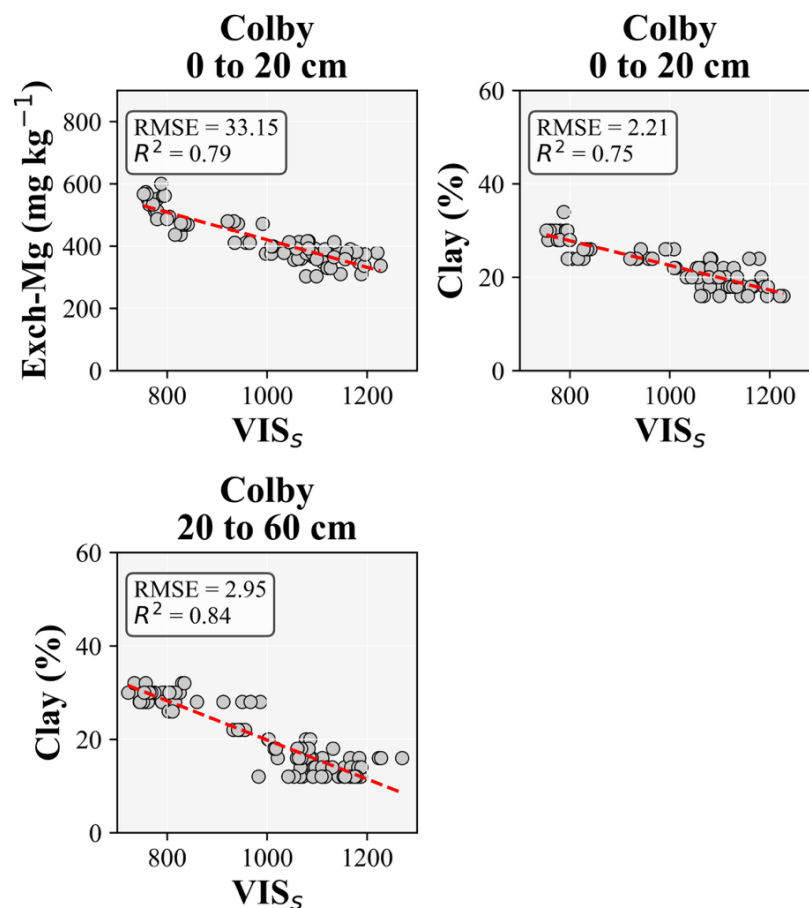


Figure 6.9. Linear regression between Visible Spectrum (VIS_s) soil sensor and significant soil variables in Colby site

In Ashland Bottoms, the Near-Infrared sensor ranged from 1323 to 2344 nm, showed a statistically significant relationship with Exch-P and SOC % across both depths. For instance, at the surface layer, NIR_s was negatively correlated with Exch-P ($R^2 = 0.53$, $RMSE = 17.7 \text{ mg kg}^{-1}$) and with SOC (%) ($R^2 = 0.45$, $RMSE = 0.09\%$). These results suggest that soils with higher phosphorus availability and organic carbon content tend to exhibit lower NIR_s reflectance. Similar trends were observed in the subsurface layer. Exchangeable P maintained a strong

inverse correlation with NIR_s ($R^2 = 0.69$, $\text{RMSE} = 12.8 \text{ mg kg}^{-1}$), while SOC (%) also showed a relationship ($R^2 = 0.69$, $\text{RMSE} = 0.30\%$). This consistency across depths indicates that NIR_s reflectance is sensitive to variations in nutrient availability, mainly in exchangeable P and organic matter content, not only in topsoil but also in deeper horizons (Figure 6.10).

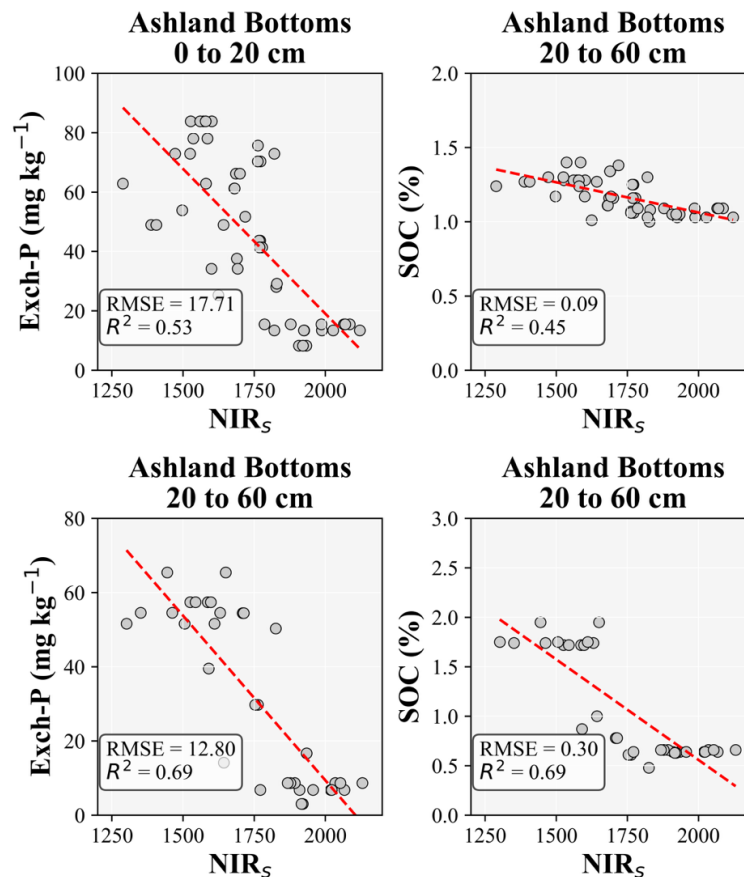


Figure 6.10. Linear regression between Near Infrared (NIR_s) soil sensor and significant soil variables in Ashland Bottoms site.

In Colby, at the subsurface depth, the NIR_s sensor ranged from 1482 to 9057 nm, showing a strong statistically significant relationship with soil organic carbon (SOC %). The regression model yielded a high coefficient of determination ($R^2 = 0.71$) and a relatively low prediction error ($\text{RMSE} = 0.10\%$), indicating a robust and reliable fit (Figure 6.11).

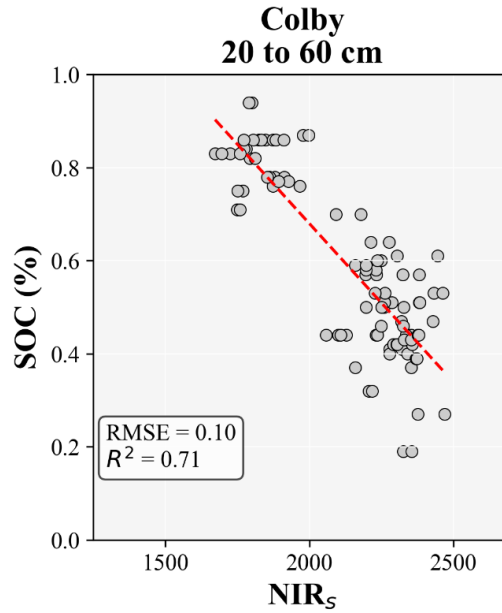


Figure 6.11. Linear regression between Near Infrared (NIR_s) soil sensor and significant soil variables in the Colby site.

These findings align with Viscarra and Hicks. (2015), who reported that VIS-NIR spectroscopy can estimate different fractions of soil organic carbon, with the visible range being sensitive to iron oxides and humic substances, and the NIR range to carbonyl, phenolic, aromatic, carbohydrate, and peptide groups. Nevertheless, Hutengs et al. (2019) demonstrated that estimating SOC in situ from VIS-NIR spectra collected with an ASD FieldSpec 4 spectroradiometer is limited by variability in moisture and soil physical properties, thereby reducing model accuracy. Surprisingly, NIR_s predictions, particularly in SOC and Exch-P, were superior in the subsurface layers. This challenges the common assumption of signal attenuation with depth. According to Zhang et al. (2024), this trend may be due to greater absorption in the surface caused by high organic matter content, while surface signals emerge more clearly due to higher mineral content. Likewise, Poffenbarger et al. (2020) found that plant-derived carbon fractions (e.g., lignin) can increase in deeper layers, not necessarily due to active accumulation

but rather due to restricted microbial decomposition (e.g., low oxygen, moisture, or temperature). This suggests that sensors capture not only texture and moisture, but also stable forms of plant-derived organic carbon. Regarding exchangeable phosphorus, despite its essentiality, predicting it using spectroscopy has yielded inconsistent results. For example, Wenjun et al. (2014) reported poor prediction of available phosphorus (AP), with $R^2 \approx 0.29$, even using advanced algorithms. In contrast, Udelhoven et al. (2003) used bidirectional reflectance measured with an ASD FieldSpec II spectroradiometer to predict Exch-P, achieving $R^2 > 0.54$, although results were highly site-dependent. Finally, according to Total Nitrogen (%), similar results were showed by Wenjun et al. (2014), who reported that total nitrogen was the best-predicted soil property using laboratory spectra and PLSR models ($R^2 > 0.87$, RPD = 2.81), attributed to the presence of N-H absorption bands and C-H + C-C combinations in organic nitrogen compounds. These findings validate that local soil conditions, whether chemical or physical, exert differentiated influences on sensor behavior. Understanding these patterns is key to improving in-field data interpretation and optimizing site-specific management strategies.

The results of the main regression equation between the sensors (transformed data) and the soil physicochemical properties, organized by site and depth, are presented in Table 6.3. These preliminary results suggest that the predictive capacity of soil variables is not solely determined by the efficiency of the Veris P4000 sensor system but is also influenced by the soil characteristics. In this regard, factors derived from management practices, soil moisture, bulk density, texture, or soil organic carbon could significantly affect prediction accuracy. Additionally, the system's response varies across sites, highlighting the importance of accounting for local soil conditions when developing predictive models.

Table 6.3. Main linear regression models between transformed sensor data and soil properties by site and depth

Site	Depth	Predictor	R ²
Ashland Bottoms	Surface	pH = 6.5016876 + 0.0276083*EC _{as}	0.52
	Surface	Exch-P = 240.61937 -0.2800388*VIS _s	0.54
	Surface	Total N = 0.3366085 -0.0003097*VIS _s	0.5
	Subsurface	Exch-P = 210.50869 -0.2547323*VIS _s	0.71
	Surface	SOC = 1.8769784 -0.0004075*NIR _s	0.45
	Surface	Exch-P = 214.21294 -0.09785876*NIR _s	0.53
	Subsurface	SOC = 4.6228245 -0.0020323*NIR _s	0.69
	Surface	Exch-P = 186.93346 -0.0887594*NIR _s	0.69
Colby	Surface	Exch-Mg = 201.86394 + 4.4260517*EC _{as}	0.59
	Subsurface	K = 144.70385 + 5.0551009*EC _{as}	0.5
	Subsurface	Clay = 1.004348 + 0.4208607*EC _{as}	0.55
	Surface	Exch-Mg = 861.6807 -0.4412009*VIS _s	0.79
	Surface	Clay = 48.958603 -0.0264033*VIS _s	0.75
	Subsurface	Clay = 61.746708 -0.0418846*VIS _s	0.84
	Subsurface	SOC = 2.042607 -0.0006814*NIR _s	0.71

Model validation

The calibration and validation results of the linear regression models are summarized in Table 6.4. The R² values for the calibration datasets ranged from 0.45 to 0.84, while those of the validation datasets ranged from 0.46 to 0.84, indicating a high degree of consistency between model training and testing phases. Model performance was generally strong for several soil properties. Notably, the clay fraction (%) showed excellent predictive performance across both depths and sites, especially in Colby, where VIS_s achieved an R² > 0.84 and an MAE of around 2.29. Similarly, Exch-P was accurately predicted in Ashland Bottoms-subsurface, with R² values ranging from 0.71 to 0.73 and MAE values >11 using VIS_s and NIR_s soil sensors. Soil organic

carbon (%) was well predicted, particularly with NIR_s, yielding R² values up to 0.72 and low MAE values (0.07-0.08), indicating strong performance of this sensor in capturing organic matter patterns. Additionally, moderate predictions were obtained for pH and Total N (%), with R² values around 0.50 and low MAE (<0.20), highlighting good model stability despite lower overall variance explained. Conversely, Exch-K and Exch-Mg predictions showed higher absolute errors (MAE > 30), particularly in Colby, even though R² values remained ~0.59, suggesting the need for additional predictors or model complexity for these nutrients. Finally, the validation results confirm that Red Visible Spectrum (VIS_s) was the most consistent and versatile sensor, particularly effective in predicting clay content (%), Exch-Mg, and Exch-P, while NIR_s performed best for SOC (%), and EC_{as} was most effective for pH and Exch-K. These results emphasize the importance of independent validation using Monte Carlo simulations to assess model generalizability under varying partitioning scenarios and to identify the most informative sensors for different soil properties and contexts.

Table 6.4. Performance of sensor-based models for predicting soil properties across sites, depths, and sensors.

Site / Depth	Soil sensor	Soil properties	Calibration dataset			Validation data set		
			R ²	RSSE	MAE	R ²	RSSE	MAE
Ashland Bottoms (0 to 20 cm)	EC _{as}	pH	0.52	0.22	0.17	0.52	0.23	0.18
Ashland Bottoms (0 to 20 cm)	VIS _s	Total N	0.51	0.02	0.02	0.52	0.03	0.02
Ashland Bottoms (0 to 20 cm)	VIS _s	Exch-P	0.54	17.16	14.3	0.55	18.4	15.2
Ashland Bottoms (20 to 60 cm)	VIS _s	Exch-P	0.71	12.12	9.69	0.73	13.1	10.64
Ashland Bottoms (0 to 20 cm)	NIR _s	Exch-P	0.53	17.4	14.5	0.53	18.5	15.5
Ashland Bottoms (0 to 20 cm)	NIR _s	SOC	0.45	0.09	0.07	0.46	0.09	0.07
Ashland Bottoms (20 to 60 cm)	NIR _s	Exch-P	0.7	12.5	10.1	0.7	13.6	11.1
Ashland Bottoms (20 to 60 cm)	NIR _s	SOC	0.69	0.25	0.25	0.69	0.32	0.27

Colby (0 to 20 cm)	EC _{as}	Exch-Mg	0.58	45.8	36.7	0.59	48.5	37.9
Colby (20 to 60 cm)	EC _{as}	Exch-K	0.5	64.5	51.5	0.51	66.1	53.3
Colby (20 to 60 cm)	EC _{as}	Clay	0.55	4.89	4.07	0.55	5.07	4.15
Colby (0 to 20 cm)	VIS _s	Exch-Mg	0.79	32.7	27.2	0.79	34.2	28.8
Colby (0 to 20 cm)	VIS _s	Clay	0.75	2.18	1.66	0.75	1.66	1.73
Colby (20 to 60 cm)	VIS _s	Clay	0.84	2.91	2.21	0.84	3.01	2.29
Colby (0 to 20 cm)	NIR _s	SOC	0.72	0.09	0.07	0.72	0.1	0.08

Influence of permanent soil properties on sensor response

Preliminary results suggest that the ability of proximal sensors to predict soil variables is not solely a function of sensor efficiency but is also strongly influenced by both dynamic and permanent soil properties. Permanent soil properties, including gravimetric water content, soil pH, CEC, bulk density, mineralogical composition (e.g., sand, silt, and clay fractions), and soil organic carbon, appear to play a significant role in shaping sensor responses. To better understand why certain sensors successfully predict specific soil properties at one site but not at others, a principal component analysis (PCA) was conducted. In this approach, selected permanent soil properties were evaluated simultaneously to explore their multivariate

relationships with sensor responses and to identify the dominant gradients structuring the data across sites (Figure 6.12-6.15).

In general, the two sites showed a consistent multivariate pattern in the relationship between EC_{as} and soil properties, where the orientation of vectors in the biplots evidenced the presence of site-dependent geochemical mechanisms. At Ashland Bottoms, the EC_{as} vector was strongly aligned with soil pH and CEC along the dominant gradient (PC1 = 41.1%), indicating a primary association with soil chemical properties. Soil organic carbon (%) showed a moderate positive alignment with EC_{as} , whereas silt content was oriented in the opposite direction along PC2 (18.5%), suggesting a secondary textural influence on signal behavior in soils with higher silt fractions. In contrast, at Colby the multivariate structure indicated a stronger influence of physical properties on EC_{as} variability. The PCA biplot showed that EC_{as} was positively aligned with bulk density and clay (%) along PC1 (41%), while CEC also loaded on the positive side of PC1, indicating a concurrent contribution of soil exchange capacity. Sand (%) and gravimetric water content were oriented in the opposite direction, suggesting contrasting physical conditions. These patterns indicate that more compact and finer texture soils with higher exchange capacity tended to be associated with higher EC_{as} responses, whereas sandier and wetter conditions showed weaker associations (Figure 6.12).

For Ashland Bottoms, the PCA biplot indicated that VIS_s was primarily associated with soil physical properties. The VIS_s vector showed a positive orientation toward bulk density along the dominant gradient (PC1 = 38.3%), suggesting sensitivity to soil structural characteristics. Gravimetric water content and sand (%) were positioned in the opposite direction, indicating weaker or inverse associations with the VIS_s response.

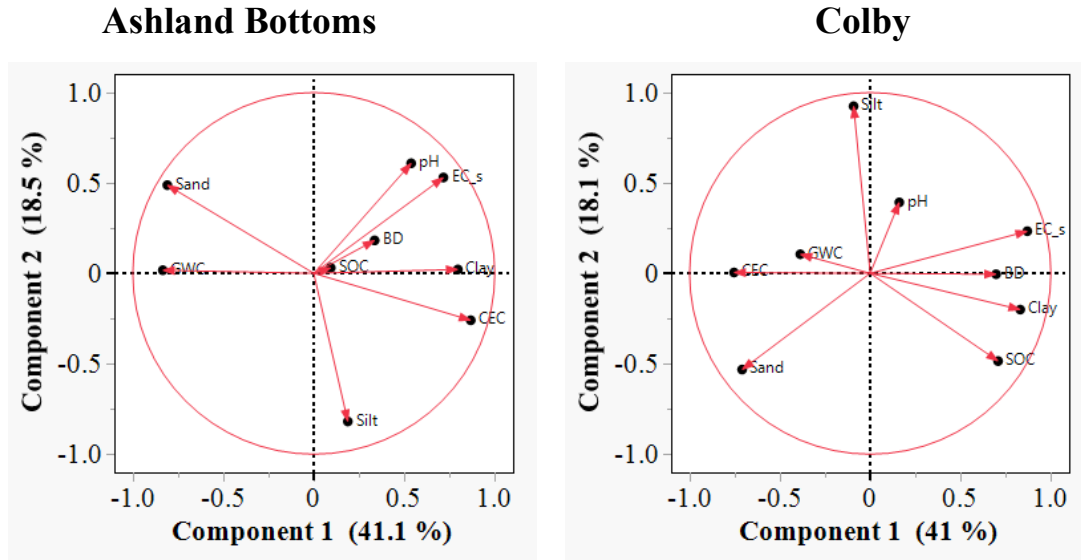


Figure 6.12. Principal component analysis (PCA) biplots showing the relationships between EC_{as} sensor response and selected soil properties at Ashland Bottoms and Colby. Vectors represent variable loadings, and their direction and length indicate the strength and nature of multivariate associations.

In contrast, clay (%), silt (%), and CEC were aligned on the positive side of PC1, reflecting a secondary contribution of finer soil fractions and exchange capacity to the VIS_s variability. Soil pH showed a moderate positive orientation, whereas SOC (%) was mainly aligned along with PC2 (21.1%), suggesting a secondary influence on the VIS_s signal. In contrast, at Colby the PCA revealed a distinct multivariate structure for the VIS_s response. Where, the VIS_s vector was strongly aligned along PC1 (42.2%) and showed positive associations with CEC and gravimetric water content, indicating sensitivity to soil physicochemical conditions that may enhance reflectance. Likewise, sand (%) showed a moderate positive orientation. In contrast, clay (%), SOC (%), and bulk density were oriented in the opposite direction, suggesting weaker or inverse relationships with the VIS_s signal under finer texture and more compact soil conditions. Soil pH was primarily aligned with PC2 (17.4%), indicating a secondary influence on VIS_s variability (Figure 6.13).

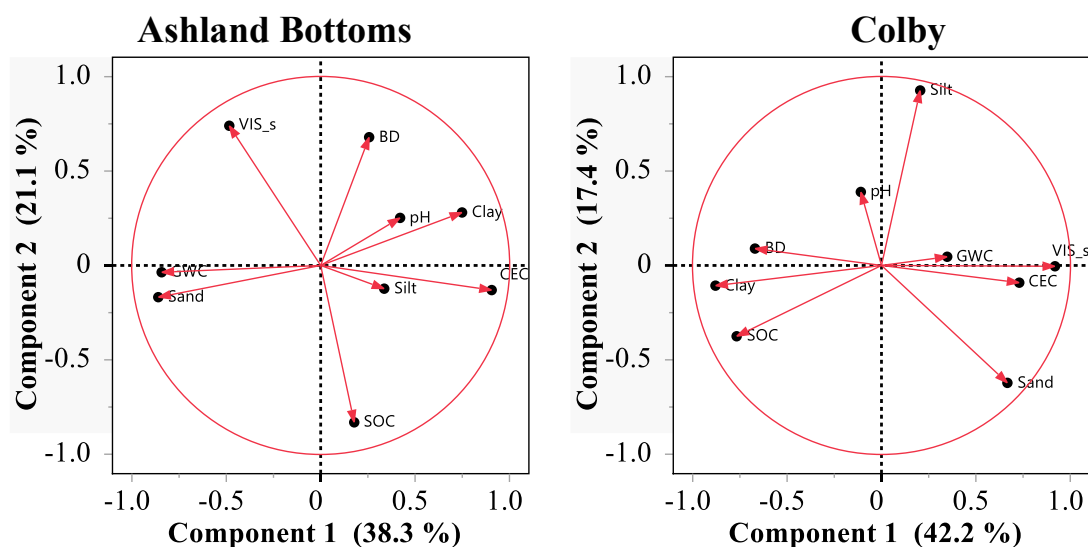


Figure 6.13. Principal component analysis (PCA) biplots showing the relationships between VIS_s sensor response and selected soil properties at Ashland Bottoms and Colby. Vectors represent variable loadings, and their direction and length indicate the strength and nature of multivariate associations.

At Ashland Bottoms, the PCA biplot indicated that NIR_s was primarily associated with soil physical properties. The NIR_s vector showed a positive alignment with bulk density along the dominant gradient (PC1 = 37.9%), suggesting sensitivity to soil structural conditions. Soil pH and clay (%) exhibited moderate positive orientations in the same quadrant, and CEC was also aligned on the positive side of PC1, indicating a secondary contribution of soil physicochemical properties to the NIR_s response (Figure 6.14). In contrast, gravimetric water content and sand (%) were oriented in the opposite direction, indicating weaker or inverse associations with NIR_s. Soil organic carbon (%) was aligned along PC2 (21.2%), suggesting a secondary influence on signal variability. In contrast, at Colby, the NIR_s vector was primarily aligned along PC1 (42.2%) and showed positive associations with CEC, gravimetric water content, and sand (%), indicating sensitivity to soil physical conditions that may enhance reflectance. Conversely, clay (%), SOC (%), and bulk density were oriented in the opposite direction, suggesting weaker or inverse relationships with

the NIR_s signal under finer texture and more compact soil conditions. Soil pH was mainly aligned with PC2 (17.4%), indicating a secondary influence on NIR_s variability (Figure 6.14).

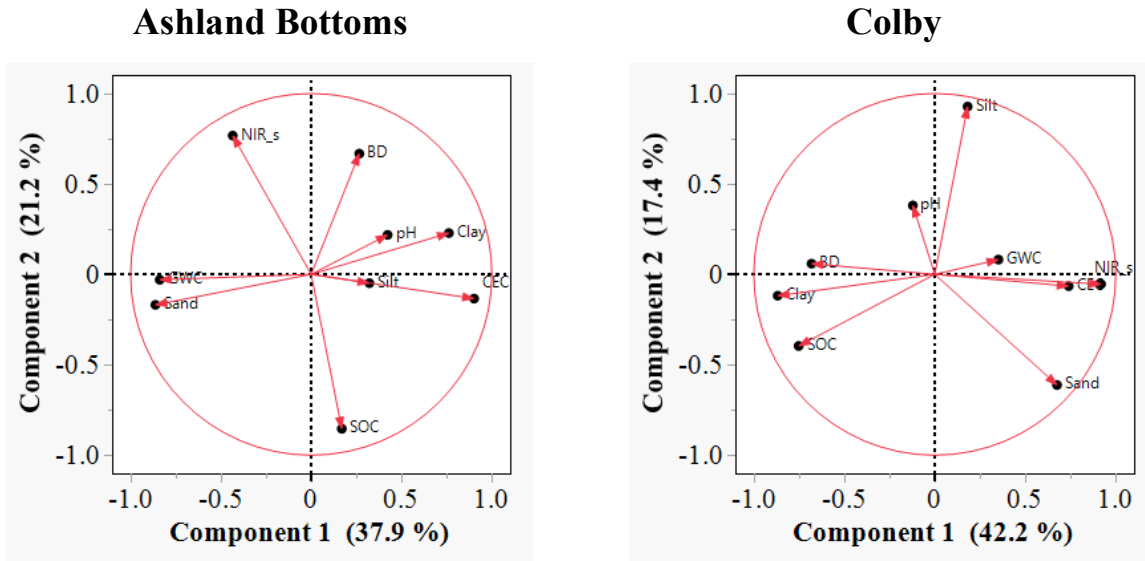


Figure 6.14. Principal component analysis (PCA) biplots showing the relationships between NIR_s sensor response and selected soil properties at Ashland Bottoms and Colby. Vectors represent variable loadings, and their direction and length indicate the strength and nature of multivariate associations.

At Ashland Bottoms, Force_s was primarily driven by dominant physical gradient (PC1 = 39.2%) and showed positive alignment with silt (%) and soil organic carbon (%) along PC2 (20.2%), suggesting sensitivity to fine-fraction and structural characteristics (6.15). In contrast, gravimetric water content and sand (%) were oriented opposite to the Force_s vector, indicating weaker associations under different moisture and coarser soil conditions. At Colby, although the response remained primarily governed by physical factors, the multivariate structure shifted, with Force_s positioned on the negative side of PC1 (37.8%) and showing inverse relationships with clay (%), bulk density, and SOC (%). Instead, Force_s exhibited closer alignment with sand (%) and gravimetric water content, indicating greater sensitivity to coarse texture and moisture conditions at this site. In sum, these patterns demonstrate that while Force_s is consistently

controlled by soil physical structure across sites, the dominant textural drivers vary according to local soil conditions. (Figure 6.15)

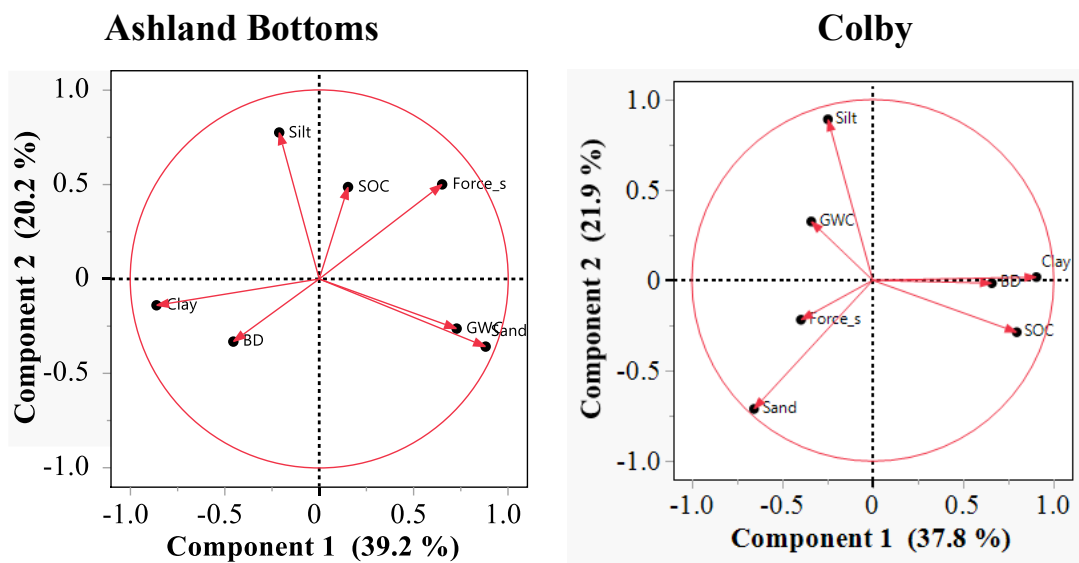


Figure 6.15. Principal component analysis (PCA) biplots showing the relationships between Force_s sensor response and selected soil properties at Ashland Bottoms and Colby. Vectors represent variable loadings, and their direction and length indicate the strength and nature of multivariate associations.

Conclusions

The results of this study confirm that Veris P4000 soil sensors can be effectively used to map soil physicochemical properties in situ, although their performance is strongly influenced by site-specific conditions. Among the four sensors, the NIR consistently showed the highest predictive accuracy for soil organic carbon and exchangeable Phosphorus across both surface and subsurface layers, often exceeding R^2 values of 0.70. The VIS sensor also demonstrated strong correlations, particularly with total nitrogen, exchangeable phosphorus, clay content, and exchangeable magnesium, especially at the sandier soils. The EC_a sensor was effective in predicting soil pH, exchangeable magnesium and potassium, and clay content (%), highlighting its sensitivity to ionic strength, salinity, and texture. Surprisingly, the subsurface layers often

showed higher predictive performance than the surface layers, particularly for SOC % and Exch-P using the NIR sensor. This observation challenges the common assumption that signal strength and model accuracy decline with depth and highlights the potential of spectral sensors to capture stable organic carbon forms in deeper horizons. Overall, factors such as soil texture, moisture, pH, SOC %, and CEC significantly influence sensor signal intensity and predictive capacity. Therefore, the successful deployment of proximal soil sensors in precision agriculture should be grounded in a site-specific understanding of soil-sensor interactions, particularly the influence of dominant clay mineralogy on signal attenuation or amplification through its control on surface charge, water retention, and electromagnetic behavior, and supported by appropriate spectral preprocessing techniques (e.g., Savitzky-Golay smoothing). This approach will ensure higher reliability and utility of sensor-based predictions across diverse field conditions.

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Chapter 7 - Summary and future work

Part I. Lead contamination and stabilization mechanisms in urban soils

Part I of the dissertation focuses on evaluating the effectiveness and mechanisms of phosphorus- and biochar-based amendments for reducing Pb bioaccessibility in contaminated urban soils under both laboratory and field conditions. Chapter 3 examines the capacity of organic and inorganic phosphorus sources to decrease bioaccessible Pb, their influence on Pb fractionation and speciation, and the relationships between soil physicochemical properties and Pb stabilization mechanisms. Chapter 4 extends this work to field conditions by assessing in situ phosphorus-based mitigation strategies, evaluating their long-term effects on soil enzyme activities, and identifying the key soil chemical properties controlling Pb bioaccessibility and their interactions with biological soil functions. Finally, Chapter 5 integrates wet chemistry and advanced spectroscopic techniques, including XANES, μ -XRF, and μ -XANES, to investigate the mechanisms of Pb immobilization and transformation, and to compare the effectiveness of phosphorus and biochar amendments across experimental scales.

The results of part I demonstrate that, prior to treatment application, Pb bioaccessibility in the studied urban soils was already relatively low to moderate, reflecting the inherent geochemical characteristics of these systems. Despite these baseline conditions, the application of phosphorus-based amendments further reduced Pb bioaccessibility by an additional 16% to 36%, highlighting their effectiveness in enhancing Pb stabilization beyond existing natural attenuation processes. However, the stabilization mechanisms varied depending on the type of amendment applied. In inorganic-P treatments, immobilization was primarily associated with modifications to soil properties that favor the precipitation of highly stable pyromorphite-group

minerals. In contrast, organic amendments, such as Class A biosolids and biochar, reduced Pb bioaccessibility primarily through complexation with organic matter and surface sorption onto organic matter and surface sorption associated with soil organic carbon and reactive sites in the soil matrix. In this context, the results indicate that Pb stabilization in urban soils is controlled by coupled changes in key soil properties, availability of Pb, including pH, phosphorus availability, organic carbon content, and clay mineralogy. These properties govern both mineral precipitation processes and organic complexation interactions, which ultimately determine Pb mobility and bioaccessibility in soils. Additionally, P treatments altered the distribution of Pb fractions, shifting from more labile forms toward more chemically stable ones. This transition was confirmed through sequential extraction procedures and synchrotron-based spectroscopic techniques. Synchrotron X-ray-based XANES and μ -XANES analyses showed that in control treatments, Pb existed primarily as Pb-sorbed-Fe species and Pb bound to fulvic acid, forms that are relatively more labile and potentially more bioaccessible compared to stable Pb precipitates. In contrast, treatments with inorganic-P sources promoted the formation of mineral phases in the pyromorphite group, particularly chloropyromorphite and hydroxypyromorphite, confirming that the precipitation of Pb phosphates was the primary long-term immobilization mechanism in P-treated soils. Treatments with Class A biosolids and biochar showed a greater proportion of Pb associated with organic fractions, particularly complexes with Pb bound to humic and fulvic acids, as well as evidence of surface sorption and associations with Fe-rich phases.

Complementarily, μ -XRF mapping revealed a heterogeneous distribution of Pb within the soil samples across treatments, thereby identifying localized hotspots where precipitation and complexation processes occurred. In addition, Pb bioaccessibility evaluated using two in vitro extraction methods, PBET and IVBA (pH 2.5), showed similar trends in detecting reductions in

bioaccessible Pb following amendment application, although PBET exhibited greater sensitivity in distinguishing treatment effects. These findings indicate that while both in vitro methods are suitable for assessing Pb bioaccessibility, PBET provides greater sensitivity for evaluating the effectiveness of phosphorus-based remediation strategies.

This study also demonstrates that the chemical stabilization of Pb may not necessarily translate into a uniform recovery of soil biological activity. Enzymatic responses were enzyme-specific and primarily controlled by the physicochemical conditions generated by the treatments, rather than by Pb immobilization alone. Among the enzymes evaluated, acid phosphatase showed the most consistent association with changes in Pb speciation, particularly with increases in Pb associated with organic fractions. Despite the effectiveness of phosphorus-based amendments, the results highlight that Pb stabilization processes strongly depend on soil geochemical conditions. Therefore, remediation strategies should consider site-specific characteristics, including soil mineralogy, organic matter composition, and soil chemical properties, to optimize treatment effectiveness and ensure the long-term stability of the mineral phases formed.

Finally, future research should focus on evaluating the persistence of Pb stabilization mechanisms under field conditions over longer time scales. Further investigation is needed into the role of Pb-Fe associations in biosolids and the potential of Fe-modified biochar to enhance Pb retention via sorption and coprecipitation. Additionally, it will be important to examine the interactions among P amendments, clay mineralogy, organic matter dynamics, and microbial activity, as these factors may influence the stability of Pb mineral phases in soils. Advancing this integrated understanding will support the development of more robust, mechanism-based remediation strategies for Pb-contaminated urban soils.

Part II. Proximal soil sensors for soil characterization

Part II focuses on quantifying the relationships between measurements derived from multiple soil sensors and selected soil physicochemical properties across two depths, and on assessing how inherent soil properties influence sensor signal intensity and predictive accuracy, with the goal of identifying the key factors controlling sensor performance in two contrasting experimental fields. In general, the results of Part II demonstrate that the sensors of the Veris P4000 system can be effectively used to map soil physicochemical properties in situ, although their performance is strongly influenced by site-specific conditions. Among the sensors evaluated, near infrared sensor consistently showed the highest predictive accuracy for soil organic carbon and exchangeable P at both surface and subsurface depths, frequently exceeding R^2 values of 0.70. The red visible spectrum sensor also exhibited strong relationships, particularly with total N, exchangeable P, clay content, and exchangeable Mg. The apparent electrical conductivity sensor proved effective in predicting soil pH, exchangeable Mg and K, and clay content, reflecting its sensitivity to ionic strength and soil texture. Interestingly, subsurface soils in several cases exhibited better predictive performance than surface soils, particularly for soil organic carbon and exchangeable P estimated using the NIR sensor. This finding challenges the common assumption that spectral signal strength decreases with depth and suggests that spectral sensors may capture relatively stable forms of organic carbon present in deeper soil horizons. Overall, the results indicate that soil properties such as texture, moisture content, pH, soil organic carbon, and cation exchange capacity play a critical role in modulating sensor signals and influencing prediction accuracy. To address these limitations, several methodological approaches can enhance predictive performance, including spectral

preprocessing techniques such as smoothing, scatter correction, and spectral transformations to reduce noise and improve signal quality. In addition, feature selection methods and robust validation strategies, such as cross-validation and Monte Carlo resampling, are essential to improve model reliability and prevent overfitting.

Overall, these findings highlight that the successful application of proximal soil sensors in precision agriculture depends on a site-specific understanding of soil-sensor interactions. Future research should focus on quantifying the type and abundance of clay minerals and incorporating this information into sensor calibration models. A deeper understanding of how clay mineralogy controls spectral and electromagnetic responses will enable the development of more robust, site-specific calibration frameworks, ultimately improve prediction accuracy and expand the applicability of proximal soil sensing technologies across diverse agricultural systems.

Appendix A - Description of urban field sites

General characteristics of urban soils

Representative view of a vacant urban lot used as a field study site, showing natural vegetation cover, minimal land management, and in situ soil sampling activities.



Vacant urban lot located adjacent to an older residential structure, illustrating typical site conditions and potential proximity to legacy sources of Pb contamination.



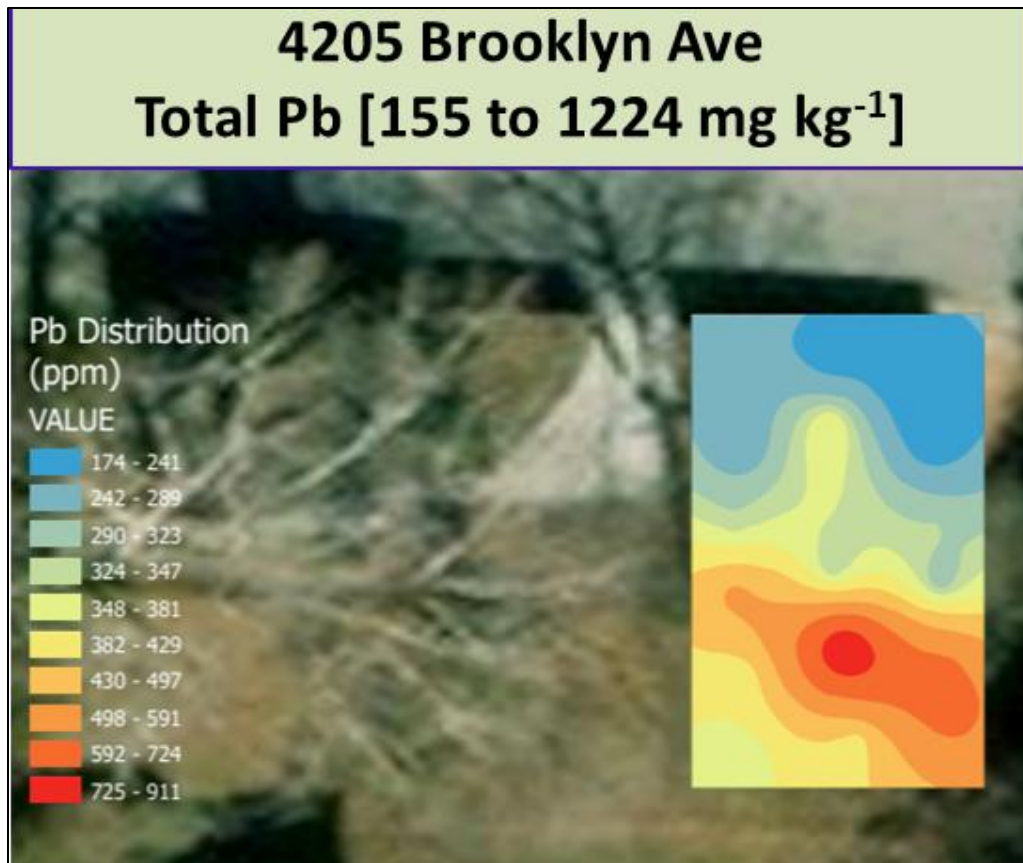
Soil sampling conducted near a deteriorated residential structure, illustrating typical conditions of urban environments with potential legacy contamination sources.



Initial site layout showing the establishment of a 2×2 m sampling grid used for in situ measurement of total soil Pb concentrations using p-XRF.



Example of spatial distribution of total Pb concentrations in an urban site, illustrating the high variability characteristic of urban soils.



Representative workflow of field treatment implementation, including site preparation, plot establishment, amendment application, and surface stabilization, illustrating the procedures used for in situ Pb remediation.

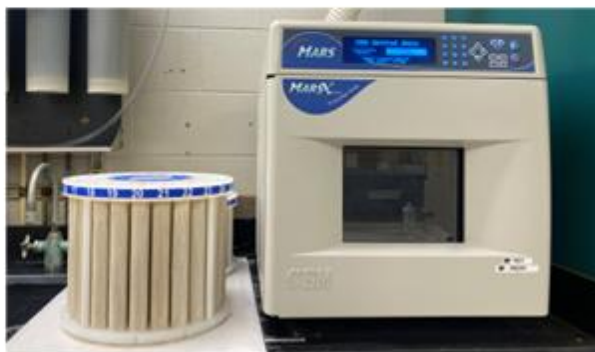
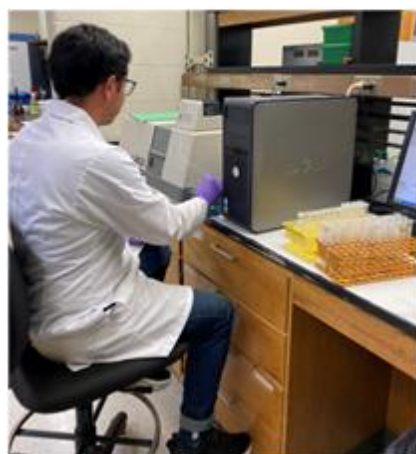
3928 Garfield Ave



Field soil sampling using a manual soil probe within established experimental plots.



Laboratory workflow illustrating soil sample preparation, processing, and analysis using standard analytical techniques to determine physicochemical properties and Pb concentrations.



Workflow illustrating synchrotron-based analyses used for advanced characterization of soil samples.

**Argonne National Lab
Advanced photon source**



Bulk-XANES



μ -XRF

