Laboratory- to field-scale investigations to evaluate phosphate amendments and *Miscanthus* for phytostabilization of lead-contaminated military sites

by

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B.S., King Saud University, 2000
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AN ABSTRACT OF A DISSERTATION

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Abstract

Potentially toxic substances can contaminate extensive areas of productive land due to military activities. The most common and widespread metal contaminant in military lands is lead (Pb). The main objectives of this study were to evaluate the feasibility of using *Miscanthus*, a second-generation biofuel crop, for photostabilization of Pb in contaminated military site soils; the effect of soil amendments on *Miscanthus* growth; and the effects of continual plant growth, nutrient removal and the soil chemical changes induced by *Miscanthus* growth on soil Pb bioaccessibility. In 2016, we established a field site on a US Army reservation in Fort Riley, KS. *Miscanthus* was planted in an area with soil total Pb concentration ranging from 900 – 1,500 mg kg^{-1} and near-neutral soil pH. Five treatments were evaluated: (i) control plots without tillage with existing vegetation, (ii) no-tillage, no additional amendments planted with *Miscanthus,* (iii) tilled soil, no additional amendments planted with *Miscanthus*, (iv) tilled soil amended with triple superphosphate (at 5:3 Pb:P molar ratio) planted with *Miscanthus*, and (v) tilled soil amended with organic P source (class B biosolids applied at 45 Mg/ha) planted with *Miscanthus*. Results from three years show that one-time addition of soil amendments to Pb-contaminated soil supports establishing and stabilizing *Miscanthus*, increasing biomass yield as well as reducing phytoavailability and bioaccessibility of Pb. Plots amended with biosolids had significantly less total Pb uptake, plant tissue Pb concentration, and Pb bioaccessibility, and more soil enzyme activities, organic carbon, and microbial biomass.

Controlled-environment greenhouse and laboratory incubation studies were conducted to test selected additional P sources. Additional sources included non-traditional, less soluble types such as struvite and apatite. The greenhouse study aimed to evaluate the effect of *Miscanthus* growth on bioaccessibility of amended and non-amended soils and the effect of soil amendments on soil-plant transfer of soil Pb over three *Miscanthus* cuttings. Soil amendments increased dry matter yield in the first cutting. Soils in *Miscanthus* pots that were amended with biosolids, had significantly lower total Pb uptake, Pb concentration in plant tissues, and Pb bioaccessibility when compared to the control across all cuttings. Overall, the results suggested that *Miscanthus* can be effectively and safely grown on Pb-contaminated soils amended with biosolids.

The incubation study evaluated the effectiveness of various in situ P treatments on reducing Pb bioaccessibility and the effects of different P sources on the speciation of soil Pb over time. X-ray absorption spectroscopy was used to understand treatment-induced changes to soil Pb speciation. Results showed that soil pH decreased slightly for all treatments. Percent of bioaccessible Pb in soils amended with biosolid were significantly ($\alpha=0.05$) less than the other amendments. The high rate of biosolids (225 Mg ha^{-1}) was the most effective in controlling the bioaccessibility of soil Pb, and the reductions in bioaccessibility ranged from 63 to 76% compared to the control. X-ray absorption spectroscopy results indicated pyromorphite (stable Pb phosphates) phases were the most dominant Pb species in both amended and non-amended soils. The results of these studies suggest that *Miscanthus* can be grown successfully in Pb-contaminated shooting range soils in combination with organic or inorganic phosphate amendments, while minimizing the associated environmental risks.

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Dedication

For their eternal love and encouragement, without which this journey could not be achievable.

To my Parents:

“God bless their soul and makes the resting place of paradise”

To my dear wife:

Fawziah Alshehri

To my children:

Moaz, Waad, Shahd, and Omar

To my brothers and sisters

To all my nephews and nieces

Chapter 1 - Introduction

Environmental contamination is a severe problem in the world. In particular, unlimited industrial and commercial practices have led to a significant accumulation of pollutants that need attention. There are many contaminated sites in urban and peri-urban areas, which limits business and real estate development. Current environmental organizations ensure environmental protection best practices in the developed world, though despite these efforts, contamination may still occur, and old contamination sites are still to be remediated or under treatment. Additionally, there are many activities that produce waste that requires treatment. For example, many governments around the world are responsible for shooting ranges, and many of these sites are contaminated due to the use of lead pellets (bullets and shot) as ammunition that have been placed on the soil of shooting ranges worldwide. For this reason, it is particularly important to take into consideration some factors, such as soil-metal concentrations, type of metal present, and amount of metal that present risks to ecological surroundings and human health, that might help determine which sites require urgent consideration (Salatas et al. 2004).

In 2006, the quantity of lead (Pb) applied in the USA for ammunition production, including shots and bullets, passed 70,000 t (USGS 2007). Common military soil contaminants include Pb, antimony (Sb), arsenic (As), copper (Cu), nickel (Ni), and zinc (Zn). Small-caliber military bullets fired at military ranges primarily consist of a Pb core (with potential As impurities) with small amount of Sb used as a hardening agent and a Cu jacket which may also contain traces of Ni or Zn. Furthermore, a jacketed bullet pellet typically consists of more than 90 % Pb (Chrastný et al. 2010; Robinson et al. 2008). Usually, Pb concentrations exceeded 1,000 mg kg^{-1} in shooting range soils (Cao et al. 2003b), while high concentrations of Pb have been reported to exceed 20,000 mg kg^{-1} (Dermatas et al. 2006). Lin et al. (1995) reported that in

Sweden, Denmark, and Finland, more than 500 t Pb year^{-1} was produced in each country for shotgun ammunition. Lead-based ammunition deposited in soil is subject to weathering over time, and the metals in the Pb pellets are oxidized to secondary minerals. Cao, et al., (2003a) reported that most of the metallic Pb in pellets will eventually be converted into particulate and ionic lead species and will be distributed into the environment to some extent. Accumulations of Pb and pellet fragments in military firing range soils can lead to a significant issue in the environment.

The US Centers for Disease Control and Prevention defines elevated blood Pb as 5ug/dL (CDC 2012). According to the CDC, elevated blood lead level (BLL) is associated with human susceptibility to a variety of health diseases (USCDC 2019). Children are particularly receptive to the toxic impact of Pb appearing in developmental impairment due to their high repetition of hand to mouth motion and enhanced intestinal absorption abilities. For example, in 2012 there were approximately half a million U.S. children ages 1 to 5 with blood Pb levels above 5µg dL^{-1} (CDC 2012). Furthermore, the Agency for Toxic Substances and Disease Registry (ATSDR) listed Pb as the second most hazardous substances in the National Priority List (NPL) sites in the US (ATSDR 2017). Therefore, it is imperative to reduce environmental risks by preventing contamination of soils at locations such as military firing ranges is imperative (Knechtenhofer et al. 2003).

Recently, shooting ranges have been under increased scrutiny due to growing concern regarding Pb contamination. Hettiarachchi and Pierzynski (2004) documented soil ingestion, inhalation, and dermal absorption of contaminated soil particles as the primary exposure pathways of soil contaminants. Military training generates soil dust and increases the trainees' and workers' exposure to contaminated soils in these areas. The toxicity and mobility of these contaminants are associated with their solubility, and in this regard, their speciation is the greatest concern to individuals tasked with determining recommendations of levels that need soil excavation. Increased concern about shooting range soil has spurred research exploring potential human health risks because of military sites established near populated areas and/or agriculture fields.

Reducing the exposure risks of Pb in contaminated military sites should allocate remediation trials that concentrate on minimizing the bioaccessibility/bioavailability of Pb. Bioaccessibility, concerning Pb or metal(lod), is defined as the fraction/percentage of the metal(loid) that interacts at an organism's contact surface and is potentially available for adsorption or absorption (McGeer et al. 2004). Thus, in this dissertation, the term bioaccessibility is utilized to represent the quantity of soil Pb that is potentially available for adsorption or absorption. Bioavailability of metal(loid)s is defined as the extent to which bioaccessible metals adsorb onto or absorb into and across the biological membrane of an organism (McGeer et al. 2004). It is often represented as a fraction of the total amount of metal(loid) that the organism is exposed to through given time and under defined conditions (McGeer et al. 2004).

It is necessary to revegetate contaminated sites to diminish further contamination of surrounding environments by erosion and runoff. Revegetation can be performed through emerging phytostabilization methods. Phytostabilization is the use of plants to create vegetation on the soil surface, reducing the risk of further environmental degradation by contaminant leaching into groundwater or spread by wind or water erosion. Biofuels crops possess required features for some phytoremediation methods, such as fast growth, high biomass yields, intense and extensive rhizome systems, and tolerance to contamination (Ali et al., 2013). Second-generation biofuel crops, such as miscanthus and switchgrass, have demonstrated potential for phytoremediation, which is known to remediate contaminated soils without using excavation techniques (Vanek et al., 2010). Two benefits of this group of biofuel crops are the production of high biomass, which is viewed as a promising source for energy, and the ability to grow as a non-food crop on contaminated soils, which resolves potential food insecurity when compared to growing maize, sorghum, oilseed crops (Kocon and Matyka, 2012).

Phytostabilization is usually combined with the addition of soil amendments to decrease contaminant bioavailability and improve the quality of the contaminated materials as a plant growth media. Soil amendments, organic and inorganic, are used to accelerate the attenuation of metal mobility and toxicity in soils. These amendments can convey soil Pb to more geochemically stable phases through precipitation, sorption, and complexation processes (Hashimoto et al., 2009). The most used amendments include phosphates and composts (Wuana and Okieimen, 2011; Zhang et al., 2010). Many studies report the use of soil amendments is much less expensive than excavation and removal of soils (Scheckel et al., 2013; U.S. EPA, 2011, 2013).

The use of soil amendments and/or chemical sorbents to bind and immobilize Pb is a common practice (Hettiarachchi and Pierzynski 2004, Scheckel et al. 2013). This approach can reduce both the mobility and toxicity of Pb, which may be favored at large sites where metal stabilization is demanded, such as shooting ranges (Wilson et al., 2006). Stabilization of lead-contaminated soil on military sites using a second-generation biofuel crop and phosphate-based soil amendments is considered one of the most cost-effective approaches in soil remediation as well as an ecologically beneficial practice (Pidlisnyuk et al., 2014). However, extensive research studies in this area are limited.

Investigations on the efficiency of phosphate (P) amendments in the immobilization of Pb have generally confirmed, that the dissolution of phosphate followed by Pb–P reactions including conversion of soluble forms of Pb into less soluble types, the formation of stable minerals, surface adsorption, complexation (Wang et al. 2008). A more common example of lead phosphate, pyromorphite, is one of the most stable forms of Pb in soils. It provides insoluble species of Pb under a wide range of environmental conditions (Lindsay, 1979; Nriagu, 1974; Lin et al., 2005). For apatite-based P treatments, this can be described by Pb penetration into the hydroxyapatite lattice and following replacement with Ca as well as precipitation of Pb with phosphate (Cao et al., 2004). Another mechanism is the dissolution of phosphate rock or other P sources and subsequent precipitation of a pyromorphite-like mineral ($Pb_{10}(PO_4)_6 X_2$, X = OH, Cl, F).

Pyromorphite-like minerals were observed via X-ray diffraction (XRD) identification in an in-situ stabilization study of Pb-contaminated mine soils amended with P sources (phosphate rock (PR), triple superphosphate (TSP), phosphoric acid (PA) (Hettiarachchi et al., 2001). Decreases in soil lead bioavailability of some treatments may be justified by the identical increased pyromorphite formation seen for the treatments. Additional research further analyzes the effect of different P sources on Pb phosphate formation (Chappell and Scheckel, 2007; Hettiarachchi et al., 2000, 2001). However, treatment results may differ due to the chemical heterogeneity of soil environments. Moreover, it is unknown whether how the Pb phosphate species formed might be affected by P source or by distance in the soil from P-containing materials, which has links for the amount of P that demands to be added and the degree of mixing that is required.

It has been recommended that the formation of Pb phosphates in soils contaminated with Pb is useful for immobilizing Pb, thereby decreasing the bioaccessibility of Pb (Ruby et al., 1994). Phosphorus treatments have, therefore, been proposed as a cost-effective remediation alternative for Pb-contaminated soils (Basta et al., 2001; Cao et al., 2004; Hettiarachchi and Pierzynski, 2004). The application of P for in situ stabilization of soil Pb is feasible if the P amendment would present long-term decreases in Pb bioaccessibility. However, there are some concerns, such as the long-term stability of the recently formed pyromorphites, the suitable quantity of P to add, and the most efficient P source. It is also not known how the growth of biofuel crops affects bioaccessibility of soil-Pb, if the Pb-contaminated sites were used for growing biofuel crops.

Biosolids were applied earlier as an in-situ amendment to decrease Pb bioaccessibility in urban soils contaminated with Pb (Attanayake et al., 2015; Defoe et al., 2014). Biosolids amendments, which are rich in P, support the formation of pyromorphite (Brown et al., 2003). Valipour et al. (2016) recorded a significant decline in the availability of Pb after the application of triple superphosphate (TSP). In this dissertation, we selected two sources of P amendments for the field study. Due to the limited land area available for our research and other logistic issues, testing different sources and rates of P amendments was not possible. In response, subsequently a greenhouse and a laboratory incubation studies were designed to test select P sources at two or three different P rates, respectively. These sources include non-traditional, less soluble P sources, such as struvite and apatite, as well as the sources used in the field study. In addition to evaluating changes in Pb bioaccessibility and extractability over time, x-ray absorption spectroscopy (XAS) was used for further observation of speciation changes of soil Pb.

In vivo animal model studies are excellent for determining the reliable bioavailability of soil contaminants; however, these studies are challenging, costly, and time-consuming. Additionally, the negative public view of animal tests raises the value of in vitro methods. In vitro procedures determine the bioaccessibility of metal(loid)s from contaminated soils. An in vitro bioaccessibility test,(also known as the physiologically based extraction test (PBET), that simulates gastrointestinal dissolution of Pb (Ruby et al., 1996) that can be applied to predict the bioaccessibility of Pb from contaminated soils as it has been proven to be well correlated with the animal model studies conducted using rats (Hettiarachchi et al, 2003), swine (Casteel et al., 2006; Ryan et al., 2004; Wragg and Cave, 2003; Zia et al., 2011). We chose a modified PBET method to evaluate the impact of soil amendments on soil bioaccessibility.

No current information concerning the effect of biofuel crop growth on speciation of Pb in soils is evident. Therefore, this new knowledge will be beneficial in developing the best management practices for growing select biofuel crops effectively and safely on shooting ranges. It is not economical to conduct detailed investigations of each site in order to determine under which circumstances treatments offer the potential to decrease the bioaccessibility of Pb significantly. However, this limitation can be overcome through an improved understanding of the fundamental mechanisms responsible for reducing bioaccessibility of Pb through treatment additions as well as Miscanthus growth. This research will be a useful addition to the recent studies promoting bioavailability-based remediation of Pb-contaminated soils.

Objectives

1- The objectives of the first study, <u>Phytostabilization of a contaminated military site using biofuel crop and soil amendments: A field-based investigation</u> (Chapter 3), were to i) to evaluate how different establishment methods and soil amendments affect miscanthus growth, plant Pb concentration and soil Pb stability, and ii) to assess the effects of continual plant growth, nutrient removal, and the soil chemical changes induced by miscanthus sp. growth on bioaccessibility and speciation of soil Pb.

2- The objectives of the second study, Effect of soil amendments and biofuel crop growth on lead uptake and bioaccessibility in a skeet range soil: A Greenhouse Study (Chapter 4), were to evaluate the effectiveness of various P sources at two different rates to mitigates the impact of vigorous miscanthus growth on the bioaccessibility and uptake of Pb in contaminated skeet-range soil.

3- The objectives of the third study, Evaluation of in situ soil amendments on speciation and bioaccessibility of lead in shooting range soil: An incubation study (Chapter 5), were to 1) evaluate the effectiveness of P source, P rate and time on reducing soil Pb bioaccessibility, and 2) elucidate the mechanisms of P-induced reductions in the bioaccessibility of Pb in a contaminated skeet shooting range soil.

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Chapter 2 - Literature Review

Military site

Definition of Shooting Ranges

Shooting ranges – enclosed gun and rifle target training areas intended for controlled shooting practice, are typically operated and maintained by military facilities worldwide. A static range, which is commonly used for military training, has targets at a known distance, a firing line, a range floor, and a primary stop butt behind the targets to contain the fired bullets. According to the Interstate Technology Regulatory Council (ITRC) (2003), the U.S. Department of Defense (DOD) oversees more than 3,000 small arms ranges for the military and the 1 million kg of ammunition expended there each year. The U.S. Environmental Protection Agency (EPA) (2003) notes that expended ammunition resulted in a metal overload in the soil, with 69% of the sites found to be contaminated; approximately 200 of the ranges are vulnerable to closure (ITRC 2003). Therefore, soils at shooting ranges might be predisposed to contamination due to overuse of weapons.

Location and climate

Fort Riley is major U.S. Army installation located in the northeast region of the state of Kansas along the Kansas River; its geographic coordinates are (39°11′30″N 96°35′30″W). Fort Riley is southwest of the city of Manhattan and north of Junction City. The base is primarily situated in Riley County with a small portion in Geary County. The installation area totals 41,141 ha. The post is also located in the Flint Hills Eco-region – part of the largest remaining section of uncultivated tallgrass prairie or grasslands in North American (Omernik, 1987; Bailey et al., 1994; Dickson et al., 2008). The chosen area for the research field was an entertainment “skeet” shooting range with lead (Pb) pellets from the 20th century. Only traces amount of Pb remain, while it ranges approximately from 900 to 1500 mg kg^{-1} over the area to ~15 cm depth, but it reaches more than 1500 mg kg^{-1} in some places within this area. Thus, the higher concentration of Pb varies from place to place inside this site.

The Fort Riley area experiences a humid continental climate with a warm, mild summer. This area also characteristically experiences widely variable annual precipitation and temperatures. Due to Fort Riley's close approximation to the continental U.S. geographic center, under a typical continental climate there will be hot summers and cold, dry winters. Winds in the Fort Riley area are considered moderate; humidity levels are considered low. During the year, the monthly average temperatures for January will be -3.0^{o}C and for July will be 26^{o}C (PRISM Climate Group, 2012). The annual mean precipitation is approximately 843mm; however, rainfall can show extremely variability on a year-to-year basis. Normally, 75 percent of the annual precipitation falls during the growing season. Violent thunderstorms often serve as the source of precipitation for the growing season as these thunderstorms are typically associated with intensive rainfalls with rainfall rates reaching approximately 60mm/hr. On an annual basis, there is an average of 55 days of rainfall in the general area (U.S. Department of Agriculture Soil Conservation Service, 1975; Knapp, 1998).

Soil taxonomy

The soil type (soil taxonomy) at Fort Riley is called mollisols. This grassland prairie soil is considered very fertile. These widely-dispersed, nutrient-rich types of soils are generally recognized as an essential source of great agricultural fertility, facilitating growth of agriculture foodstuffs and economically important plants. This type of soil is defined as silty clay loam, which derives from parent materials that are accumulated on-site through precipitation by wind and rain. Using simplified soil classifications (USDANR, 2012), it has been found that the majority of the soil composition is clay upland. These are combined with a loamy upland that includes loamy lowlands and limy soils. The (USDANR, 2012) reported that soil permeability differs from extremely drained sandy lowland soils to compact clays with quite low permeability.

Vegetation

The Fort Riley area is a mix of native prairie and introduced vegetation (Dickson et al., 2008). This vegetation includes C3 and C4 grasses plus forbs. Fort Riley's eastern part is quite characteristic of the Flint Hills region, with its primary composition of annual and perennial forbs and highly productive, warm-season C_4 grasses. According to Quist et al. (2003), Fort Riley's western portion is primarily composed of plant communities that are now undergoing a recession back to the original native prairie condition from previous agricultural cultivation that occurred in the 1960s. Our study area, located in the east part of Fort Riley, showed that the area's prairie grasslands are now dominated by grasses and a mixture of annual weeds. These plants include switchgrass (*Panicum virgatum*), cheatgrass (*Bromus tectorum* (L.), Canadian horseweed (*Conyza canadensis* (L.) and Conyza canadensis var. canadensis), little bluestem (*Schizachyrium scoparium*), green foxtail (*Setaria viridis*), large crabgrass (*Digitaria sanguinalis*), and West Indian nightshade (*Solanum ptycanthum Dunal*). There is other vegetation that includes forbs and grasses, but these occur in much lower abundances.

Military Training and Environmental Impacts

As one of the most important military training bases in the U.S., Fort Riley is a major site for combined arms exercises. This combat training includes mechanized infantry tactics, tank, unit field maneuvers, small arms, mortar and artillery fire, combat aircraft sorties or flights, and supporting arms (Quist et al., 2003; Althoff et al., 2006). Starting in the 1980s, U.S. Army units have involved continuous maneuver-based training exercises at all U.S. military installations

(U.S, Army, 1994). However, Quist et al. (2003) and Althoff et al. (2006) note that such training exercises at Fort Riley are concentrated in the northern 75 percent of the installation's area. These training exercises take place at 17 out of the 18 designated training areas. These training areas cover approximately 26,000 ha. The tallgrass prairie vegetation in these regions have seen significant disruption from mechanized and military vehicle traffic and maneuver warfare activities due to the frequent and highly intensive military training maneuvers conducted in these areas throughout the year (Guretzky et al., 2006). This activity has been cited as the direct cause for environmental damage. This extensive damage includes high concentrations of toxic metals, compositional shifts in plant communities, reduced plant cover, increased instances of bare soil and compacted soil conditions (Whitecotton et al., 2000; Quist et al., 2003).

Lead

Lead Characteristics and Appearance

Lead (Pb) is positioned in a group IVA number four of the periodic table and has an atomic number of eighty-two. Pure Pb is commonly seen as a silvery-white or soft gray metal, with a specific gravity of 11.34. The valence shell electron configuration of Pb is $6s^2$ $6p^2$, which enables it to have three possible oxidation states: Pb (II), Pb (III) and Pb (IV). The Pb (IV) state is a powerful oxidizer and is rarely on Earth's surface. Over the wide range of environmental conditions, Pb^{+2} is the most stable oxidation state, which is present in soils. Mean soil Pb concentrations worldwide range from 10 to 84 mg kg^{-1} (Kabata and Pendias, 2000; McBride, 1994).

Features and Common Uses

Due to its many unique physical and chemical characteristics, society exploits this abundant metal for many distinct purposes such as the material for manufacturing individual letters, numbers, and symbols in the printing industry. Lead is known for having a low melting point of about 327.5^{o}Cs, a high density of approximately 11.35 g cm^{-3}, and low ductility. Its low melting point makes possible the production of small arms ammunition and shotgun pellets, while its high density allows Pb to be used to make fishing net weights, scuba diving belts, anchors, as well as a sound absorber and in radiation shields during x-rays. The metal was also used in the production of construction pipes/sheeting and detail casting materials because it is a very soft and malleable metal. The most widely-known chemical property of Pb is that it is an anti-corrosive, leading to the production of Pb-acid batteries for automobiles in the U.S.

Sources of Lead Contamination

Lead-based paints

It was very common for Pb (II) Carbonate – “white lead” – and Pb chromate – “chrome yellow” – to be used as a pigment in paint to sustain a fresh appearance and resist moisture that could lead to corrosion (Minca and Basta, 2013). Because of its anti-corrosion characteristic, these paints were used to coat construction structures such as bridges, metal pipes, and in households. In 1978, the use of Pb-based paints was banned in U.S. households. According to the U.S. Consumer Product Safety Commission U.S. CPSC (1977), adults and children living in houses built prior to the banning still face exposure risks. In 2000, the number of U.S. houses containing indoor lead-based paint was estimated to be about 38 million (Jacobs et al., 2002). Jacobs et al. (2002) reported that approximately 63% of these houses had an abundance of lead-based paint hazards; their research also showed that 5% of the 63% of households with lead-based paint hazards housed low-income families with children under the age of six years old. (CDC, 2004; Jacobs et al., 2002).

Lead in gasoline

From the mid-1920s to the mid-1980s, Pb was added to gasoline (Kirt and Othmer, 1992). Tetraethyl Pb, or TEL, was used as an inexpensive gasoline additive to increase gasoline octane number in internal combustion (IC) engines. Increasing the octane number to the increase in the “anti-knock” property of the gasoline, resulting in better fuel economy and higher IC engine power output. About 75 percent of gasoline Pb (about 0.3 gm/liter of Pb contained in gasoline in 1980) polluted the air of traffic ways and entered the atmosphere in the form of a fine dust from exhaust pipes (Mielke, 1999). In 1986, Pb gasoline was phased out of use in U.S. motor vehicles due to environmental damage and health risks (Mielke, 1999).

Lead-based bullets

Due to its high-density, Pb has been used in the production of munitions for several centuries, giving bullets greater strength to long ranges and greater power than similar weapons of less dense materials. Lead’s low melting point allows it to be dissolved and molded to make bullets and shot. Additionally, Pb is favored because its anti-corrosive and high flexibility characteristics makes little corrosion inside of the gun pipe, and its relatively low cost. There is no other proper metal that couples these properties to create bullets (Thornton et al., 2001).

Soil contamination at these sites depends on the intensity of the shot operational timeframe (Dermatas et al., 2006; Murray et al., 1997). Small-caliber military bullets (5.56 and 7.62 mm) fired at military ranges primarily consist of a Pb core with small amount of antimony used as a hardening agent; arsenic, which is an impurity in Pb; and a copper jacket, which in addition to copper contains zinc or nickel as an alloy (Laporte-Saumure et al., 2010). An average shot originated from these weapons at these sites contains approximately 97% metallic Pb, 2% antimony, 0.5% arsenic, and 0.5% nickel; however, bullets are produced with Pb (90–99%), antimony (2-5%), and copper (0.1%) (Johnson et al., 2005; Moon et al., 2013b; Sanderson et al., 2015b). The corrosion of these materials has the potential to release significant quantities of metal into the environment. Among the range of metals commonly associated with range activities including Pb, copper, antimony, arsenic, and zinc, which are used in the production of bullets. Lead is the most important due to its concentration mass and because it is the principal component of the bullets in shooting range soils. It must be noted that there may be other metals that need to be appropriately treated.

Rainwater plays a significant function because it works as an electrolyte with bullets, which encourages the electron exchange between the two metals, leading to corrosion. Since Pb has a significantly lower electromotive force (-0.126) than copper (-0.337), these metals together are liable to corrosion. The Pb will be more soluble than copper, prompting environmental concern (Bricka et al., 1998). Military weapons from skeet shooting have little potential to contaminate soil by bullets utilized in skeet shooting and do not break into fragments.

Yearly usage of Pb in the United States differs within 1.4 and 1.5 million metric tons, presenting above 4.5 kg (10 pounds) of Pb per person per year (Carlin et al., 2006). It should be noted that more than a million metric tons of Pb are reused yearly through the battery recycling processes (Carlin et al., 2006). Lead, in the form of bullets and pellets from firing ranges, end up seeping into the environment. For example, there are approximately 3,000 small-arms ranges (SAR) in the U.S. operated by the DoD (USEPA, 2005). As previously mentioned, approximately 69% of the shooting ranges are contaminated (USEPA, 1997) with about 200 of those ranges subjects to closure due to ammunition expended resulting in a metals overload in the soil (ITRC, 2003). The environmental risks related to shooting ranges have been well documented, particularly in countries where shooting ranges are widespread (USEPA, 2001; Laporte-Saumure et al., 2010; Lin et al., 1995; Sorvari et al., 2006). The quantity of Pb applied in bullets and shots yearly ranges from 72,600 metric tons in the U.S. (USEPA, 2001), 2,000 metric tons in Canada (Scheuhammer and Norris, 1995), 800 tons annually in Denmark (Jorgensen and Willems, 1987), 500 – 600 tons in Sweden (Lin, 1996), 530 tons a year in Finland (Mukherjee, 1994), and 400 tons in Switzerland (Johnson et al., 2005). According to the United States Geological Survey (USGS), approximately 75,100 and 73,900 tons of Pb were used in manufacturing ammunition, shots, and bullets in the U.S. in 2011 and 2012, respectively (quoted. in Guberman, 2016).

Lead contamination problems in the shooting range soils are well documented as the second-largest contaminator after the battery industry (USGS, 2002; Li et al., 2015; Sanderson et al., 2012). Researchers have concentrated on shooting ranges as one of the leading causes of Pb contamination attributable to the increased use of small arms ammunition for shooting activities (Kelebemang et al., 2017; Sanderson et al., 2018; Sehube et al.,2017; Vandebroek et al., 2019). Many studies focus on total Pb concentration and deposition in shooting range soils, which do not provide an accurate test of the environmental contamination (Sehube et al., 2017). Total Pb concentration in shooting range soils on its own does not represent an obvious understanding of the mobility, dissolution, bioavailability, bioaccessibility, fate, and ecotoxicity of Pb to biota (Kelebemang et al., 2017). To obtain a clear understanding of the potential environmental contamination risk represented by Pb accumulation in the soil, studying speciation of Pb is necessary to provide extensive information on the partitioning of the various chemical species of

Pb in shooting range soils (Bandara and Vithanage, 2016; Kelebemang et al., 2017). Additionally, the significance of contamination risk indicator in establishing the environmental contamination risk has further enhanced the appreciation of the degree of the hazard created by Pb accumulated into shooting range soils.

Toxicity and Human Health

The negative health impacts posed by exposure to Pb are well documented. According to Sigel et al. (2017), Pb has been known to be extremely toxic to humans; however, the harmful effects of Pb caused by biological mechanisms remain poorly understood. Sokol (2005) explains how Pb affects the human reproductive system. Similarly, Schoeters et al. (2008) discusses how Pb causes disruptions and abnormalities of endocrine development in children. Levin et al. (2008) adds that young children are open to significant exposure to Pb unknown or accidental ingestion of Pb-contaminated soil. The Pb toxicity usually has marked adverse effects on young children because children's bodies are more efficient at absorbing Pb as they can absorb up to 50% of Pb compared to adult's 10% (Greenway and Gerstenberger, 2010). Needleman (2004) has stated that children are more adversely affected because their intestines absorb Pb more readily, and because their developing central nervous system (CNS) is more vulnerable than that of adults. In children, Pb toxicity can affect the brain as well as cause stunted growth, trouble hearing, weakened vision, kidney breakdown, learning limitations, and behavior problems (Bruell et al., 1999). Additionally, Pb can drive to anemia, weaken children's immune system, and harm reproductive organs, liver, and cardiovascular system. According to the USEPA (2005), low levels of Pb, as little as10 μg/l, can be highly toxic to children.

In adults, exposure to Pb can create pregnancy difficulties, reproductive challenges such as delivering birth to an undersized child, congenital disabilities, and low productivity. Research shows links between high concentrations of Pb and generative dysfunction, kidney problems, digestive problems, and brain impairment (USEPA, 2005). Due to these health risks, it is of supreme importance that a precise understanding of the chemistry that takes place on Pb bullets is approved. Previously, the U.S Center for Disease Control and Prevention (CDC) decreased the allowable threshold level of blood Pb concentrations from 25 µg dL^{-1} to be 10 µg dL^{-1} (CDC, 1991). In 2012, the CDC suggested reducing the definition of elevated blood Pb in children from10 µg dL^{-1} to 5 µg dL^{-1}. There are nearly half a million U.S. children ages 1 to 5 with blood Pb levels above 5 µg dL^{-1} (CDC, 2012). The CDC (2012) identifies 5 µg dL^{-1} as the entrance for elevated blood Pb while stating that "no secure blood Pb level in children has been distinguished.".

Distribution and Accumulation of Pb in Shooting Range Soils

Studies in recent years have focused mainly on shooting ranges as one of the primary causes of Pb contamination due to the overloaded use of small-arms ammunition for shooting activities (Kelebemang et al., 2017; Sanderson et al., 2018; Sehube et al., 2017; Vandebroek et al., 2018). Through firing activities, Pb-based bullets and fragments accumulated in the stop butt of shooting ranges and generally remain near the surface of the soil for numerous years (Duggan and Dhawan, 2007). However, once a bullet has penetrated and precipitated into the soil, it could undergo a set of chemical and physical processes leading to enhances in the mobility and bioavailability of Pb in the soil. Over time, these transformations can increase concentrations of Pb in shooting range soils. Lead in shooting range soils is usually concentrated in the surface soil (0–10 cm) (Dermatas et al., 2006). Lead concentrations in the surface soils of shooting ranges soil have been reported up to 397840 mg kg^{-1} from intact spent bullets (Dermatas et al. 2006).

The distribution of Pb has been the main focus of studies describing shooting range soils (Table 2.1).

Recent studies quantified the amount of Pb contamination in the soils of shooting ranges. It is apparent in (Table 2.1) that no region avoided Pb contamination from ammunition. Lead concentration as high as 97,600 mg kg^{-1} was found in a shooting range soil in the South America (Clausen and Korte, 2009), 42,854 mg kg^{-1} in the USA (Perroy et al., 2014), 28,000 mg kg^{-1} in Finland) (Selonen and Setala, 2015), 24,484 mg kg^{-1} in the U.S. (Bannon et al., 2009), 12,167 mg kg^{-1} in Australia (Sanderson et al., 2014), 7,996 mg kg^{-1} in South Korea (Moon et al., 2013), and 4,600 mg kg^{-1} in Poland (Rauckyte et al., 2009). Lead accumulation in shooting range soils and the surrounding environment significantly increased in past years, approaching highs of 200,000 (Rooney and McLaren, 2000) and 300,000 mg kg^{-1} in rim soils of a shooting range (Van Bon and Boersema, 1988). The physicochemical properties of the soil such as the soil pH, organic matter, soil moisture, soil type, and CEC may significantly affect the accumulation and distribution of Pb in shooting range soils (Chen and Daroub, 2002). Considerable research also pointed out that the repetition of shooting practices affects the increase in concentration of Pb existing in shooting range soils (Sehube et al., 2017; Rooney and McLaren, 2000). Sehube et al. (2017) determined that 38,386 mg kg^{-1} of Pb occurred at a 22-year-old shooting range used by a military group for target practice, while another 30-year-old range showed only 6,163.15 mg kg^{-1}. They state that the age and type of shooting range can impact the concentration of Pb accumulated in the soil.

The transformation of metallic Pb through oxidation, carbonation and hydration processes to secondary Pb minerals has been confirmed, that be controlled by soil conditions such as moisture content, organic matter, cation-exchange capacity (CEC), and soil pH (Ma et al., 2007). The transformation of Pb was recorded that only 15.6% of metallic Pb had been transformed into secondary minerals in 25 years (Lin et al. 1995). It is important to note that to complete the transformation of metallic Pb to secondary minerals may take up to 300 years (Jorgensen and Willems, 1987). For example, Lin et al (1995), determined that only 15.6% of metallic Pb at their study site transformed into secondary minerals over 25 years. Thus, delayed transformation may be a possible cause of metal appearance over many years, which may lead to contaminated surface and groundwater through leaching and runoff. Additionally, the longer bullets settle on shooting range soils the longer the oxidation processes can act on the metallic Pb to transform them into secondary mineral forms such as cerussites ($PbCO_3$) and hydro cerussites ($PbCO_3H_2O$) (Sehube et al., 2017). Hydro cerussite ($Pb\ (CO_3)_2(OH)_2$) (Cao et al., 2003a, b; Chen et al., 2002), cerussite ($PbCO_3$), massicot, and litharge (PbO) (Dermatas et al. 2006b) were identified as the most common secondary mineral forms of Pb in shooting range soils. As previously mentioned, the distribution of Pb in shooting range soils is concentrated in the stop butt and usually settles close to the surface of the soil (Duggan and Dhawan, 2007); however, studies have also identified high concentrations of Pb in the subsurface in organic matter-rich soil (Dermatas et al., 2006).

There are three different sections of the shooting range soils: stop butt (berm), firing line/firing bay, and target line. The highest concentration of Pb is found at the berm, because bullets and shots fall and are absorbed by the berm after penetrating the targets (Cao et al., 2003; Sehube et al., 2017; Mariussen et al., 2018). There are several possibilities for the existence of Pb in the berm such as 1) Pb bullets staying relatively intact, 2) bullets fragmenting into small portions, 3) shells in the form of aggregate, and 4) shots transforming into oxidized forms of Pb such as Pb carbonate and oxide (Bannon et al., 2009).

Table 2.1 Studies from the past 10 years investigating Pb contaminated shooting range soils

Location and year of research	Number of years in operation	Total Pb concentration (mg kg^{-1})	Reference
USA (2009)	-	990–97,600	Clausen and Korte,2009
USA (2009)	16	340	Scheetz and Rimstidt,2009
Poland (2009)	-	640–4600	Rauckyte et al., 2009
USA (2009)	-	4549–24,484	Bannon et al., 2009
Australia (2012)	42–52	399–10,403	Sanderson et al., 2012
Switzerland (2012)	-	466-644	Evangelou et al., 2012
Czech Republic (2013)	40	4800	Ash et al., 2013
South Korea (2013)	-	7996	Moon et al., 2013
Australia (2013)	45–55	233–12,167	Sanderson et al., 2014
USA (2014)	34	42,854	Perroy et al., 2014
Australia (2015)	-	612–4697	Sanderson et al., 2015b
Finland (2015)	22–28	19,000–28,000	Selonen and Setala,2015
Spain (2016)	30	82.4–724.9	Rodríguez-Seijo et al.,2016
South Korea (2017)	30	3436	Islam and Park, 2017
Belgium (2018)	28	23.4–2167	Vandebroek et al., 2018

Weathering

Weathering is the process of bullets breaking down into soil due to being exposed to air and water. Weathering of bullets results in Pb-contaminated soils (McLaren et al., 2009). Together, weathering source materials and soil physical-chemistry explain the nature of contamination and its distribution. The weathering of bullets is associated with lifetime, physicochemical features of the stop butt material (pH, water content, clay and other mineral surfaces, and organic matter), erosion of bullets leading to firing speed, and climate (rainfall, temperature) (Johnson et al., 2005; Rooney et al., 2007). The existence of high levels of Pb secondary minerals such as carbonates in shooting range soils indicates weathering of consumed bullets. Lin et al. (1995) and Mellor and McCartney (1994) reported Pb concentrations of 3,400 to 5,000 $mgkg^{-1}$ in skeet shooting ranges of northern England and central Sweden. In most range soils, X-ray diffraction displays the presence of cerussite ($PbCO_3$) or hydro cerussite ($PbCO_3H_2O$). Average Pb oxidation and the resulting weathering products are changeable and site-specific. In acidic conditions, Pb is more soluble than at neutral or alkaline conditions. Phosphate, carbonate, and sulfide as anion ligands, are especially efficient Pb solubility due to the formation of less soluble Pb compounds, leading in low Pb concentrations in water. Chen et al. (2002) reported that the potential weathering operation of metallic Pb from bullets could be as follows:

$$Pb + O_2 + H_2O \leftrightarrow PbO + 2OH^- \text{ Oxidation of Pb to massicot} \quad (1)$$

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \text{ Formation of cerussite} \quad (2)$$

$$PbO + HCO_3^- \leftrightarrow PbCO_3 + OH^- \quad (3)$$

$$3PbCO_3 + 2H_2O \leftrightarrow Pb_3(CO_3)_2(OH)_2 + H_2CO_3 \text{ Formation of hydro cerussite} \quad (4)$$

$$PbCO_3 + 2H^+ \leftrightarrow Pb^{+2} + H_2O + CO_2 \quad \text{Dissolution of cerussite} \quad (5)$$

$Pb_3(CO_3)_2(OH)_2 + 6H^+ \leftrightarrow 3Pb^{+2} + 4H_2O + 2CO_2$ Dissolution of hydro cerussite (6)

During electrochemical corrosion processes of steel shells, a rust layer is created on the external shell of the bullet. The metallic Pb is easily oxidized (PbO) by O_2 (Eq. 1) and directly carbonated in the presence of CO_2 and H_2O into cerussite (Eq.3). The Pb carbonate crystals slowly form a layer, which can preserve the inner Pb from additional weathering and naturally minimize the weathering rate of Pb. However, pores may exist in this protective layer an expose the inner metallic layer, and this allows it to be attacked. Hence, slow but continuous corrosion can happen. Cerussite reactes with H_2O of wet soil and, consequently, hydro cerussite is formed on the Pb layer (Eq. 3 and Eq. 4) (Ma et al., 2007; Rooney et al., 2007). Cerussite and hydro cerussite are dissolved into the soil matrix (Eq. 5 and Eq. 6). The dissolution of these minerals relies upon soil conditions such as pH, which in turn, could be a significant factor in limiting the migration of Pb in soils (Eq. 5) (Astrup et al., 1999). Cao et al., (2003b) pointed out that weathering of Pb has a natural liming influence because Pb oxidation-dissolution causes elevation of pH (Eq. 5).

Soil pH

Soil pH is a dominant factor in determining soil weathering results (Ma et al., 2007; Rooney et al., 2007; Sehube et al., 2017). Lower pH enhances the rate of transformation of Pb bullets to secondary minerals during the dissolution (Ma et al., 2007). Afterward new metallic Pb surfaces are exposed for more weathering, where the transformation to hydro cerussite was accelerated at acidic pH while at an alkaline pH, and with controlled Pb oxides and carbonates (Ma et al., 2007). Ma et al. (2007) determined that in alkaline soil (8.5) the quantity of metallic Pb remaining in the soil after incubation of used Pb bullets was higher than for lower pH (4.5) treatments. Rooney et al. (2007) found that treatments at pH 4.5 and 5.5 had a lesser amount of crust material compared with treatments at pH 6.5 and 8. Acidic soils with high moisture content can dissolve the Pb-minerals formed on the surface of the Pb bullet, promoting the leaching of Pb into the subsoils (Sanderson et al., 2012). For instance, Sanderson et al. (2012) confirmed that approximately 7.5 and 46% of Pb transferred from surface soils into the subsoils at shooting ranges with acidic soils (pH 5.4–6.4) compared to only 6–18% in a shooting range with alkaline pH of 9.3.

The influence of pH on the mobilization of Pb in soil was detailed in Chen et al. (2002). Soil samples obtained at 300 m from the shooting range were found to be acidic with pH values of approximately 5.0, while soils sampled less than 36 m from shooting range berm which included high concentrations of Pb showed elevated pH values of around 6.7 (Chen et al., 2002). Stabilizing the Pb carbonates and Pb oxides in shooting range soils at alkaline pH results in higher concentrations of Pb absorbed into the soil, while acidic conditions enhance the dissolution and mobility of Pb in the soil delivering less Pb into the soil (Cao et al., 2003a). Dermatas et al. (2006) conducted a study concerning the immobilization and slow transformation of Pb pellets in alkaline shooting range soils. Similarly, Ma et al. (2007) examined the influence of a large-scale soil pH on the abrasion and transformation of metallic Pb. Following incubating a Florida soil sample at pH 4.8 for nine days with 5% metallic Pb powder, Ma et al (2007) observed that Pb rapidly converted into hydro cerussite. X-ray diffraction (XRD) of shooting range soils with pH reaching 8.5 illustrated the metallic Pb peaks of hydro cerussite, the slowed rate of weathering metallic Pb (Ma et al., 2007). The weathering and transformation of metallic Pb into secondary minerals reflects the crucial role of the surface area to volume ratio of metallic Pb bullet fragments.

Influence of soil organic matter

Soil organic matter is a significant factor in the mobility, solubility, and fate of Pb in shooting range soils. Organic matter influences the transformation of metallic Pb (Jorgensen and Willems, 1987; Ma et al., 2007). According to Dermatas et al. (2006) the weathering rate of Pb is higher in organic soils compared to soils with low organic C contents. Total extractable Pb in forest samples at a shooting range was high, intimating that the rate of weathering was significant due to the acidic conditions and organic matter (Duggan and Dhawan, 2007). The existence of organic matter could accelerate weathering of metals such as Pb in the soil due to the formation of soluble organo-Pb complexes (Cao et al., 2003b). Pb formed on the surface of metallic Pb may bind with humic acids and fulvic, leading to accelerating weathering by exposing new surfaces (Jorgensen and Willems, 1987) and thereby dominating the solubility, mobility, bioavailability, bioaccessibility, and toxicity of Pb in shooting range soils (Xiong et al., 2013). However, soil pH represents a crucial role in the binding capability of humic substances to Pb. Consequently, the availability of Pb^{+2} in shooting range soils is dominated by factors such as the type of organic and mineral structure of the Pb complexes, Pb saturation rate in the organic complex, the biodegradation of the Pb-organic complex, and the sorption of the Pb complex in the soil. Microbial organisms activate underlying mechanism by breaking down organic matter to CO_2 to support the conversion of metallic Pb to secondary minerals. Some soil factors such as iron (Fe) and Al (hydro) oxides, clay, and organic matter do not produce chemical compounds with Pb but present a large surface to sorb it. Therefore, Pb mobility and leachability in soils with high quantities of these components or high cation exchange capacity (CEC) tend to be low (Cao et al., 2003b). Soil organic matter serves a vital role in indexing soil quality and is a significant building block for the soil structure by comparing to other chemical characteristics of the soil.

Climate

Conversion of metallic Pb is influenced by climate such as temperature and rainwater. Throughout the year, high temperature and rainfall create a suitable Pb-transforming environment (Chen et al., 2002a). McLaren et al (2009) found that in increasing temperatures there was an increase in labile Pb in terms of exchangeable and carbonate fractions. Pb concentrations were 5–12% at low temperature (10°C) solution compared with solution at moderate heat (30°C), and the quantity of weathering crust remaining was much less. Higher soil moisture content stimulates weather; thus, soil moisture content has a significant influence on the transformation of Pb bullets into more reactive composites such as cerussite. Ma et al. (2007) conducted a study at field capacity where most metallic Pb converted to secondary minerals in a week compared to an incubation study with 80% field capacity at which significant quantities of metallic Pb stayed. McLaren et al. (2009) indicated that low moisture limits the production of possibly soluble corrosion products, potentially leading to the decreased existence of electrolyte for the elimination of metal by corrosion.

Solubility and Stability of Pb Minerals in Soils

Lead containing complexes and minerals with their equilibrium reactions are presented in Table 2-2. Lindsay (1979) showed that the dissociation constant in the following half-cell reaction is an explanation of why the metallic Pb (Pb°) is seldom found as a pure metal in nature.

$$Pb^{2+} + 2e^{-} \leftrightarrow Pb\ (c) \qquad\qquad \text{Log } K^{\circ} = -4.33$$

Table 2.2 Equilibrium reaction of various Pb minerals at 25° C (adapted from Lindsay, 1979)

No.	Equilibrium Reaction	Log K^o
1	PbO (yellow) + 2H+ ↔ Pb^{2+} + H_2O	12.89
2	PbO (red)+ 2 H^+ ↔ Pb^{2+} + H_2O	12.72
3	$Pb(OH)_2$ (C) + 2 H^+ ↔ Pb^{2+} + H_2O	8.16
4	Pb_3O_4 (C) + 8 H^+ + 2e- ↔ 3 Pb^{2+} + 4 H_2O	73.79
5	PbO_2 + $4H^+$ + 2^{e-} ↔ Pb^{+2} + $2H_2O$	49.68
6	$PbSO_4$(anglesite) + $2H^+$ ↔ Pb^{2+} SO_4^{-2}	-7.79
6	PbS (galena) ↔ Pb^{2+} + S^{2-} + H_2O	-27.51
6	$PbCO_3$ (cerussite) + $2H^+$ ↔Pb^{2+} + CO_2 (g) + H_2O	4.65
7	$Pb_3(CO_3)_2(OH)_2$ (hydro cerussite) + $6H^+$ ↔ $3Pb^{2+}$ + $2CO_2$ (g) + $4H_2O$	17.51
8	$Pb_5(PO_4)_3OH$ (c)(hydroxypyromorphite) + $7H^+$ ↔ $5Pb^{2+}$ + $3H_2PO_4^-$	-4.14
9	$Pb_5(PO_4)_3Cl$ (c)(chloropyromorphite) + $6H^+$ ↔ $5Pb^{2+}$ + $3H_2PO_4^-$ + Cl^-	-25.05
10	$Pb_5(PO_4)_3Br$ (c) (Bromo pyromorphite) + 6H+ ↔ $5Pb^{2+}$ + $3H_2PO_4^-$ + Br^-	-19.49
11	$Pb_5(PO_4)_3F$ (c) (Fluorpyromorphite) + $6H^+$ $5Pb^{2+}$ + $3H_2PO_4^-$ + Fl^-	-12.98
12	$PbAl_3(PO_4)_2(OH)_5H_2O$(c)(Plumbogummite)+$9H^+$ ↔ Pb^{2+} $2H_2PO_4^-$ +$3Al^{3+}$+ $6H_2O$	9.80†

† Calculated using data from Nrigu (1984) and Lindsay (1979).

Galena (PbS) is the most common Pb ore mineral under reduced condition, where sulfur is present. Measured by weight, PbS is 87 % Pb content. PbS is the only mineral that includes adequate quantities of Pb to provide an economic source of Pb. Additionally, PbS is easily transformed into other common forms of Pb. These forms will include the pyromorphites ($Pb_5(PO_4)_3$ X; X = Cl^-, F^- and OH^-), cerussite ($PbCO_3$), and anglesite ($PbSO_4$). For example, when exposed to the atmosphere or in any oxygen-rich water, pyromorphites, cerussite and anglesite occur upon oxidation from sulfide to sulfate in an oxidized environment.

Lead oxides, carbonates, and sulfates

Lead oxides are possibly the initial products released from the direct oxidation of metallic Pb via oxygen. Oxides are also possible primary products; they are not expected to persist for long times in the environment and should quickly respond with water to produce lead hydroxide Pb $(OH)_2$ or other chemical components. Pb hydroxides (Pb $(OH)_2$), basic Pb carbonates ($Pb_3(CO_3)_2(OH)$) and Pb carbonates (cerussite ($PbCO_3$)), are the most stable known forms of Pb. They are in an oxidized system and operate at high pH. Under low pH conditions, Pb also produces many mixed minerals, including carbonate, sulfate, oxide, and chloride. These minerals have moderate solubilities, and some have very low solubility to be significant in soils. For example, anglesite ($PbSO_4$) will have a lower solubility when compared to the hydroxides and carbonates of Pb under low pH conditions and higher sulfate levels. According to Lindsay, (1979) reported that the sulfate activity is equivalent to 10^{-3} M, since normal levels of sulfate ranges from 10^{-4} to 10^{-2} M for the most soils. At this sulfate activity, the solubility of Pb in equilibrium with Pb sulfate is such that the Pb activity would be well above the suggested upper limit of Pb in drinking waters (15 $\mu g\ L^{-1}$) as recommended by USEPA. This supports that Pb sulfate is more soluble to continue under reasonable levels of sulfate activity in soils. Hence, Pb is supposed to be in an extremely bioavailable form when pH is low.

Jorgensen and Willems (1987) found that changes in the pH, adsorption with clay minerals, organic complexes, Fe-Mn- and Al-hydrous oxides affect the solubility and stability of Pb and its associated minerals in soil environments. Cao et al. (2008) investigated phosphate-induced Pb immobilization from different Pb minerals in soils containing varying pH levels.

They determined that the three soils that were spiked with anglesite ($PbSO_4$), litharge (PbO), or cerussite ($PbCO_3$) were equilibrated with $Ca(H_2PO_4)_2.H_2O$ and KCI in a pH range from 3.0 to 7.0. Pb solubility followed PbO-soil > $PbCO_3$ > $PbSO_4$ at a pH range of 3.0 to 5.0. Cao et al. (2008) reported that at a pH 7.0 the reading was $PbSO_4$ > PbO > $PbCO_3$. While adsorption of Pb is significant in dominating Pb solubility in mildly-contaminated soils, precipitation of Pb minerals is essential in controlling Pb solubility in highly contaminated soils.

Lead phosphates

The Pb phosphates and, in particular, the pyromorphite minerals [$Pb_5(PO_4)_3(OH, Cl, F)$], are the most stable and extremely insoluble forms of Pb under a wide soil pH (Hettiarachchi, Pierzynski et al., 2004; Nriagu, 1974; and Lindsay, 1979). High concentrations of soluble inorganic P supported the development of pyromorphite, but there is also a significant concentration of Pb in the soil solution (Scheckel et al., 2013). Tang, et al. (2004) showed that pyromorphite complexes might not only be less soluble but also less bioavailable because of the measured parameters associated with the conditions of the human gastric system. Lindsay, (1979) reported that the pyromorphite species are more stable over a broader range of pH values than other Pb mineral forms. The typical Pb phosphorus reduction in solubility is expressed as the following:

$b(H_2PO_4)_2$ (c) > $PbHPO_4$ (c) > $Pb_4O(PO_4)_2$ (c) > $Pb_5(PO_4)_3F$ (c) > $Pb_5(PO_4)_3OH$ > $Pb_3(PO_4)_2$ (c) > $Pb_5(PO_4)_3Br$ (c) > $Pb_5(PO_4)_3Cl$ (c).

This comparison was constructed assuming 10^{-3} M Cl^-, 10^{-4} M F^-, and 10^{-5} M Br^- (Lindsay, 1979). Nriagu (1974) displayed the stability relationships of various Pb minerals at 25°C and 1 atm of pressure; he described the important field of chloropyromorphite stability under aerobic conditions. Moreover, Nriagu proved that the chloropyromorphites are stable not only in aerobic conditions but also in moderately lower environments of the conditions applied to determine the boundaries. For instance, when soil pH is approximately 9.0, the change of chloropyromorphite to galena happens at an Eh of about -270 mV.

Lead reactions with soil

Lead released into the soil from bullets undergoes many reactions with various soil components, altering their solubility, mobility, and availability to organisms. These reactions involve specific adsorption to the mineral matrix, ion exchange onto surface-active minerals (Naidu et al., 1996; Moreno et al., 2006), precipitation of sparingly soluble compounds (Altin et al., 1999), and complexation with soil organic matter and inorganic components (Sauvé et al., 1998). Soil constituents in this specific adsorption contain aluminum, iron, and manganese oxides, organic matter, and clay minerals (McBride, 1994; Moreno et al., 2006). The Pb typically is adsorbed onto clay and oxide surfaces and hydrolyzed in general environmental pH ranges (pH 3–9) (Thorbjornsen and Myers, 2007). Strawn and Sparks (2000) and Bradl (2004) studied the adsorption of Pb on soils and soil constituents. Adsorption or precipitation of Pb metal is possible under ideal conditions, which would remove dissolved ion from solution and cause it to be less bioavailable (Bradl, 2004; Hettiarachchi and Pierzynski, 2004). Strawn and Sparks (2000) pointed out that the divalent cation such as Pb^{+2} could be adsorbed on the clay surface via inner or outer-sphere complexation depending on ionic strength and pH. At low concentrations, specific adsorption to soil components predominates (Moreno et al., 2006) and an insignificant influence of pH is anticipated (Harter and Naidu, 2001; Naidu et al., 1994). At high concentrations, nonspecific adsorption, including ion exchange, could be controlled (Moreno et al., 2006).

Phosphates, chloride, and carbonate impact Pb reactions in soils either through precipitation of minerals with limited solubility or by decreasing adsorption during complexation (Rickard and Nriagu, 1978). Precipitation of Pb compounds such as carbonates and phosphates may control Pb bioavailability (Badawy et al., 2002). Lead could precipitate in soils if soluble concentrations passed about 4 mg L^{-1} at pH 4 and about 0.2 mg L^{-1} at pH 8 (Rickard and Nriagu, 1978). Additionally, Soil organic matter (SOM) is a significant constituent of heavy metal preservation in soils (Nriagu et al., 1978; Sauvé et al., 1997; Rooney et al., 2007). Organic matter in the soil is present as solid and dissolved phases. Organic matter in the solid phase contributes to metal adsorption and, in the dissolved phase, contributes to metal complexation (Rooney et al., 2007; Sauvé et al., 1997). Strawn and Sparks (2000) investigated the effects of SOM on sorption and desorption behavior by treating the soil with sodium hypochlorite to remove the SOM fraction.

Soil organisms such as plants and microbes may affect metals that can reach the soil. Kucharski et al. (2005) indicated that stabilization of metals may occur by adsorption, accumulation, and precipitation in the root zone. It is important to note that microbes affect the toxicity and mobility of trace elements in the soil through different mechanisms, including sorption and oxidation (Adriano et al., 2004). The adsorption of Pb by solid phases in the soil decreases its availability for chemical reactions and uptake by living organisms.

The separation of the chemical processes in solid phases and the soil solution controls the availability of Pb in the soil. Moreno et al. (2006) pointed out that the fundamental chemical methods influencing mobility and availability of Pb in soils are those associated with adsorption from the soil solution to the solid phase. Soil characteristics, such as texture pH, clay content, iron, organic matter, and manganese oxides, help modify those processes (Dayton et al., 2006).

Additionally, it is important to consider that the soil solution parameters are also effective, such as competing ions, ionic strength, pH, and loading rate (Naidu and Bolan, 2008). Bolan et al. (1999) indicated that several physical and chemical characteristics of soil are directly or indirectly managed via the nature and quantity of surface charge and the variation of surface charge with soil solution features. Bolan in his previous study also explained that permanent charges are generated through isomorphous substitution in the lattice structures of silicate minerals, and that variable charges are developed through the dissociation of functional groups, varying mostly with the pH of the soil.

Soils controlled via changeable charge constituents have developed negative surface charge and a high affinity for metals owing to the number of positions available for adsorption reactions under high pH (Dayton et al. 2006). At high pH, cationic trace element adsorption by oxide surfaces increases to almost 100% (Basta et al. 2001). Harter and Naidu, (2001) stressed that the reverse is true at low pH. Therefore, soil pH impacts metal hydrolysis, ion-pair formation organic matter solubility, as well as the surface charge of iron and aluminum oxides organic matter, and clay edges (Appel and Ma, 2002).

Lead in Plants

Lead contaminated lands produce a wide range of results toxic to living organisms in these areas. This metal harms plant maturity, grain germination, root elongation, seedling growth, transpiration, chlorophyll manufacture, and cell separation (Gupta et al., 2009, 2010; Maestri et al., 2010). However, the expanse of these influences differs and depends on the Pb concentration, the continuance of exposure, the strength of plant stress, the phase of plant growth, and the selective organs investigated. Some advanced, internal methods of responding to toxic metal exposures include inner detoxification mechanisms that transact with metal toxicity from metal uptake, excretion, complexation by specific ligands, and classification (Maestri et al., 2010; Gupta et al., 2009). Rooney et al. (1999) confirmed elevated Pb concentrations are photo available to various species of plants that reside in shooting range soils. Root uptake from soil is the main pathway to accumulate metals (Sharma and Dubey, 2005), where high levels of Pb concentrations are mostly absorbed in the roots of plant species such as *Festuca rubra* (Ginn et al., 2008), *Vigna unguiculata* (Kopittke et al., 2007), *Lactuca sativa* (Uzu et al., 2009), and *Funaria hygrometrica* (Krzesłowska et al., 2010) on shooting range soils (Rooney et al., 1999; Mellor and McCartney, 1994). Once Pb reaches the soil, it tends to accumulate on the surface layer, and the concentration drops down with soil depth. Pb is easily taken up by plants from the soil and accumulated in different organs, but significantly less Pb is transferred to the leaves due to precipitation of Pb in the cell walls of plant roots (Adriano, 2013). When Pb is adsorbed onto the surface of the rhizoderm root, Pb may negatively access the roots and keep track translocating water streams. The largest Pb concentrations can be identified in root tops, where root cells are young and have small cell walls that promote root uptake (Seregin et al. 2004). Furthermore, the top area is the area where rhizodermic pH is the lowest, which enhances the solubility of Pb in the soil solution.

Various pathways serve as Pb's entry into the roots through ionic channels. Wang et al. (2007) pointed out that even though Pb uptake is a non-selective occurrence it relies on the process of an H^+/ATPase pump to support a greater negative membrane potential in rhizoderm cells. Calcium acts to inhibit the absorption of Pb (Kim et al., 2002) and is linked with competing with these two cations for calcium channels (Huang and Cunningam, 1996). Authors such as Wang et al. (2007) show that the primary road Pb penetrates the roots through is Ca^{2+} membrane channels).

Preventing Pb from entering the food chain by reducing and absorbing Pb in vegetable parts is crucial. Yet, lowering uptake and transfer of Pb to elevated plant parts becomes a significant issue when plants are applied to remediate contaminated soils. Lead concentration in plant leaves could usually be approximately high, not only because of the uptake of Pb by plants but also because of contaminated soil particles that adhere to the plant surface or are embedded in the waxy outer layer of plants (Zimdahlet al.,1979). Arshad et al. (2008) showed that plants with a transfer agent higher than one are classified as hyperaccumulators and transfer agent less than one are considered non-accumulators of Pb. Soil remediation needs hyperaccumulators that can catch high Pb levels and transfer it to elevated plant parts with little to no toxicity. Measuring "transport agent" – the ratio of Pb concentration in the plant to the level of Pb in the soil – indicates how much Pb is transmitted from the soil to plants. The transfer agent will vary for each plant species and will evolve as soil physical and chemical properties are changed (Liu et al., 2010).

Hyperaccumulator plant species such as *Pelargonium* can transfer higher concentrations of Pb to elevated plant parts without acquiring a loss to their essential metabolic jobs (Arshad et al., 2008). Maestri et al. (2010) reported that, in a particular, hyperaccumulator species can accumulate more than 1000 mg kg^{-1} of Pb. Furthermore, hyperaccumulators can afford higher concentrations of Pb ions due to their different detoxification mechanisms, which can involve selective metal uptake, excretion, complexation by specific ligands, and classification. Transfer of Pb to elevated plant parts increases with existence of organic chelators such as ethylenediaminetetraacetate (EDTA) (Barrutia et al., 2010) or specific kinds of micro-organisms (Punamiya et al., 2010). Verbruggen et al. (2009) and Liao et al. (2006) reported that transferring metals from plant roots to shoots needs a support movement during the xylem and, when it happens, it is motivated by transpiration. In an X-ray mapping study conducted by Arias et al. (2010), mesquite plants showed high Pb deposition in xylem and phloem cells. After entering into the central cylinder of the stem, Pb can be transported through the apoplastic pathway; Pb then moved to leaf areas through vascular flow (Krzesłowska et al., 2010), whereas Pb can produce complexes with amino or organic acids in moving within the xylem (Maestri et al., 2010). Moreover, Pb can also be transported in inorganic structures such as cadmium. Some studies utilized a transferring agent (Pb in aerial parts/Pb in roots) to display the level of Pb translocation (Uzu et al., 2009; Liu et al., 2010). They pointed out that the numeric value is rather low when the transferring agent is applied, indicating that Pb was been isolated in the roots.

Soils contaminated with Pb produce obvious reductions in crop productivity, resulting in a severe issue for agriculture (Johnson and Eaton, 1980). It is vital to understand the mechanisms included in plant uptake, removal, and poisoning. The sustained use of Pb in many anthropogenic processes has increased Pb concentration to toxic levels in all environmental compartments.

Remediation of Soil Lead

Lead Remediation in Shooting Range Soils

Due to anthropogenic activities and the associated potential human and ecological risks, Pb contaminated soil needs remediation. Remediation treats the land so that it may be utilized again for a different objective or readopted for its current task with decreased environmental and health risks. Remediation may be needed, depending on ecological goals, treatment technologies accessible, and practical economic factors. Various therapeutic methods involve excavation and landfilling of shooting range soils/smelter/mine contaminated soil/waste material s, but lately less invasive and costly in situ solutions have been chosen to reduce metal bioavailability (Iskandar and Adriano, 1997). Trace elements such as Pb are difficult to destroy and break down compared to organic contaminants; thus, long-standing remediation strategies concentrate on replacing excavated contaminated soil with "clean" soil, or simply capping the contaminated area (Hettiarachchi et al., 2000). Further, these treatments are often ineffective at entirely removing Pb or reducing metal bioavailability, and are incapable of restoring soil productivity (Gradwohl, 1994). According to the U.S. EPA (1993), large-scale, traditional remediation of Pb contaminated areas, usually consisting of excavation, disposal, and replacement of the soil, can be costly to complete. Presently, the high cost of this common procedure, coupled with limited resources to reclaim contaminated sites, motivated researchers to explore alternative and less invasive measures (Kumpiene et al., 2008). Additionally, Adriano (1997) promoted economical in situ remediation procedures focus on decreasing metal bioavailability. Soil chemical stabilization has been observed as a cost-effective alternative procedure that renders metals less mobile and less available to receptor organisms and is also less disruptive to the environment (Kumpiene et al., 2008).

Phytoremediation

Phytoremediation is a management manner extensively employed in the recovery processes of contaminated soils due to its low technological and economic requirements; it applies plant species that use either naturally occurring or genetically engineered plants (Flathman and Lanza, 1998), to eliminate pollutants from different soils and promote their transition to immobilize contaminants or render them to less bioavailable forms (Mench et al., 2009; Vamerali et al., 2010). This management is a cost-reasonable alternative to the more accepted treatment methods used at seriously contaminated sites (Zhang et al., 2009). Plants should be able to endure site circumstances (e.g., low pH, high metal concentration, and salinity), have a fast growth rate, high biomass production, resistance to diseases and pests (Watanabe, 1997), and have relatively dense roots that serve as extra surface area for metal precipitation or adsorption (Berti et al., 1997). According to Pulford and Watson (2003), hyperaccumulators can accumulate 50 ~ 100 times more metals compared to other plants and ambient metals concentration. Natural metal hyperaccumulators should accumulate and tolerate higher metal concentrations in shoots than those typically located in non-accumulators without apparent traits. Additionally, hyperaccumulators ought to have a metal accumulation passing a threshold value of shoot metal concentration of 0.1% Pb of the dry weight shoot biomass (Baker and Brooks, 1989).

Phytoremediation technologies include various applications such as phytoextraction (Prasad and Freitas, 2003), phytovolatilization, phytodegradation, and phytostabilization (Long et al., 2002; Stoltz and Greger, 2002). Extensive studies reviewed several significant stages of this story plant-based technology (Salt et al., 1995, Meagher, 2000; McGrath et al., 2002; Prasad and Freitas, 2003; Ghosh and Singh, 2005).

Phytoextraction, also termed phytoaccumulation, is the process of uptake and translocation of metal contaminants from the soil by plant roots and accumulating contaminates in the shoots that are subsequently harvested to remove the pollutants from soils (Salt et al., 1995).

Phytovolatilization uses plants to uptake contaminants from the soil and convert them into volatile forms and transpire them into the atmosphere (USEPA, 2000).

Phytodegradation, also called phytotransformation, remediates organic compounds by breaking them down through plate produced enzymes such as dehalogenase and oxygenase that support catalyze degradation. This process usually involves inorganic pollutants, herbicides, insecticides, and chlorinated solvents (Pivetz, 2001).

Phytostabilization is the use of plants to stabilize trace element contaminated soil by reducing windblown dust, decreasing soil erosion, limiting the mobility and bioavailability in the soil.

Phytoremediation technologies are not sophisticated but may be beneficial for less contaminated areas, where extended treatment times could be essential to treat contaminants. Some concerns about phytoremediation technologies include improving the accumulation of metals by plants, promoting strategies to extract metals from plants, and controlling correlations between soil constituents and bioavailability. However, for shooting range soils, economical in situ remediation methods such as in-place inactivation through soil amendments and phytostabilization were chosen to minimize ecosystem health risk (Adriano, 1997). Among these strategies, phytostabilization is the most promising procedure to remediate Pb from contaminated shooting range soils. Kamal et al (2004) also mentions technique that couples fast screening of plant species that controls the tolerance and capacity to accumulate heavy metals with agronomic manners that improve shoot biomass production and develop metal bioavailability in the rhizosphere.

Phytostabilization

Phytostabilization, also known as in-place with in situ inactivation, uses certain plant species combined with in situ inactivation or chemical stabilization via the addition of soil amendments to immobilize contaminants in soil, through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone and physical stabilization of soils

(USEPA, 2000). Hence, contaminant mobility and bioavailability in the soil is limited by this application.

This process has been productive for remediating Cd, Cu, As, Zn and Cr, and is efficient when accelerated immobilization is required to maintain ground and surface waters (USEPA, 2000). Phytostabilization can support re-establishment of a vegetative cover at sites where native grasses are lacking due to high metal concentrations (Tordoff et al., 2000). Phytostabilization has advantages over other soil remediation practices because it is less costly, easier to achieve, and preferable aesthetically (Berti and Cunningham, 2000). When cleaning approaches are ineffective due to the range of the contaminated area or the lack of sufficient funding, phytostabilization is advantageous (Berti and Cunningham, 2000). Additionally, this procedure may provide the capability of plant roots to change soil conditions such as pH and soil moisture content by exudation (Susarla et al., 2002).

Properties of plants suitable for phytostabilization include tolerance to high concentrations of the contaminant(s) of concern; high production of root biomass able to immobilize these pollutants through uptake, precipitation, or reduction; and retention of relevant contaminants in roots to avoid transferring to shoots and avoid special handling and disposal of shoots. Yoon et al. (2006) assessed the potential of 17 species growing on a contaminated land and observed that plants with a high level of bio-concentration factor (BCF) – metal concentration ratio of plant roots to soil – and low translocation factor (TF) – metal the concentration ratio of plant shoots to roots – have the potential for phytostabilization.

Native plant species that can survive in that particular contaminated area are preferred for phytostabilization. The plants selected for phytostabilization include grasses or other rapid-growing plants that provide coverage with numerous shallow roots to stabilize Pb, access to soil water, and are simple to take care of once the growth is established. The most promising and wide-spread phytostabilization strategy combined use of plants and soil amendments in remediating Pb-contaminated shooting range soils.

In situ remediation

In situ remediation strategies include phytoextraction, solidification/stabilization, vitrification, electro kinetic remediation, phytostabilization, soil flushing, and chemical stabilization. Ultimately, the in-situ remediation procedure is dependent on three therapeutic approaches: isolation, extraction, and stabilization. Isolation technologies apply to those that are performed to decrease contaminant availability by decreasing the exposed surface area, minimizing the soil permeability, and/or decreasing the contaminant solubility. Extraction technologies apply to those that are used in situ to extract metals from a contaminated soil matrix through the practice of physical and chemical methods (Zhang et al., 2010). For the objectives of this dissertation, we will focus on chemical stabilization using biofuel crop growth (*Miscanthus*) assisted with soil amendments. To our understanding, the influence of biofuel crops on bioaccessibility of soil Pb has not been reported.

In situ stabilization

Chemical stabilization strategies involve those that include the usage of the chemical amendment(s) and plants to decrease the bioavailability of metals in contaminated soils. In situ stabilization, like other procedures such as in situ capping and in situ confinement, is cost-effective and less disruptive to the ecosystem compared to ex-situ processes (Smith et al., 1998). Chemical stabilization is less expensive than situ remediation method where amendments and organic materials are applied as amendments to contaminated soil to decrease solubility and stabilize toxic metal contaminants. Phosphate-based amendments, biosolids, composts, manures, alkaline materials and are extensively applied to immobilize Pb in contaminated soil (Brown et al., 2003, 2005, 2012; Haering et al., 2000). This method could mimic long-term remediation behavior through the formation of low solubility minerals or precipitates. The common mechanisms of stabilizing trace elements through in situ stabilization are primarily dependent on decreased trace element mobility and availability through enhanced precipitation, adsorption, complexation and cation exchange. The favorable outcome of chemical stabilization can only be estimated by assessing its capacity to decrease bioavailability or reduce the solubility of possibly toxic elements such as Pb, and, consequently, decrease human and ecological risk.

By selecting suitable plant species combined with appropriate soil amendments, it may be possible to stabilize Pb in the soil and decrease the interaction of metal with associated biota (Susarla et al., 2002).

Bioenergy Crops

Greenhouse gas concentrations are continually increasing due to the burning of nonrenewable fossil fuels; thus, there is an increasing demand for cleaner and renewable fuel sources. Biofuel crops have long been served as anther option to fossil fuels due to these crops burn cleaner and possibly can decrease greenhouse gas emissions (Wang, M et al., 2012). One of the greatest significant concerns surrounding biofuel production is the displacement of primary food crops by biofuel crops, which can lead to higher food costs in some cases and likely lower food security in the long-term (Ra et al., 2012). Van der Weijde et al., (2013) reported that if biofuel crops can provide high yields in marginal soils that are not currently utilized for food production, some barriers to growing biofuel plants can be avoided. Some plants should be capable of giving high yields with minimal inputs to decrease prices and to continue sustainable in the long-term.

Rhizomatous grasses have appeared among the highest candidates for biofuel production because of their high productivity and long-life extent, usually as long as 10-20 years (Jing et al., 2012). VanLoocke et al., (2012) reported that many of bioenergy species support considerable quantities of resources into belowground biomass (and transfer their nutrients to their rhizomes each winter, they favor to have large nutrient-use efficiencies, which decreases their demand for large fertilization (Heaton et al., 2008). Tonini et al., (2012) reported that there is no need to till soils each year with perennial rhizomatous grasses, which decreases soil erosion and nutrient leaching while concomitantly improving carbon sequestration in the soil. *Miscanthus* species has received remarkable attention as a qualified energy crop and is considered as one of the most favorable for phytoremediation (Pidlisnyuk et al., 2014b).

Miscanthus **as a second-generation biofuel crop**

The first-generation biofuels as defined by Eisentraut, 2010 is primarily used food crops to produce biofuel, met with concern because they directly displace food crops, and negatively affect food security. Second-generation biofuel crops as described by Rosillo-Calle et al., (2006) that are generally nonfood crops, or crop by-products, are preferable because they are not in direct conflict with food crops and may not directly affect food prices (Pidlisnyuk et al., 2014 b). Second-generation biofuels are generated from hemicellulose, cellulose, or lignin, i.e. biomass that can be obtained from natural ecosystems like forests, grassland, or aquatic ecosystems, or by cultivation of bioenergy crops (Naik et al., 2010).

Miscanthus genus is one of biofuel crops and non-food crop, woody, rhizomatous, C-4 grass that originates from tropical and subtropical areas of southeast Asia (Lewandowski et al., 2000; Chung and Kim, 2012). Most *Miscanthus* are commonly ranging from 1.5 to 4 m in height with 1to 2 cm stem diameter, but some species like *M. lutarioriparius and M. floridulus* can reach 6 to 7 m high (Yan et al., 2012). Pudelska, (2008) reported that *M. sacchariferous (M.x sacc)* reaches 1.5–3 m high, with silvery-white panicles (20–25 cm), and leaves that convert to a rust color, appending apparent interest in the fall season. Sun et al., (2010) explained that *M. lutarioriparius* and *M. floridulus's* leaves have a noticeable white midvein, with the size ranging from 20 to 100 cm long and 1 to 3 cm wide according to the species. Also, Sun et al., (2010) reported that flowers are mostly developed between July and September. The inflorescence composes of a fan-shaped crest built up of tall branches connected to a pivotal axis.

Miscanthus is considered an appropriate bioenergy resource for producing greater biomass per unit area, and can be used for thermal applications (e.g., heating of buildings) or transmutation into valuable products such as composites and fiberboard. Heaton et al. (2008) indicated that *Miscanthus* generated more than twice the biomass on less than half the predicted area requirements for both switchgrass and corn. *Miscanthus* straw is used as bedding for animal husbandry (Van Weyenberg et al., 2015; Daraban et al., 2015; Muthuraj et al., 2017). It can be used for auxiliary material in structural construction along with wood and concrete to construct ecological houses (Park et al.,2012). Visser et al., (2001) reported that other possible applications for *Miscanthus* insert as a composition construction and building material, for bioremediation of heavy metal contaminated areas, as feedstock for fermentation and as an ingredient of composting.

Miscanthus is a sterile rhizomatous perennial grass, which is able to grow in different climates from tropical to temperate regions and can adapt to a wide range of soil conditions including marginal lands with no fertilizer requirement. (Lewandowski et al., 2003). Lewandowski et al., (2000) indicated that in Europe, some of *Miscanthus species* such as *Miscanthus giganteus (M.x g), M. sinensis (M. sin),* and *M. sacc* grow well in the Mediterranean, warm, temperate areas that match optimal soil and water conditions. They also occur in high latitudes up to 57°N, but favorable productivity is restricted by the short growing season. Stewart et al., (2009) reported that some *Miscanthus* such as *M. sin* can grow on neutral or acid-sulfate soils (pH = 4 to 6) that often have relatively high Al levels.

Miscanthus in Contaminated Soil

Miscanthus grows well in mildly contaminated soil and on sites where soil quality is poor (Pidlisnyuk et al., 2014a; Schmidt et al., 2015). Following Pidlisnyuk et al.’s (2014a) extensive research on the growth of *Miscanthus* in contaminated soil, there is an ongoing effort to fill a knowledge gap to develop profitable *Miscanthus* for phytoremediation technology. Few previous studies have evaluated the potential of energy crops such as *Miscanthus* to grow in contaminated soils (Barbosa et al., 2015; Hartley et al., 2009: Jeżowski et al., 2017; Kocoń et al., 2017; Nsanganwimana et al., 2014; Pidlisnyuk et al., 2016,2018, 2014b; Pogrzeba et al., 2017). *Miscanthus* has been known as a good candidate for stabilizing heavy metals within soils. Peng et al. (2006) studied heavy metal uptake in *Miscanthus floridulus*, determining that the plant was metal-resistant because it was able to restrict metal uptake and prevent those minerals from moving up from the roots to the stem and leaves. In a study performed by Nsanganwimana et al. (2014) using a non-food crop (*M.xg*) to mitigate environmental and health risks caused by pollutants, and at restoring ecosystem services, He found that *Miscanthus* is a potential phytostabilization crop candidate. Subsequently, the cultivation of the sterile hybrid *Miscanthus* was proposed to be a sustainable management option to preserve local farmers' activities on Metaleurop-contaminated agricultural soil.

A study by Clifton-Brown et al. (2017) on the development of seed-based hybrids determines they can be used to establish *Miscanthus* in contaminated soil. They also provide a descriptive goal towards the establishment of *Miscanthus* in contaminated soil and reducing the cost of planting. A greenhouse study (Pidlisnyuk et al., 2018) conducted at a military site in Sliač, Slovakia explored potential metal uptake of *Miscanthus species* through two seasons; the results indicated that the accumulation of metals by stems and leaves was much lower than by roots. The arsnic, Zr, and Ti were not accumulated by stems and leaves during both periods and the accumulation of Cu, Fe, Mn, Zn, and Sr was not fundamental, determining that biomass of *Miscanthus* species possibly treated for the energy goal (Pidlisnyuk et al., 2018).

Previous research reported the presence of small quantities of Pb in *Miscanthus* stems and leaves grown in particularly metal-contaminated soil (Pidlisnyuk et al., 2014a; Pidlisnyuk et al., 2014b; Pidlisnyuk et al., 2016). Arsenic is also present in small quantities and was also not taken up through stems and leaves (Pidlisnyuk et al., 2016). However, both manganese and zinc were transported into stems and leaves in higher quantities (Pidlisnyuk et al., 2016; Kokon and Jurga, 2017). Unlike previous studies, Pogrzeba et al. (2017) reported the effect of microbial treatments that proportionately increase Pb and Zn concentrations in the leaves. The inclusion of fertilizer reduced the pH, directly affecting the concentration of Zn in the leaves (Pogrzeba et al., 2017; Nsanganwimana et al., 2015). Both Cu and cadmium were found in small quantities in stems and leaves of *Miscanthus* (Pidlisnyuk et al., 2014b; Nsanganwimanan et al., 2015).

In contrast, Nickel is taken up in larger quantities into the stems and leaves of *Miscanthus* compared to cobalt, which is not taken up by stems and leaves of *Miscanthus* (Pidlisnyuk et al., 2014b; Bosiacki, 2015). Nickel was tested at varying concentrations of 75, 150, and 600 mg/L added to two soils, which resulted in good growth of *M.xg* at 75 and 150 mg/L. The highest level of Nickel for *Miscanthus* was reported in the soil mixture with concentrations of more than 400 mg kg^{-1} dry soil. Another independent study also reported smaller uptake of Nickel by *Miscanthus* in the soil (Kocon and Jurga, 2017).

The presence of arbuscular mycorrhizal fungi (AMF) has a synergistic effect on the growth of *Miscanthus* in metal-contaminated soils leading to increased biomass yields (Firmin et al., 2015; Nsanganwimana et al., 2015). In saline soils, *Miscanthus Sinensis* helped salt extrusion and reduced salt concentrations in leaves, protecting photosynthesis. Electric conductivity of up to 2.5 S/m is beneficial for *Miscanthus* production. If the electric conductivity is more than 3 S/m, the inclusion of *M. Sinensis* and *M. Sacchariflorus* are recommended to enhance the yield of *Miscanthus*. Additionally, the bioconcentration factor of metals plays a role in plant biomass prediction for *Miscanthus*. This factor is smaller for Pb followed by Cu, cadmium, and Zn (Kocon and Jurga, 2017). These results are compatible with several other studies on uptake and *Miscanthus* production (Pidlisnyuk et al., 2014a).

Establishment of *Miscanthus*

Most of the challenges connected with *Miscanthus* occur from its sterility and associated propagation requirements. Recent practice is to excavate rhizomes from surviving plantations and replant them in new fields. Rhizomes should, therefore, be taken from the edges of the field, so the cultivated field is not affected. Pidlisnyuk et al. (2014a) pointed out that there is no need to disturb the soil when establishing *Miscanthus* because it is considered a perennial grass. Another way to cultivate *Miscanthus* is to use existing plants called “plugs,” produced in greenhouses. This approach may be beneficial, but some studies indicate that plug plants have a reduced survival rate because rhizomes system may be small in the first season (Boersma, 2011).

Miscanthus is a fast-growing crop that meets energy goals. To maximize agricultural production, the site must be cultivated properly. These locations are not intended to produce food crops. *Miscanthus* can be planted on a wide range of soils, thriving in everything from sandy soils to soils with high organic content. Demo et al (2013) indicated that, for energy crops to establish themselves after planting, a flat or slightly sloping area is desired. Land preparation is considered essential for excellent establishment, easy crop administration, and high biomass. *Miscanthus* establishment plantations are typically based on different planting materials. *Miscanthus* plantations are formed through propagation and established by rhizomes division or as a seedling from in vitro cultivation (Atkinson et al., 2009; Filová, 2015); however, the dominant method is propagating via rhizomes (Boersma and Heaton, 2014).

A study (Jeżowski et al., 2017) was conducted on three blocks of waste originating from Adamów, an open lignite mine near Turek. This study aimed to test growth potential of *Miscanthus* when used to restore open mining areas. In addition to the biomass in the contaminated environment, the study focused on the impact of organic and inorganic fertilization on crop growth. In areas where the establishment of *Miscanthus* was successful by a considerable percentage, approximately 99% of it survived the first winter of 2012-2013. Vital differences were visually observed between plant development and third-year biomass production. Jeżowski et al. (2017) recommended that because of the favorable biomass and inevitable results of *Miscanthus* on the degraded soil, long-term plantations are established on contaminated sites. Pyter et al. (2010) conducted field trials at the Landscape Horticulture Research Center at the University of Illinois at Urbana-Champaign to study *Miscanthus* growth in the U.S., determining optimal root size and appropriate depth of cultivation. This study was conducted using different rhizome masses at different depths on the same planting grid. Pyter et al. (2010) reported that rhizome survival across all treatments had a varying success rate over the following three years: 100% in 2005, 92% in 2006, and 75% in 2008. They suggested that in order to have a high amount of above-ground biomass production of *Miscanthus* in the establishment year rhizomes should be 60-75 g, plant depth should be 10 cm, and roots should be preserved in cold storage for as short of time as possible to maintain vitality. *Miscanthus* production from warm perennial grasses can take 3 to 5 years of the establishment before reaching full maturity, assuming appropriate cultivation and establishment success (Karp and Shield, 2008). A study assessing rhizome quality at different depths was conducted in Missouri, U.S. at two locations: the University of Missouri South Farm located near Columbia, and a long-term USDA-Agricultural Research Service study site near Centralia (Randall et al., 2016). Approximately 8 percent (8 of 96) rhizomes did not emerge on landscapes of *Miscanthus* establishment and winter kill at the Columbia site, compared to approximately 16% (14 of 90 rhizomes) that did not show across landscape positions at Centralia. The authors suggested that larger rhizomes should be propagated in order to have a good establishment outcome.

Tillage and no-tillage

Tillage requirements before planting rely on the soil type, condition of the field, previous crop and/or site use, and the accessibility and availability of planting tools. Tillage can positively or negatively change soil properties such as soil structure and porosity either and this, in turn, influences soil, air, and water relations. Tillage helps to keep perennial weeds under control. Various tillage practices include no-till, reduced-till, or methods with continual deep tillage. Tillage tools include a disk, a cultivator, a moldboard plow, and sometimes a harrow, depending on the goal of tillage.

Tilling a *Miscanthus* establishment the first year may improve soil structure, enhance nutrient and moisture retention, decrease wind and water erosion, and enhance drainage. Slight increases in SOC following *Miscanthus* establishment can create useful effects on soil quality

(Borjesson, 1999; Lal, 2009). *Miscanthus* crops only need soil tillage in the establishment year because soil erosion will be reduced when long periods occur without tilling. Rhizome systems of *Miscanthus* can recycle nutrients, which reduces nutrient input requirements. *Miscanthus* cultivation poses a significant challenge, due to the continuous long-term changes of various soil properties following the establishment of *Miscanthus* (Christian et al., 2006). For *Miscanthus*, tillage may be required when the soil is harsh and the planter is unable to put the rhizome at the required 5–10 cm depth, or when it's unable to completely cover the rhizome which makes it susceptible to drying out; facing these issues, plowing to a depth of 15 cm is desirable. During *Miscanthus* establishment, a no-till planting system would significantly minimize the risk of soil erosion. Soils under no-till have better retainment of diverse plant biomass on an undisturbed surface, which results in moist, low-temperature soil with efficient microbial activity. Better aggregate structure and considerable improvement in soil properties, particularly N content, SOM and SOC content, CEC (Cation exchange capacity), and a decreased C/N ratio have been observed in no-till soil (Madejon et al., 2009; Naudin et al., 2010; Derpsch et al., 2010; Moussa-Machraoui et al., 2010; Benitio, 2010; Celik et al., 2012; A'lvaro-Fuentes et al., 2012) compared to conventional tillage (CT) soils. The carbon accumulation that occurs from no-till is enhanced within microaggregates which, in turn, forms macroaggregates. The shift of soil organic carbon within microaggregates is beneficial for long-term carbon storage in soil (Erkossa, 2011). This is how no-till farming reduces the soil bulk density in the upper soil layer (He et al., 2011).

Water and Rainfall

Water availability is one of the most valuable factors for cultivating bioenergy crops. Drought harms *Miscanthus* crop, resulting in lower yield and raised mortality (Heaton et al., 2004). As a C_4 grass, *Miscanthus* can obtain more productive use of water than C_3 species

(Lewandowski and Schmidt, 2006). Improvements in productivity led to an increase in water demand. For instance, *Miscanthus* is capable of using high amounts of water, up to 900 mm/year, to gain greatest yields (Heaton et at., 2004). This indicated that *Miscanthus* productivity increased with increased precipitation. Yield production is associated with seasonal precipitation and can decrease under water-stressed conditions. *Miscanthus* can be planted in areas with total annual precipitation ranging from 600-1500 mm (Price et al., 2004). *Miscanthus* is characterized by its deep roots that can penetrate the soil and extract water to a depth of more than 2m. Additionally, *Miscanthus* has good water-use efficiency when estimating the amount of water needed per unit of yield.

Soil Quality

Soil is a significant factor in the productivity of *Miscanthus*. *Miscanthus* is acclimatized to a wide range of soil conditions from sands to high organic matter soils. A study by Heaton et al. (2010), determined that the biomass yield of *Miscanthus* is limited in shallow, dry, and waterlogged soils which are known to reduced plant height. *Miscanthus* grows well and produces the best biomass in the site that has well-drained soil. Miscanthus is tolerant of a wide range of soil pH (5.5 and 7.5) with medium to high fertility (Caslin et al., 2010; Heaton et al., 2011). Caslin (2010) also mentioned that *Miscanthus* growth is weaker on soil with a pH higher than 8. Through previous experiments and studies, we conclude that *Miscanthus* works well on a range of soil conditions when water is not limited. If the water is limited, *Miscanthus* can achieve a good growth when planted in clay soils, and lousy growth when planted in sandy soils, perhaps due to the higher water holding capacity of clay soils. In a study by Jones and Walsh (2013), they found that an excellent soil aggregate structure –as shown by pore volume, size distribution – is more important than the soil type or soil pH.

Rhizome Density and Spacing

Rhizome systems of *Miscanthus* can recycle the nutrients, reducing the nutrient inputs for their productions. To achieve optimal yields from year three onwards and effective weed inhibition through competition, rhizome planting density in Europe is one to four rhizomes per m^2 (10,000–40,000 rhizomes /ha) but may vary from site to site (Lewandowski et al., 2000). Knowing that higher planting densities did not justify additional planting costs, the value of the establishment could be lessened by successfully reducing cultivation density. So, a density of one plant per square meter is recommended. The optimal number of rhizomes depends on several environmental conditions – soil moisture and anticipated precipitation – and yield aims. Christian and Haase (2001), mentioned that high-density planting helps the crop by developing competition for resources with weeds and gaining high yields faster than when using low planting densities.

Equal spacing around the plant allows better growth with success than planting at a higher rate within the row as is done with many annual crops (Vogel and Masters, 2001). This spacing will benefit weed control and also allow *Miscanthus* to grow in the spaces between the rows and become capable of inhibiting soil erosion. Heaton et al. (2011) indicated that the recommendation in the Midwest is to plant using 30-inch rows with 30-inch spacing between plants. Closer rows result in earlier canopy narrowing in the second year, which will increase yield and reduce weed pressure.

Planting Date

Planting too early can cause harm due to increased weed competition and planting too late can also have detrimental effects to stand success in drier years. The best planting time for rhizomes is from March to April, due to early planting allows to get the benefits of soil moisture and extends the first season of growth (DEFRA, 2007; Heaton et al., 2011). This gives a better chance for larger rhizomes to grow strong and lets the crop avoid drought and frost better. Planting through May and early June is accepted, but soil moisture may be lower at these dates (Pyter et al., 2009).

Organic Phosphorus as Biosolids and Inorganic Phosphorus

According to Vadas et al. (2008), one major concern of using plant biomass as a source of energy is that bioenergy crops will displace food crops in farmers' fields. Contaminated land is not suitable for food production because the food may have heavy metal toxicities (Raut and Islam, 2010). Heavy metal toxicities in soil lead to reduced agricultural yields and make producing crops on this land unprofitable. Therefore, as long as the highest-quality land is reserved for food production and bioenergy crops are grown on contaminated lands this will not be a problem.

Contaminated soil quality can be bettered through the use of soil amendments. The majority of commercially available fertilizers only address the issue of poor nutrient quality. These fertilizers typically provide an over-application of phosphorus (P) which can be detrimental to aquatic ecosystems (Costello, 2009). In this study, two different products were used, one municipal wastewater product (organic Biosolids) and the other one inorganic as triple superphosphate (TSP).

Biosolids are a nutrient-rich, organic material that come from the treatment of sewage sludge (US EPA, 2012). Municipalities face an increasing amount of wastewater as urban populations in the U.S. increase, which, in turn, means there are more biosolids for disposal. The three options for disposal include incineration, burial in a landfill, or application as fertilizer. Currently, only 50% of biosolids are used for beneficial purposes such as application as fertilizer

(US EPA, 2012). The EPA supports the safe recycling of biosolids, meaning that they meet the pollutant content and treatment criteria to be used as fertilizer for crops or to reclaim abandoned mine lands (US EPA, 2009).

Using organic P (biosolids) as amendments to support fertilizing energy crops can help crop nutrition and improve soil quality because of the increased organic matter content. This leads to higher capacity for water retention, improved soil structure, and better soil infiltration rates. Energy crops induce long-term phytoremediation and can speed up the breakdown of organic matter in topsoil. In Ireland, organic by-products and distillery effluents were applied to *Miscanthus* plots to investigate the potential to dispose of these effluents while improving soil quality (Galbally et al., 2012). Kilpatrick (2012) reported the benefits of using biosolids from wastewater treatment to increase *Miscanthus* productivity on marginal (low pH and low nutrient) land in southern Ohio, U.S.

Inorganic phosphorus

Phosphorus is one of the most restrictive nutrients on agricultural lands. To better understand the most efficient quantities of P fertilizer need for giant *Miscanthus* crops, researchers studied the uptake and remobilization of P in above- and below-ground biomass (Andersohn, 1996; Beale and Long, 1997; Heaton et al., 2009; Strullu et al., 2011). The best time to apply P to giant *Miscanthus* is prior to planting or during planting when P can be applied by banding and be incorporated during planting. According to Jones and Walsh (2013), giant *Miscanthus* can survive for a minimum of 10 to 15 years. Appropriate P fertilizer application and incorporation or banding before crop establishment may be important, particularly in low P-testing soils, because the incorporation of P after planting is not practical as P would be immobile in soil. Propheter and Staggenborg (2010) reported that the annual amount of P removed by a giant *Miscanthus* ranged from 2 to 5 kg P ha-1 yr-1, which was similar to other perennial grasses including switchgrass and big bluestem (*Andropogon gerardii Vitman*) but less than the removal from annual crops such as corn and sorghum which ranged from 20 to 54 kg P ha-1. Studies monitoring giant *Miscanthus* nutrient cycling found that nutrient concentrations reach their peak at the start of the growing season then become diluted as above-ground biomass increases, and then continue to decline as the plant senesces (Beale and Long, 1997; Himken et al., 1997). According to Himken et al. (1997), 36-50% of P in leaves and stems can be remobilized to the rhizomes, and through this remobilization process, the giant *Miscanthus* can store their nutrients for dormancy and recycle the stored nutrients to the growing plant tissue during the next growing season. For *Miscanthus* to be a sustainable bioenergy crop, there must be an understanding of the effects of the soil amendments and how to use them to develop the best management practices.

Stabilizing Pb using Phosphate in shooting range soil

Researchers have extensively investigated the use of P additions in Pb-contaminated soils as a potential means of in situ remediation by immobilizing Pb through the formation of less soluble pyromorphite ($Pb_5(PO_4)_3X$, X= OH^-, Cl^-, F^-) (Hettiarachchi et al., 2000; 2004; Cao et al., 2003a; Lin et al., 2005; Bolan et al., 2003; Yoon et al., 2007). The stability of pyromorphite under a wide range of environmental conditions render Pb immobilization via P additions a successful mechanism since accidental pyromorphite ingestion will not lead to bioavailable Pb (Arnich et al., 2003). Common P amendments used to stabilize soil Pb are phosphate rock (Brown et al., 2005; Cao et al., 2004), phosphoric acid/hydroxyapatite (HA) ($Pb_5(PO_4)_3OH$) (Hettiarachchi et al., 2000; Cao et al., 2008), and biosolids (Brown et al., 2003, 2005, 2012). With adequate quantities of P, Pb can be immobilized as (HA) or chloropyromorphite

(CAP)($Pb_5(PO_4)_3Cl$) (Nriagu, 1974; Islam et al., 2010). Low pH conditions may contribute to rising metal and phosphate solubility. For example, an increase in pH from 5 to 8 results in a decrease of HA solubility of two orders of magnitude (Shi and Erickson, 2001). According to Zhang and Ryan (1998, 1999) the transformation of anglesite, cerussite, and PbS to CAP or HA happened between pH 4 and 5, which also optimizes the rate of HA dissolution. Mignardi et al (2012) states that HA and FAP (phosphate rock) may significantly decrease Pb availability in terms of water solubility in contaminated soils while reducing soil acidification and possible risk of eutrophication related to the treatment of highly soluble phosphate sources.

Organic phosphate sources

Soil organic matter has been proven to have a positive effect in many sustainability aspects as well as in ecosystem processes and the remediation of heavy metal contaminated soils (Clemente et al., 2005). Organic matter may increase soil P availability through decomposition and mineralization of contained organic-P, or by abiotic activities such as ligand-exchange. Poor soil properties such as low organic C, P, N, and low/neutral pH are characteristics of mine tailings. High CEC is found in organic matter and can adsorb metals through pH-dependent exchange sites, which form strong complexes with Pb, Cu, or Zn contaminated soils. Several organic amendments that include an elevated concentration of P or Fe or have a high pH can lead to immobilizing soil metals. Various organic materials – biosolids, composts, manures – are commonly used to improve soil properties such as water holding capacity, enhanced water infiltration, increased nutrient supply, improved aggregation, proper aeration, and higher microbial activity. According to Sauve et al. (2003) and Baker et al. (2009), when organic C is consumed by microbial communities, large amounts of organic amendment needs to be completed over a certain time period, establishing a new equilibrium where contaminants are retained and the functions of the ecosystem can be restored to normal. Additionally, Sauve et al (2003) demonstrated that heavy metals can adsorb organic materials that are 30 times higher than clay minerals. The stability complex has the potential to influence the bioavailability and extraction of heavy metals by plants. They may also affect acidity and the redox properties of the soils (Walker et al., 2003). In recent years, attempts to remediate heavy metal contaminated soils have been done using biosolid applications.

Biosolids

Biosolids are frequently utilized in agricultural land applications, horticulture, land and mine site reclamation. Providing additional OM, N, P, and Fe, biosolids play a key role in metal immobilization through sorption and complexation of the metal fractions associated with OM, metal oxides, or carbonates (Walker et al., 2003). Biosolids are an effective alternative source of P due to the low cost and ability to reduce soil Pb hazards at several industrial sites and residential communities associated with smelter. Some locations include a Pb smelter in Joplin, MO (Brown et al., 2004), a Pb smelter and mine pile in Katowice, Silesia, Poland (Stuczynski et al., 2000), and multiple Superfund sites in the U.S. (Hettiarachchi and Pierzynski, 2004; Brown et al., 2003, 2005, 2012). Application of biosolid amendments has been successful in former smelter sites (Brown et al., 2003; Stuczynski et al., 2007) and urban gardens (Farfel et al., 2005; Attanayake et al., 2014). Brown et al. (2003) indicated that the addition of biosolids reduced in vitro Pb bioaccessibility in contaminated soil by 43%.

Struvite

In addition to traditional organic amendments applied for nutrient cycling, Struvite – a crystalline phosphate mineral that frequently accumulates on equipment surfaces during anaerobic digestion and post-digestion processes in the wastewater industry – is a useful alternative for phosphorus (P) and nitrogen (N) recovery in wastewater, which can then be used as P and N fertilizers in agricultural soils (Huang et al., 2015). The solubility of struvite is low in water; however, Naidu et al. (2012) reported that struvite dissolves slowly over time in soil environments. Struvite can supply three nutrients essential to plant growth, as shown by the chemical formula $MgNH_4PO_4 \bullet 6H_2O$; this can form through precipitation in wastewater as well as anion exchange sorbent eluant usually conducted by the addition of high concentrations of $MgCl_2$ and then raising the pH using a base (e.g. NaOH or KOH). Another method is allowing the pH to increase naturally through the addition of Mg in the form of MgO or $Mg\ (OH)_2$. When ground and blended into soils, the fertilizer performed similarly to conventional acidulated fertilizer products (Ackerman et al., 2013; Antonini et al., 2012; Degryse et al., 2017; Le Corre et al., 2009; Sengupta and Pandit, 2011). However, performance can be compromised if applied as granules like a dry fertilizer in agronomic situations (Ackerman et al., 2013; Degryse et al., 2017, Everaert et al., 2017, Talboys et al., 2016).

Inorganic phosphate sources

The chemical stabilization of Pb in the soil is influenced by many factors which control metal solubility. The most stable form for environmental Pb comes from Pb phosphates under many conditions (Nriagu, 1974, Ruby et al., 1994). In alkaline environments, Pb precipitates into many different forms including phosphates, hydroxides, and carbonates. Therefore, Pb solubility decreases at a high pH. Common P amendments that stabilize Pb in the soil are phosphate rock (Cao et al., 2004; Ma et al., 1993; Brown et al., 2005), phosphoric acid/hydroxyapatite (Cao et al., 2009; Hettiarachchi et al., 2001), and biosolids (Brown et al., 2003, 2005, 2012).

Different P sources were examined and compared. Ma and Rao (1999) conducted a study that checked the ability to use Florida PR to immobilize Pb from 13 Pb-contaminated soils.

These mining and smelting soils from a wide cross-section of the U.S. contained Pb arsenate. About 4 g of each soil with 0, 0.5, or 1 g of PR in 40-mL centrifuge tubes containing 25 mL of deionized water were reacted with one another. The suspension of samples underwent shaking for 2 to 96 hours. The concentrations of the Pb in the soil ranged from 198 to 40,100 mg kg^{-1} and the pH ranged from 5.53 to 8.59. Adding PR significantly increased Pb immobilization. For instance, immobilization of Pb ranged from 22-94% with 0.5g of PR, but the addition of 1 extra gram of PR the immobilization increased from 33-100% (Ma and Rao, 1999).

Triple superphosphate (TSP) acts as a soluble form of P. TSP is made by reacting PR with orthophosphoric acid (Hettiarachchi et al., 2001) and is commonly used as a fertilizer in agricultural practices. The main constituent is monocalcium orthophosphate [$Ca(H_2PO_4)_2 \cdot H_2O$]. Hettiarachchi et al (2000) discovered that P added as TSP or even PR to Pb-contaminated soils could decrease bioaccessibility of Pb in PBET extractions. The reduction of the bioaccessibility ranged from 15-41%. These soils had limited bioaccessibility that ranged from 18.3-36.6%. Several P treatments reduced Pb bioaccessibility in a study by Brown et al (2004). Brown et al. (2004) measured the reduction through the in vitro PBET method and by tall fescue Pb uptake. Adding 1% P as rock phosphate showed a reduction in vitro Pb; it was not significantly different from the control which was 0% P. Adding 1% P as TSP did not reduce in vitro Pb or Pb uptake by tall fescue. The highest dose of TSP at 3.2% was the only treatment that seemed effective. Most investigations, however, noted little variation in Pb bioaccessibility over time (Hettiarachchi et al., 2000). For instance, in an incubation study conducted by Hettiarachchi et al. (2001), bioaccessibility was reduced between 0 and 3 days after the treatment and no further reductions were observed after one year. In four out of five soils, rock phosphate was equally or more effective than TSP or phosphoric acid (PA) at reducing Pb bioaccessibility. A significant reduction in bioaccessible Pb happened when increasing the amount of P from 2500 mg kg^{-1} to 5000 mg kg^{-1}. Overall, a 25–38% reduction in bioaccessibility was observed in all five of the soils that were tested (Hettiarachchi et al., 2001).

Lead apatites have been heavily researched over the past 25 years, due to development of new reclamation procedures on in situ immobilization of Pb in soils, sediments, and waste (Laperche et al., 1997). The apatite group of minerals includes pyromorphite $Pb_5(PO_4)_3Cl$, hydroxylpyromorphite $Pb_5(PO_4)_3OH$, fluorpyromorphite $Pb_5(PO_4)_3F$, brompyromorphite $Pb_5(PO_4)_3Br$ (Pan and Fleet, 2002; Pasero et al., 2010). Ma et al. (1995) demonstrated that phosphate rock PR, mostly fluorapatite (Ca_{10} (PO_4) $6F_2$), significantly immobilized Pb from aqueous solutions, with Pb immobilization ranging from 39-100%. Pb immobilization's primary mechanism is dissolution of PR followed by precipitation of fluorpyromorphite-like mineral (Pb_{10} $(PO_4)_6F_2$); precipitation of Pb as hydro cerussite also occurred in some situations. Pyromorphite-like materials formed from ionic exchanges involving phosphate amendments and soil Pb. These materials were observed to have low solubility and bioaccessibility (Hettiarachchi et al., 2001; Scheckel and Ryan, 2004).

Bioavailability assessment of Pb

Previously, total elemental concentrations were used to determine environmental risks posed by toxic elements. However, scientific advances show that bioaccessibility tests are a more accurate predictor of environmental risk. The elemental concentrations overestimate the risks of human exposure by assuming 100% bioavailability (Bruce et al., 2007). Following removal from the soil under physiological conditions, soil bioavailability defines the amount of a chemical or compound absorbed into an organism's circulatory system. The bioaccessible fraction refers to the total fraction released from the soil matrix that is available for gastrointestinal absorption.

Humans are not used as test subjects due to ethical concerns. The bioavailability of Pb is observed through other animals such as rats, juvenile swine, and monkeys (Schoof et al., 1995; Casteel et al., 1997; Roberts et al., 2002; Hettiarachchi et al., 2003; Juhasz et al., 2007). A strong correlation (r^2=0.92) between an in vivo study and an in vitro analysis following a simplified physiological based extraction test was observed.

Swine models are time-consuming, costly studies that require elaborate set-ups and strict ethical guidelines. According to Juhasz et al. (2013), these tests measure contaminant concentrations that are solubilized to mimic human gastrointestinal (GI) extraction and the potential for absorption into the systemic circulation. Over the last decade, the development and success of in vitro methods have been used as a guideline for determining the relative bioavailability of contaminants in soils. Ruby et al (1996) advanced an in vitro method evaluating the Pb solubility from different mine wastes. Pb concentrations observed in soil materials – mine composites, tailings, former Pb – ranged from 1,388 to 10, 230 mg kg^{-1}. Hettiarachchi and Pierzynski (2004) indicated that soil Pb bioavailability depends on the solubility of Pb solid phases and other site-specific soil chemistry. Soil pH, organic matter, CEC, and mineralogy of the soil affect the retention of metals on the solid phase of the soil (Takac et al., 2009). Soil pH can affect solubility and soil reactions, making it critical in determining bioavailability (Muhlbachova et al., 2005). It is important to consider factors that affect the bioavailability of Pb through soil reactions such as complexation, sorption/desorption, and redox reactions (Hernandez-Soriano and Jimenez-Lopez, 2012).

Although many studies utilized soils with a history of mining and smelting, bioaccessibility tests are not restricted to these soils. To evaluate Pb bioaccessibility, a physiological-based in vitro method developed by Oomen et al. (2002) was used on soils contaminated with pottery flakes containing varying Pb concentrations (50 to 2400 mg kg^{-1}). In a pottery flake, the bioaccessibility of Pb was less (0.3 ± 0.2%) than Pb in soil not containing pottery flakes (42 to 66% at the same site, and 28 to 73% at other sites in the same town) (Oomen et al., 2002). This demonstrates that the bioavailability of Pb is not related to the total concentration but instead is affected by the portioning in the soil medium. Fayiga and Saha (2016) reported that the bioavailability of Pb in shooting ranges is higher than other metal-contaminated soils because of its very low residual Pb (<1%).

Use of X-Ray Absorption Spectroscopy on Pb Contaminated Soil

To further understand the mechanisms of sorption in soils, researchers explored X-ray Absorption Spectroscopy (XAS) (Kelly et al., 2008; Scheidegger and Sparks, 1996). The primary techniques include X-ray Absorption Near Edge Structure (XANES) and Extended X-ray Absorption Fine Structure (EXAFS). X-ray Absorption or X-ray Absorption Fine Structure (XAFS) Spectroscopy gives a pictorial view of interatomic distances between the sorbed ions and displays the central atom in the complex. The information analyzed by XAFS helps conclude the coordination number of central atoms present in the complex and the oxidation state of the bound metal ion.

The X-ray absorption or fluorescence of a given sample is measured as a function of the wavelength to obtain the XAFS spectra (Manceau at al., 1996). The spectral scan is an element-specific, bulk-sensitive, and nondestructive method when performed in the vicinity of an X-ray absorption edge of a chosen target element; however, it is difficult to determine the chemical form of trace metals (Manceau et al., 1996) and conduct EXAFS studies for soils with low elemental concentrations (Manceau et al., 2000; Scheckel et al., 2005). This structural technique has the required sensitivity, specificity, and detection limit equal to ~ 100 mg kg^{-1} (Manceau et al., 2000).

Scheckel and Ryan (2004) conducted XAFS studies on a residential soil adjacent to a lead smelter amended with various P sources. In 32 months of residency, pyromorphite concentration varied from 0% in control soil to 45% for a 1% phosphoric acid amendment (Scheckel and Ryan, 2004). Pb mainly existed as a Pb-sulfur species (53%). Detecting Pb bearing minerals and gaining insight into its phases provides toxicity recommendations with more credibility. Developing an understanding of the speciation of this element is critical for relating the effectiveness of the treatments in shooting range soils to their bioavailability potential. Wet chemical analysis and plant uptake data are essential in explaining mechanisms and trends because of inherent problems associated with Pb XAFS.

Soil Health

Soil health is crucial for sustainable food production and key to feeding the increasing human population. Soil health is defined as the continued potential of soil to function as a living system within natural ecosystem boundaries; it is necessary to sustain biological productivity, maintain water quality, enhance air quality, and support the health and habitation of the human race (Shukla et al., 2006; Karlen et al., 2008). Assessing soil health is challenging because there are no minimum data sets. Currently, evaluations are made from management-induced differences in soil characteristics (Bhardwaj et al., 2011). Soil health assessment research focused on the chemical and physical properties since only simple methods of analysis were available (Carter et al., 1997; Bhardwaj et al., 2011). Research on soil biological measurements was difficult to interpret because of the sensitivity of soil to dynamic daily changes in the soil environment (Pankhurst et al., 1997; Bhardwaj et al., 2011). Recent studies demonstrated the need to include soil biology as an indicator of soil health; soil biology plays an important role in soil aggregation, structural development, and nutrient cycling (Bhardwaj et al., 2011; Biswas et al., 2014). Currently, enzyme activity, fatty acid analysis, organic carbon, microbial biomass, potentially mineralizable nitrogen, and soil respiration are all researchable health indicators of soil (Kennedy and Papendick, 1995). According to Pankhurst et al. (1997), Karlen et al. (1997), and Bhardwaj et al. (2011), soil function is associated with recommended soil health indicators.

Soil enzymes

Soil enzyme activities are essential for knowing how alterations in microbial communities affect soil processes. Enzyme activities are significant indicators of soil microbial activity. Four enzymes were examined in this research: acid and alkaline phosphatase and arylsulfatase and Beta-D-glucosidase. These enzymes are produced mostly by microorganisms, plant roots, and soil fauna, and are important in soil nutrient and carbon cycling (Aon and Colaneri, 2001; Acosta-Martinez et al., 2007).

Organic amendments improve overall enzyme activity (Moeskops et al., 2010), but activities of particular enzymes may alter depending on the form of the treatments and the relative availability of nutrients, soil type, soil features such as texture and pH (Stursová and Baldrian, 2010). Soil enzyme activities could improve the availability of the most limiting nutrients in order to meet microbial metabolic requirements (Allison et al., 2011).

Phosphatase is a wide group of enzymes that can stimulate the hydrolysis of esters and anhydrides of phosphoric acid (Schmidt and Lawoski 1961). Phosphatases are thought to play significant tasks in P cycles in soil ecosystems as indication confirms that they are correlated to P stress and plant growth (Speir and Ross, 1978). Aon and Colaneri (2001) found that the activity of acid and alkaline phosphatases correlates with organic matter. In addition to working as helpful indicators of soil productivity, phosphatase performs essential roles in the soil system (Dick et al., 2000). Acid phosphatases increased with high P stress (Li et al., 2002). For instance, when there is a signal showing P insufficiency in the soil acid phosphatase excretion from plant roots is increased to improve the solubilization and remobilization of phosphate, affecting the capability of the plant to deal with P-stressed conditions (Kai et al., 2002; Karthikeyan et al., 2002).

Alkaline phosphatase breaks down organic P compounds into organic carbon and mineralized P. Phosphate is an important plant nutrient that limits nutrients for microbial growth in soil; the enzyme is an important indicator of soil ecological health (Acosta-Martinez et al., 2000). The activity of alkaline enzymes relies, in part, on the organic-P levels of the soil. Higher soil pH indicates the presence of alkaline phosphatase functions (Makoi and Ndakidemi, 2008). Metal contamination contributes to decreased enzyme activity, resulting in soil stress (Khan et al., 2010; Aon and Colaneri, 2001).

Arylsulfatase is a natural soil enzyme that acts to cleave sulfur from organic compounds, leading to mineralized S and a remainder organic C compound. Mineralization of sulfur is essential for plant nutrition (Makoi and Ndakidemi, 2008).

Beta-d-glucoside is a natural soil enzyme that decomposes more complex carbon compounds, cleaving glucosides of larger C compounds to generate glucose. Makoi and Ndakidemi (2008) reported that Beta-d-glucoside is sensitive to metal contamination and correlates with organic carbon availability and the microbial population.

Phospholipids-linked fatty acid (PLFA) composition is a widely-used approach, an essential part of cell membranes, and the exact chemistry of the cellular phospholipids varies from organism to organism. It allows for the description of the microbial community, and by describing the phospholipids in the soil it is easier to identify the species of microbes that exist in the soil. Lipids linked with the organic form are broken down into neutral, glycol-, and phospholipids on silicic acid columns (Vestal and White, 1989). Fatty acids are main cell membrane components, and their diverse structures vary between many microbial groups (Hinojosa et al., 2005).

Microbes

Microbes and fauna mineralize organic matter and input large quantities of nutrients into the soil. Ecosystem fertility relies on the rate of organic matter turnover mediated by soil microbial biomass (Brookes, 1995). Microbes interact with metals and metalloids that can be beneficial or detrimental to the environment (Ehrlich, 1997). Microbes contribute to plant growth. A microbial community is crucial for cycling nutrients, maintaining organic matter, and producing of growth-promoting compounds (Bolton et al., 1985). In particular, microbial communities are the primary driving force in acid mine drainage, making them essential for solubilization, transport, and bioavailability of heavy metals (Lovely, 1991).

In general, a small percentage of organic matter (1-3%), the activity of the soil microbial biomass greatly influences the fertility of the soil. Soil microbial biomass is a fundamental part of the biogeochemical cycle for all major plant nutrients. In particular, microbes aid soil organic matter cycling, nutrient transformation, and building of aggregates; these are essential for plant nutrition, water storage and movement, and proper soil aeration (Robert and Chenu, 1992). However, the presence of heavy metals causes abiotic stress to soil microbes, which affects the growth, morphology, and metabolism of microorganisms. These pollutants can also disrupt functionality, destroy cell membranes, and alter the size and structure of the microbial community (Aceves et al., 1999; Smejkalova et al., 2003). Due to their long half-lives, heavy metals remain in the soil for extended periods— up to several thousand years. Sandaa et al. (1999) and Kelly et al. (1999) demonstrated heavy metals influence on the structure of microbial communities. However, Kelly et al (1999) also determined that bacteria can develop a tolerance to heavy metal contamination. Heavy metals are some of the most harmful inorganic pollutants as they can damage microbial communities.

Heavy metal ions must penetrate the cell to have a physiological or toxic effect. In soils, Pb occurs in its ionic form (Pb^{2+}). Unfortunately, microbial cells confuse the ionic form of Pb with similar ions needed for proper growth and function, allowing Pb to be transported within a cell's internal system. PLFA analysis confirmed shifts in microbial populations of contaminated soils (Kelly et al., 2003; Hinojosa et al., 2005). Hinojosa et al. (2005) observed that Cu, Cd, and Zn have the largest effect on microbial community structure and pH, affecting the availability of these metals. Studies show that PLFA decreases the pattern of toxicity of metals in the order of Cd = Ni > Zn = Cu > Pb (Frostegård et al., 1993). The PLFA can be a great tool to measure changes in microbial community structure resulting from management practices (Frostegård et al., 1993).

Currently, there are very few studies analyzing the direct toxic effects of Pb on microbial communities. Lead may not be toxic to most microbial species due to low soil solubility. Moreover, studies have shown Pb tolerant bacteria that precipitate Pb-phosphates in their cell walls (Levison and Mahler, 1998). Konopka et al. (1999) demonstrated that in a soil with high concentrations of Pb there was a microbial community shift of approximately 10,000 mg kg^{-1}. In another study conducted by Marzadori et al. (1996) concluded that there was a significant reduction in phosphatase enzyme activities when soil Pb content was 5,000 mg kg^{-1}. However, the increase in Pb additions was not proportional to the decrease seen in enzyme activity.

Physiochemical characteristics of the soils (pH, organic matter content, and moisture) are the most likely causes for variation of the effect of Pb on microbes.

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Chapter 3 - Phytostabilization of a Contaminated Military Site Using Biofuel Crop and Soil Amendments: A field-based investigation

Abstract

Toxic substances can potentially contaminate productive land due to military activities. The most common and widespread metal contaminant in military sites is lead (Pb). A field experiment was begun in 2016 at Fort Riley, KS in an area with soil Pb concentration ranging from 900 to 1,500 mg kg^{-1} with near neutral pH. The main objectives of the study were to determine the feasibility of using *Miscanthus* sp. as biofuel crop, for the phytostabilization of the contaminated military site and also to evaluate the effect of soil amendments on *Miscanthus* growth, soil-plant Pb transfer, bioaccessibility of soil Pb, and soil health. A randomized complete block design with five treatments and four replications formed the experimental design. Five treatments were: (i) control plots without tillage and left with existing vegetation, (ii) no-tillage, no additional amendments and planted with *Miscanthus*, (iii) tilled soil, no further amendments and planted with *Miscanthus*, (iv) tilled soil amended with inorganic P (triple superphosphate applied at 5:3 Pb:P) and planted with *Miscanthus*, and (v) tilled soil amended with organic P source (class B biosolids applied at 45 Mg/ha), and planted with *Miscanthus*. Soil and plant samples were analyzed each year. Tilling and soil amendments increased dry matter yield in 2016 with no differences in the harvested biomass in subsequent years (2017, 2018). Total Pb uptake, Pb concentration in plant tissues, and bioaccessibility of Pb in soils were significantly less in the *Miscanthus* plots amended with biosolids compared to the *Miscanthus* plots with no added P across all years. Enzyme activities, organic carbon, and microbial biomass were also

greater in biosolids-treated plots than in untreated plots. Our results show that one-time addition of soil amendments to Pb-contaminated skeet-range soil support establishing *Miscanthus*, stabilizing and reducing bioaccessibility of soil Pb, and reducing concentration and uptake of Pb by *Miscanthus*.

Introduction

Increase in human activities coupled with extreme demographic pressure has led to the degeneration of many agricultural lands. This has led to soil erosion and flooding, which minimized the use of agricultural lands for agriculture (Pidlisnyuk et al., 2014a). The presence of soil contaminants and accumulation of metals in the soil has an adverse impact on environmental health, crop development, and food quality leading to possible health crisis in humans (Bermudez et al., 2011; Pidlisnyuk et al., 2014a). Different anthropogenic activities such as industrial, agricultural, mining, and urban activities plays a major role in accumulation of high concentration of lead (Pb) in the soil, one of the major contributors of environmental pollution. Other activities of military importance such as training, testing, and the deployment of weapons which occurs in the shooting ranges have resulted in both severe and chronic soil contamination in the environmental system at military sites and also in surrounding areas.

Around the globe, firing ranges are operated for recreation and military training, which annually deposits millions of kilograms of Pb into the soil. Lead contamination from firing activities is a widespread issue, with unanticipated results to both wildlife and human health (USEPA 2005; Sorvari et al., 2006; Laporte-Saumure et al., 2010). The Agency for Toxic Substances and Disease Registry (ATSDR) listed Pb as the second most hazardous substance in the U.S. on the National Priority List (NPL) (ATSDR, 2017). The United States Environmental Protection Agency (U.S. EPA (2005) reported that the increased regulation under the Clean Water Act and the Resource Conservation and Recovery Act catalyzed exciting developments in levels of Pb contamination in active firing ranges such as phytoremediation (Wilde et al. 2005) and stabilization (Bruell et al. 1999), as remediation options for shooting range soil. The lack of controls governing “safe” military soil levels where the shooter may practice, in addition to variations of opinion on exposure criteria to humans, is a cause for concern. Reducing the exposure risks of Pb in contaminated shooting ranges should use remediation efforts that focus on minimizing the bioaccessibility or bioavailability of contaminants. Phytoremediation is known as a cost-effective, sustainable, and environmentally friendly strategy for an effective mitigation with substantial benefits for the large-scale cleanup of contaminated sites (Pilon-Smits, 2005). Phytostabilization of metals in soils using plants and their associated microorganisms is the most promising technique applicable to remediation of trace element contaminated soil (Mench et al., 2010). It is essential to revegetate these contaminated sites to minimize further contamination of the surrounding soil by erosion and runoff. Plants have a range of possible mechanisms at the cellular level that might be included in the detoxification and tolerance to trace element stress. Various phytotechnologies make use of different plant features (Hall, 2002; Pilon-Smits, 2005).

Phytostabilization is the use of plants to stabilize trace element contaminated soil, thereby reducing the risk of further environmental degradation by contaminant leaching into groundwater or dispersion by windblown dust. Generally, perennial grasses present suitable features for phytoremediation, such as fast growth, high biomass yields, deep root systems, and tolerance to contamination (Yang et al., 2005; Nsanganwimana et al., 2015). Second-generation crops, such as *Miscanthus* and switchgrass, show good potential for phytoremediation or phytostabilization in Eastern Europe and the United States (Pidlisnyuk et al., 2018; Andrejić et al., 2019). There are two benefits of this group of biofuel crops: first, they produce high biomass, making them a promising biomass crop for energy; second, growing *Miscanthus* and switchgrass as non-food crops on contaminated soils will not conflict with food security, which will limit contaminants from accessing the food chain (Meers et al., 2010). In comparison, growing other crops such as maize, sorghum, oilseed crops conflict with agricultural processes and safety (Pidlisnyuk et al., 2014a; Kocon and Matyka, 2012).

Miscanthus is a C-4, high-yielding, non-food perennial grass, perceived as an encouraging biomass crop for energy, bio-based products, and raw materials for various industrial activities (Lewandowski et al., 2003; Acikel, 2011). Few previous studies have evaluated the potential of energy crops such as *Miscanthus* to grow in contaminated soils (Kocoń et al., 2017; Pidlisnyuk et al., 2018; Pogrzeba et al., 2017). Rhizome systems of *Miscanthus* can recycle nutrients, which reduces nutrient input requirements (Heaton et al., 2009; Dohleman et al., 2012). Since *Miscanthus* only needs soil tillage in the establishment year, chances for soil erosion will decrease over time. But information on the effect of *Miscanthus* growth on soil contaminant bioavailability is lacking.

Phytostabilization is usually combined with soil amendments to decrease contaminant bioavailability and promote the health of the contaminated soil to support plant growth. The use of soil amendments and chemical sorbents to bind and immobilize Pb is a common practice (Hettiarachchi and Pierzynski, 2004; Scheckel et al., 2013). The geochemical nature of Pb favors the utilization of phosphate addition as a chemical sorbent due to its ability to immobilize Pb even at low pH. Phosphate amendments showing high Pb immobilization include moderately soluble P fertilizers made by reacting phosphate rock with orthophosphoric acid, such as triple superphosphate (TSP); less-soluble P fertilizers, such as hydroxyapatite (HA) and phosphate rock (PR) ; and wastewater-derived products, such as biosolids (Brown et al., 2005, 2012; Cao et al., 2009; Hettiarachchi and Pierzynski, 2002). Baker (2011) examined the effectiveness of organic amendments (manure compost) at two different rates (45 and 269 Mg ha^{-1}) with and without lime and bentonite, on mine waste materials at two field sites in southeast KS. Two to three weeks after the plot was established, they planted switchgrass (*Panicum virgatum*) in plots to examine the chemical changes as well as changes in microbial community structure and function. They found that high rates of compost additions significantly changed the organic carbon (OC), total nitrogen (TN), and available N, P, and K concentrations along with pH values. Higher arylsulfatase, β-glucosidase, and phosphatase activities were observed in high rates of compost added plots compared to all other treatments. The high compost treatment effects on biomass and enzyme activities disappeared over the course of 8yr study, suggesting that reapplication of compost is necessary (Gudichuttu, 2014).

Organic P sources, such as biosolids, play a crucial role in metal immobilization through sorption and complexation of the metal fractions associated with organic matter (OM), metal oxides, or carbonates (Walker et al., 2003). Brown et al. (2003) indicated that the addition of biosolids had reduced in vitro Pb bioaccessibility in contaminated soil by 43%. The purpose of using recyclable, nutrient-rich organic materials to support the phytostabilization of metal contaminated soils is to generate a self-sustaining environment where nutrients are cycled internally by healthy microbial and plant communities with no additional inputs.

Inorganic P is one of the most restrictive nutrients on agricultural lands. In order to better understand the most efficient quantities of P fertilizer that need to be applied to *Miscanthus*, researchers have studied the uptake and remobilization of P in above- and below-ground biomass (Heaton et al., 2009; Strullu et al., 2011). By selecting phosphate amendments as a method of in situ stabilization of Pb, transformation of Pb into insoluble Pb-phosphates, namely pyromorphites [$Pb_5(PO_4)_3X$, X= OH^-, Cl^-, F^-], can be induced (Hettiarachchi, et al., 2000; Cao et al., 2003). However, before applying this technique, many issues need to be taken into consideration including the long-term stability of the newly formed Pb phosphates, the suitable amounts of P to add, P source, and the influences of P on other metals usually associated with Pb in contaminated soils (e.g., Zn and Cd) (Hettiarachchi et al.; 2001). Because, this in situ technique does not alter the total Pb concentration in soil, information on the amount of time required for decreasing soil Pb bioaccessibility after P application as well as the long-term impacts of P on soil Pb bioaccessibility is very important. Hettiarachchi et al. (2000) have also shown that phosphorus added as soluble P (TSP, phosphoric acid) or low soluble (phosphate rock, PR) sources to five Pb-contaminated soils, could decrease the bioaccessibility of lead in PBET extractions.

To address some of the above mentioned research gaps, we designed a study with following objectives: i) to evaluate how different establishment methods and soil amendments affect *Miscanthus* growth, plant Pb concentration and soil Pb stability, and ii) to assess the effects of continual plant growth, nutrient removal, and the soil chemical changes induced by *Miscanthus* growth on bioaccessibility and speciation of soil Pb.

Materials and Methods

Study Location

Fort Riley is a United States military base located southwest of the city of Manhattan and just north of Junction City, along the Kansas River in Geary County in northeast Kansas. Its geographic coordinates are (39°11′30″N 96°35′30″W) spanning an installation area which totals 41,141 ha. (Omernik, 1987; Dickson et al., 2008). The climate in Fort Riley is mostly considered temperate continental. A continental climate is extremely inconsistent but can be described as having hot summers, cold, dry winters, mild winds, low humidity, and a very noticeable peak in rainfall late in the spring and the first half of summer. Average monthly temperatures range roughly from -2.7 C in January to 26.6 C in July (Althoff et al., 2007; PRISM Climate Group, 2012). The average annual precipitation is approximately 843 mm; droughts are frequent in this area (Abrams and Hulbert, 1987) but precipitation varies widely from year to year, with almost 75% of precipitation occurring during the growing season (Hayden, 1998).

The chosen area for the research field had been a recreational “skeet shooting” range with Pb pellets deposited during the 20th century. Relatively small amounts of Pb remain, approximately, 1,000 mg kg^{-1} over the area to ~15 cm or more depth, but can reach upto 1,500 mg kg^{-1} in some places in the study site (Figure 3.1).

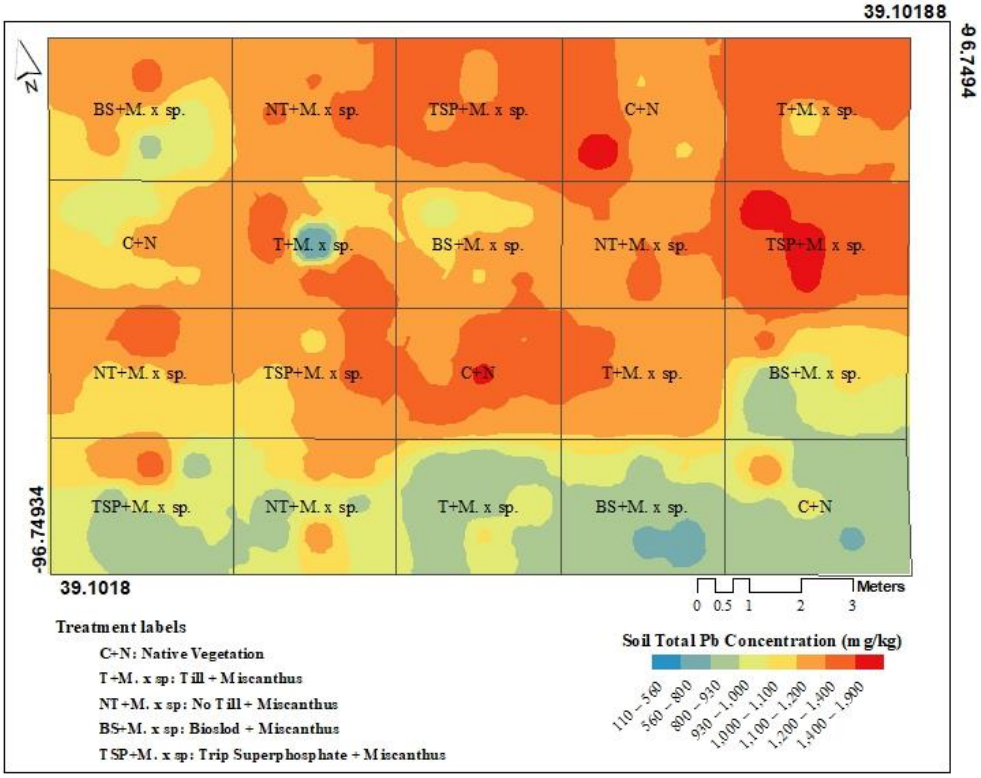

Figure 3.1 Distribution of soil total Pb concentrations in test plots at sample points at the skeet range of the military site, Fort Riley,2016.

Before plot establishment, the site was under continuous grasslands dominated by grasses and a mixture of annual weeds. These plants include switchgrass (Panicum virgatum), cheatgrass

(Bromus tectorum (L.)), Canadian horseweed (Conyza canadensis (L.) and Conyza canadensis var. canadensis), little bluestem (Schizachyrium scoparium), green foxtail (Setaria viridis), large crabgrass (Digitaria sanguinalis), and West Indian nightshade (Solanum ptycanthum Dunal) (Towne 2002). Other vegetation included forbs and grasses but in much lower abundances.

The soil at Fort Riley site is classified as a Wymore-Irwin association of deep, silt loams and silty clay loams which derive from parent materials that are accumulated on-site by wind and rain (Althoff et al., 2009; Jantz et al., 1975). At April 2016, the soils at the site were screened using an XL3T Niton handheld X-ray fluorescence (XRF) analyzer (Thermo Scientific, Billerica, MA) to detect the concentrations of potentially toxic trace elements, including Pb, with readings collected for 120 seconds. The results confirmed the elevation of soil Pb, but concentrations of other toxic trace elements were low. Soil samples were collected at random locations (six samples) of the study area using a soil probe (5 cores) and combined for a representative sample. Existing and dispersed plant materials in the study area were removed before placing soil samples in air-tight Ziploc bags. Soil digestion method EPA 3051A was used to determine total metals by ICP-OES to verify XRF results.

Soil Characterization

Before initiating the study, the soil was tested for selected physicochemical parameters (Table 1). An additional sample was digested via microwave digestion (EPA 3051A) and analyzed for trace metals by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Soil pH was determined in 1:10 soil: Milli-Q water using an Orion glass electrode (Thermo Orion, model A111). Soil texture was determined using the hydrometer procedure (Bouyoucos, 1962). Soil samples were taken before and after amending the soil, and soon after biomass harvest. All soil samples were air-dried and sieved (< 2.0 mm) for analysis of soil pH, total organic carbon (TOC), total Pb, available N, and extractable P and K. Available N (NO_3^-N and NH_4^+N) was extracted by shaking with 1M KCl solution (Keeney and Nelson, 1987), and extractable K was determined by extracting with 1 M ammonium acetate (Helmke and Sparks, 1996). Extractable P was determined by Mehlich-3 (Mehlich, 1984). Total OC and N in soils were measured using dry combustion method in a LECO CNS-2000 analyzer (LECO Corporation, St. Joseph, MI). Cation exchange capacity (CEC) was estimated by ion displacement (Jaynes and Bigham, 1986). Extractable Pb was determined by the Mehlich-3 extraction (Minca et al., 2013), followed by analysis using ICP–OES.

Plots Preparation and Experiment Setup

Before planting, herbicide (glyphosate) was applied according to standard agricultural practices approximately 3 weeks before plot establishment as a foliar spray to to reduce weed pressure throughout the growing season in all plot areas except control plots. This step improved *Miscanthus* establishment by reducing competition from existing vegetation.

The site was a rectangle (12.8 m x 10.4 m) comprising four randomized complete blocks as shown in appendix A Figure A.1. A block was divided equally into five experimental units (1.83m x 1.83m) with a spacing between blocks (0.46 m) to facilitate activities such as weeding, and harvesting. A plastic border (20 cm high) was installed around the perimeter of amended plots to reduce movement of amended soil. The experiment evaluated five treatments: (i) control plots without tillage and left with existing vegetation; (ii) no-tillage, no additional amendments, and planted with *Miscanthus*; (iii) tilled soil, no additional amendments, and planted with *Miscanthus*; (iv) tilled soil amended with inorganic P (triple superphosphate (TSP) applied at 5:3 Pb : P molar ratio based on the ratio of Pb and P in pyromorphite) and planted with *Miscanthus*; and (v) tilled soil amended with organic P source (class B biosolids (BS) applied as wet weight basis at 45 Mg/ha) and planted with *Miscanthus*. The biosolids was obtained from Oakland Wastewater Plant (Topeka, KS). Chemical properties of the biosolids are listed in (Table 3.1). Both, organic (BS) and inorganic (TSP) treatments were surface broadcast on plots and tilled to a depth of ~15 cm using a mechanical tiller to mix thoroughly into the soil prior to planting.

Planting Material

Miscanthus as second-generation biofuel crop, was selected for this study at the military site. Details on *Miscanthus* species that were used in this research were provided in Appendix A. In March 2016, dormant rhizomes were obtained from digging rhizomes of 10-yr old existing study ((2007- 2016) at the Kansas State University Agronomy Research Farm in Manhattan, Kansas (39°11´ N, 96°35´ W) (Propheter et al., 2010). The rhizomes were washed with tap water to remove soil attached to roots, then placed in large plastic bags inside a bucket and stored in cold-storage conditions (<4^{o}C). In each plot, rhizomes weighing 60-90 g/plant were planted by hand at a depth of 15 cm. There were 16 rhizomes per plot, totaling 320 rhizomes with approximately 3-4 rhizomes (according to the weight) with 0.46 m plant spacing. Field servicing was performed using a push mower and hand weeding to reduce weed pressure and plant loss. Plants did not receive any additional fertilizer or herbicide after adding amendments before planting and were allowed to grow until the end of each season undisturbed.

Plant Harvest

Aboveground biomass was harvested in early December in all three years (2016, 2017, and 2018). Senescence of *Miscanthus* and the drop of most leaves were used to determine the harvest time. Harvesting was done by hand cutting a 0.836 m^2 wide swath (using a 3/4" diameter PVC pipe) at 5 cm above the soil surface from the center (four plants) of each plot. The border plants were removed using a sickle-bar mower. Harvested material and subsamples were weighed in the field and placed in separate labeled bags. Subsamples were dried at 65 °C in forced-air dryers to constant weight, then weighed to determine dry matter content, which was used to calculate the total aboveground dry biomass per plant. Aboveground dry biomass per plot was calculated and expressed as aboveground biomass per plot area (Mg ha^{-1}). The biomass of each sample was subsequently ground to pass through a 1 mm sieve before analysis. The ground plant samples were stored in labeled polyethylene vials until analysis.

Plant Sample Analyses

The ground plant materials were digested in triplicate. For digestion, 0.5 g sample was placed in 55 mL Teflon tubes (CEM, Mathews, NC). A 10-mL aliquot of trace metal-grade concentrated nitric acid (70% v/v) was carefully added, and the contents allowed to sit for 15 minutes in the fume hood to allow venting. Teflon tubes were capped, placed on the carousel, and digested in the CEM MARS unit (Matthews, NC). The tubes were ramped up to 200°C and held for 15 minutes (1600 W @ 100%, 15-minute ramp time, 15-minute cooling time). Following digestion, the tubes were removed from the unit and left to cool in the fume hood for an hour or until the contents were at room temperature. Teflon tubes were uncapped slowly, and the solution was filtered through Whatman No. 42 filter papers into screwed plastic scintillation vials under the fume hood. Samples were then stored at 4 °C until the analysis. The National Institute of Standards and Technology (NIST) standard reference materials (SRM apple leaves: 1515) were digested and analyzed alongside the plant materials. Plant samples were analyzed on an AA240Z graphite furnace atomic absorption spectrometer (Varian Inc., Australia) with Zeeman background correction. All plant samples were prepared in triplicate.

Soil Chemical Analyses

Soil samples were air-dried and sieved to < 2 mm size for chemical analysis. Extractable P, TOC, and soil pH were analyzed as described above. Total Pb and other trace element concentrations in soils were determined by EPA method SW846-3051A (USEPA, 2007). About, 10 mL of trace metal-grade concentrated HNO_3 was added to 0.5 g of soil and digested in a microwave digestion unit (MARS Xpress, CEM Corporation, Matthews, NC). In the first step on the CEM MARS unit, the temperature of the soil-acid mixture was increased to 165 °C in 5.5 min. The temperature was further increased to 175 °C in 4.5 min in the second step, and the mixture was kept at 175 °C for 5 min. After digestion, the tubes were transferred and left to cool in the fume hood until the contents were at room temperature. Soil solution was filtered through Whatman No. 42 filter papers into screwed plastic scintillation vials under the fume hood.

Bioaccumulation Factor (BF) of Pb in *Miscanthus* tissues for 2016, 2017 and 2018 were calculated. After each harvest of biomass, the ratio of the concentration of metal in biomass to the concentration of these metals in the soil was calculated using the following equation:

$$\text{BF} = \frac{\text{metal concentration in aboveground (mg kg}^{-1}\text{)}}{\text{metal concentration in soil (mg kg}^{-1}\text{)}}$$

A blank (trace-metal grade HNO_3 acid) and standard reference materials (NIST 2711a-Montana II soil) were digested and analyzed along with each batch of soil materials for quality assurance and quality control (QA/QC). Samples were then stored at 4 °C until chemical analysis. The total concentrations of Pb and other trace elements were determined using the ICP-OES (Varian 720-ES).

Bioaccessible Pb

Bioaccessible Pb was determined by performing a modified physiologically-based extraction test (PBET) to evaluate contaminated soil risks at the shooting range site (Ruby et al.,1996) on soil samples collected from each treated plot. The gastric solution was prepared by adding 1.25g of pepsin, 0.50 g citrate, 0.50 g malate, 420 µL lactic acid, and 500 µL acetic acid to 1 L of deionized water and acidified to pH 2.5 with trace metal-grade HCl. The solution was heated to 37 °C before adding it to the soil. One-hundred ml of the gastric solution was combined with the 1 g of <250 µm soil fraction in a 250-mL wide-mouth HDPE bottle and tied up tightly. Bottles containing the mixture were shaken for one hour at 150 rpm on a Queue orbital shaker (Parkersburg, WV) preserved at 37 °C. The pH of the soil-gastric solution mixture was adjusted to 2.50 ± 0.02 using trace metal grade HCl. The supernatant solution was filtered using 0.45-µm syringe filters into plastic scintillation vials and analyzed for Pb by GF-AAS. A soil standard reference material (Montana II) at pH 2.5, and blanks were routinely included in PBET analysis.

Microbial enzyme method

Soil enzyme activities were measured within two weeks after soil sampling. Until then, the moist soil samples were stored at 4 °C. The soil microbial enzyme analyses included: activities of arylsulfatase (pH 5.8, 37 °C), β-D-glucoside (pH 6, 37 °C), acid phosphatase (pH 6.5, 37 °C), and alkaline phosphatase (pH 11, 37 °C). After the enzymatic assay, samples were extracted using a mixture of toluene and organic acids, as described by Tabatabai (1994). The amount of p-nitrophenol released was measured calorimetrically using a Beckman Coulter DU 800 spectrophotometers and results were on a dry-weight basis; moisture content was determined from loss in weight after drying at 105 °C for 48 h.

Phospholipid fatty acid analysis

Phospholipids ester-linked fatty acids (PLFA) can function as an index of soil health and environmental stress to supply more information on the diversity of the microbial community

(White and Rice, 2009). The PLFA was analyzed using a modified protocol of White and Ringelberg (1998). Within one week of sampling /sieving, roughly 20 g of moist material was frozen at -20 °C and lyophilized. Total lipids were determined by adding 5 mL of chloroform,10 mL of methanol, and 4 mL of phosphate buffer (pH 7.4) on 5 g freeze-dried soil. Chloroform and water were added three hours after extraction to separate the mixture into polar and nonpolar fractions, while total lipids remained in the nonpolar phase. Using silicic acid chromatography columns (Disposable BAKERBOND® SPE Columns, J.T. Baker®), phospholipids were separated from neutral lipids and glycolipids and then, removed using with methanol. The fatty acids were then saponified by KOH (0.2 M KOH in methanol) methylated to form fatty acid methyl esters (FAME) (Allison and Miller, 2005). The resulting FAMEs obtained from PLFA were analyzed with a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Scientific, USA) equipped with a DB5-MS column (30 m x 250 μm in diameter x 0.25 μm film thickness) Agilent gas chromatograph (GC) using helium as the carrier gas. Standard nomenclature was used to explain the PLFAs. The total PLFA represents microbial biomass. A total of 30 biomarkers were presented from all soil samples. Microbial groups were determined based on features of biomarkers: (1) gram-positive bacteria (Gram +ve) were determined using the following biomarkers: i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0; (2) gram-negative bacteria (Gram -ve) were determined using the following biomarkers: 16:1 2OH, 16:1ω7c, 16:1ω9c, cy17:0, 17:1ω8c, 18:1ω7c, and cy19:0 (Liang et al., 2014; Bossio and Scow, 1998; Zogg et al., 1997); (3) Fungi were determined using the fatty acid biomarker 18:1ω9c and 18:2 ω 6c; (4) Arbuscular mycorrhizal fungi (AMF) was represented using 16:1ω5c; (5) Actinomycete abundance was determined using the 10Me16:0, and 10Me18:0. Total fungi were calculated by summing 16:1ω5c, 18:1ω9c, 18:2ω6c (Vestal and White, 1989). The Fungi: Bacteria (F: B) ratios were determined using fatty acid percent proportion of fungi relative to bacteria as following as described by (Malik et al., 2016):

$$F : B = \frac{fungal\ biomarkers}{\sum(\mathrm{Gram}\ +\mathrm{ve}), (\mathrm{Gram}\ -\mathrm{ve}\ )\mathrm{and\ actinomycete}}\ x\ 100$$

Statistical Analysis

Statistical analyses of the data were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, 2017) for Analysis of Variance (ANOVA). The main plot factor was treatment and was arranged in a randomized complete block design. The data were analyzed using a PROC MIXED procedure with treatments considered a fixed effect and replications considered a random effect. Repeated measures analyses were used to determine the effects of the years. Tukey's Honestly Significant Difference (HSD) test was used for multiple mean comparisons at a ($\alpha = 0.05$) probability level of significance.

Results and Discussion

Growing Conditions

Weather data were collected from Kansas Mesonet Kansas State University using data from the nearby weather station, Ashland Bottoms (Figure 3.2). Ashland Bottoms station was approximately 14.5 km from the experimental site. In 2016, there were no drought conditions, and annual precipitation was moderate,with a total rainfall of 948 mm. Annual precipitation was less in 2017 to 597 mm. In the third year of study, 2018, annual precipitation increased slightly during the growing season (April-October) to 700 mm, with a period of limited water stress in summer 2018. Greater precipitation occurred in May, June, July, and August of 2016 compared to 2017 and 2018, the peak months of the growing season. Temperatures were also higher in 2016 during the growing season (27.7°C) compared to 2017 and 2018 (26.9, and 26.7 °C, respectively). Some *Miscanthus* species (*M.×g*) thrive in regions with a minimum monthly average winter air temperature of -3.5 °C and annual precipitation of 600 mm (Xie, 2012).

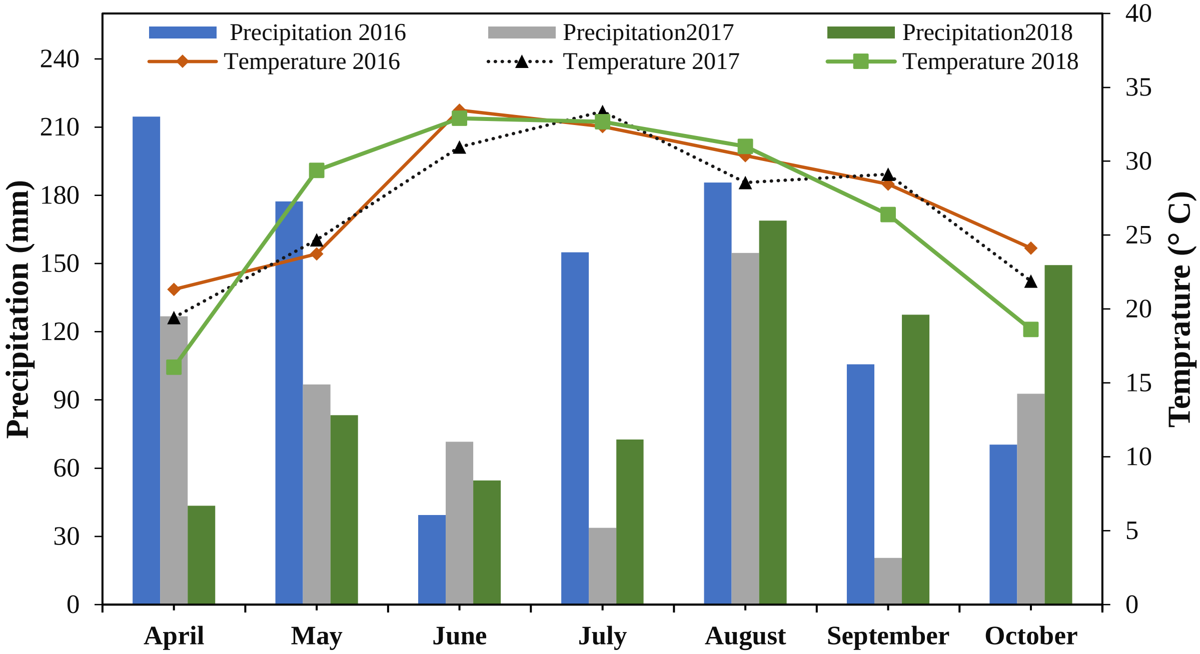

Figure 3.2 Total monthly precipitation (mm) and average annual temperature at the skeet-range site from 2016-2018. This data was collected from the weather data library Kansas Mesonet of the Kansas State University, accessed at http://mesonet.k-state.edu/.

Soil Characterization

The main physicochemical properties of the test plots are summarized in Table 3.1. The soil texture was a silty clay loam (11.3 % sand, 59.8 % silt, and 28.9 % clay). The CEC was 19.5 cmol$_c$ kg^{-1}, which is considered moderate . Soils contained relatively moderate levels of TOC. Mehlich-3 extractable P was 41 mg kg^{-1}, which can be classified as medium. Total P level in the skeet range test site was 502 mg kg^{-1} and was within the normal range of total P concentrations of 200 to 5,000 mg kg^{-1} in surface soils (Lindsay, 1979). The available nitrogen (N) was low at the beginning of the study.

Generally, Pb was concentrated in the surface soil (0-15 cm), and its concentration decreased with soil depth. Lead-based ammunition deposited in the soil had been subjected to weathering, which is influenced by soil properties such as pH, moisture, and temperature (Rooney et al., 2007). The Pb released from the ammunition is undergoing potential retention by reaction with the soil matrix. The distribution of Pb at a skeet range is primarily related to firing activities at the range. There is a considerable variation in surface Pb concentration, which may be due to the heterogeneous deposition of Pb (Figure 3.1).

The basic selected physical and chemical properties of the biosolids used at the site are presented in Table 1. The soil pH was slightly acidic. Total N, P, and Fe of the biosolids were 7.6 %, 13017, and 11200 mg kg^{-1}, respectively. The trace element concentrations (Cd, Sb, Pb, and As) in biosolids were low.

Table 3.1 Chemical and physical properties of the soil (0-15 cm) at the skeet range of the military site, Fort Riley,2016 and the biosolids used in the study.

Parameters	Soil	Biosolids
pH (1:10 soil: water)	6.9	5.5
CEC †, $\text{cmol}_c\ \text{kg}^{-1}$	20.0	-
Sand	11.3	-
Silt	60.0	-
Clay	29.0	-
TOC‡, %	2.0	-
Total N %	0.2	7.6
Mehlich-3 P, mg kg^{-1}	41.0	-
Ext. K, mg kg^{-1}	590.0	3,270.0
Total P mg kg^{-1}	502.0	13,017.0
Pb mg kg^{-1}	1,231.0	43.0
Fe mg kg^{-1}	12,898.0	11,200.0
As mg kg^{-1}	10.0	3.0
Zn mg kg^{-1}	38.0	266.0
Mn mg kg^{-1}	322.0	272.0
Cu mg kg^{-1}	10.0	91.0
Cd mg kg^{-1}	0.4	1.0
Sb mg kg^{-1}	2.0	1.3

†CEC: Cation Exchange Capacity
‡Total Organic carbon

Prior to the establishment of test plots, the soils were under continuous grass (training area) cover. In 2016, before and after a certain time of applying amendments about five days, some soil variables of the skeet range site were measured (Table 3.2). The nutrients needed for plant growth were within sufficient concentrations in the soil. In plots that did receive phosphate amendments, concentrations of available N and Mehlich-3 P did increase after planting. The majority of available N was in NO_3^- form in the biosolids-added soils, whereas NH_4^+ was the significant available source of N in other treatments. The greatest Mehlich-3 extractable P concentration was observed in biosolids-added soils, followed by TSP-added soils. Initially, soil pH was slightly lower after adding biosolids (~ 6.2). In contrast, there were almost constant or minimal changes in pH with TSP addition. Although, TSP usually has an acidulating effect, the application rate might not have been high enough to exert that effect on soils (Hettiarachchi et al., 2001). Soil fertility parameters can affect the Pb uptake and Pb concentration in biofuel crops. When soil/growth medium is rich with essential plant nutrients, the potentially toxic trace elements must compete with those nutrient ions to be absorbed into plants (Gothberg et al., 2004), leading to lower absorption of non-essential, potentially toxic trace elements. On the other hand, the total plant biomass could be high with sufficient nutrients, which could dilute the already absorbed/accumulated contaminants in plant tissues (Attanayake et al., 2014; Ekvall and Greger, 2003).

Table 3.2 Soil variables of the skeet range site measured in 2016, before and after applying amendments.

Time	Treatments	pH	Mehlich-3 P	Ext. K	Available		Total	
					NH_4^+-N	NO_3^--N	P	Pb
			----------mg kg^{-1}----------					
Before amendments	Control	6.9 ± 0.0	50.6 ± 4‡	517.0 ± 16	7 ± 1.1	2 ± 0.8	373 ± 20	1231 ± 188
	M.x sp.†+ No-Till	6.7 ± 0.1	43.6 ± 2	540.0 ± 8	6 ± 0.6	1 ± 0.4	374 ± 33	1374 ± 139
	M.x sp. + Till	6.9 ± 0.0	41.1 ± 7	539.0 ± 15	6 ± 0.5	2 ± 0.5	348 ± 20	1118 ± 106
	M.x sp. +TSP	6.8 ± 0.0	33.0 ± 8	553.0 ± 20	55 ± 0.3	1 ± 0.3	351 ± 13	1069 ± 242
	M.x sp. + BS	6.9 ± 0.0	43.4 ± 10	523.0 ± 17	6 ± 0.2	1 ± 0.5	354 ± 10	1090 ± 137
After amendments	Mx sp. +TSP	6.7 ± 0.0	1523.0 ± 10	592.3 ± 12	7.1 ± 0.5	13 ± 0.8	535 ± 21	1426 ± 41
	Mx sp. +BS	6.2 ± 0.0	664.0 ± 27	586.6 ± 16	19.1 ± 3.5	121 ± 15.0	1851 ± 14	1256 ± 176

†*Miscanthus specie.*

‡Values are means of four replicates ± standard error

Effect of Soil Amendments on Soil Properties and on Pb Extractability

As expected, concentrations of nutrients in biosolids plots were significantly higher than in soils that did not receive biosolids (Table 3.3). Biosolids-applied plots significantly ($p < 0.05$) increased total N in 2016 compared to no-till and the control treatments. At 2017 biosolids-emended plots had higher levels of total N compared to the control treatments. There were no significant differences in total N in the third growing season 2018. The addition of organic amendments (biosolids) significantly ($p < 0.05$) increased TOC in 2016 and 2017 compared to the control treatments. There were no significant differences in TOC in the third growing season 2018. Available P decreased through the growing seasons in phosphate amended plots. In soils that did not receive phosphate amendments, concentrations of available P did not decrease throughout the growing seasons. The increase of available P in biosolids-treated skeet-range soil compared to the other treatments suggests that biosolids can maintain high available P levels. The addition of biosolids led to a significant decrease in soil pH. Furthermore, as one would expect, biosolids addition improved the concentration of soil TOC (Table 3.3). However, no significant differences in pH were seen with the tillage or no-tillage at any season. We observed high biomass production in biosolids-added soils. For example, in 2016, the average yield harvest in biosolids-added plots weighed 13.5-Mg ha^{-1}, but it was 9.6-Mg ha^{-1} in the plots that did not receive biosolids. We also observed that *Miscanthus* in biosolids-added plots grew faster, was healthier, and produced more biomass contents than the *Miscanthus* in plots that did not receive biosolids, especially in first growing season (Table 3.4).

Differences in extractable Pb between treatments through the first and third years were not significant except, between control and other amendments in the second growing season

(2017), there was an increase of 34% for no-tillage and 44% for tillage compared to the control (Table 3.3).

Table 3.3 Chemical properties of the skeet-range soil after each harvesting growing season (2016, 2017, 2018).

Year	Treatments	pH	Mehlich-3 P	Ex. Pb†	Total N	TOC
			mg kg^{-1}	-------------------%-----------------		
2016	Control	6.7 a‡	31.4 c	49 a	0.16 b	1.9 b
	M.x sp. -No-till	6.8 a	43.2 bc	62 a	0.20 b	2.4 ab
	M.x sp. -Till	6.7 a	41.9 bc	52 a	0.22 ab	2.5 ab
	M.x sp. -TSP	6.6 ab	111.1 b	62 a	0.21 ab	2.4 ab
	M.x sp. -BS	6.5 b	642.1 a	53 a	0.28 a	3.0 a
2017	Control	6.8 a	50.6 b	57 c	0.22 b	2.1 c
	M.x sp. -No-till	6.8 ab	53.1 b	76 ab	0.24 ab	2.6 ab
	M.x sp. -Till	6.8 a	41.8 b	82a	0.24 ab	2.6 bc
	M.x sp. -TSP	6.7 bc	96.1 b	68 abc	0.23 b	2.6 ab
	M.x sp. -BS	6.6 c	533.8 a	61 bc	0.28 a	3.0 a
2018	Control	6.8 ab	69.2 bc	67 ab	0.29 ab	2.7 a
	M.x sp. -No-till	6.9 a	42.8 bc	69 a	0.29 ab	2.8 a
	M.x sp. -Till	6.8 ab	37.6 c	73 a	0.27 b	2.8 a
	M.x sp. -TSP	6.7 ab	82 b	76 ab	0.29 ab	2.9 a
	M.x sp. -BS	6.6 b	446.3 a	53 b	0.33 a	3.1 a

† Mehlich-3 extractable Pb

‡ Means followed by different letters are significantly different between the treatments according to Tukey's HSD test with $P < 0.05$

Establishment, Survival and Dry Matter of Miscanthus

Soil amendments and weed control only improved biomass yield in 2016 at the skeet range, resulting in increased plant survival (Table 3.4). According to Zapater et al. (2017), *Miscanthus* yield displays high variability between sites and between years. In our study, the late frosts didn't show near the experimental fields, but dry summer growing seasons caused slow biomass development. Therefore, it can be supposed that through hot and dry years, the primary influence on *Miscanthus* biomass was a limited amount of water.

Miscanthus survived the first winter of 2016/2017, with 99% overwintering survival rate. Winter temperatures were not low enough to severely affect the survival rate at the site; the November to March average temperature at the site was 5°C. In the middle of June 2016, nearly one month after planting, plant density was calculated and just one plant was missing (99 % survival).

Across the three growing seasons, we observed increasing plant height. The final average height of *Miscanthus* in the third year reached 233 cm, which relatively conforms with Knörzer et al. (2013) indications. In the first and third year of cultivation (2016, 2018), the greatest increase in height was evident in the plots amended with biosolids. All *Miscanthus* plots showed greater increases in height in the second year (2017) than the first year, but there were no significant differences in height relating to the amendments. A clear tendency of biosolids treatment to increase *Miscanthus* height up to 251 cm was in accord with the findings by Lisowski and Porwislak (2010).

The two main factors determining establishment success were the rhizomatous material and the planting density. The optimal number of rhizomes depends on several environmental conditions such as (soil moisture, anticipated precipitation) and yield aims. Christian and Haase, (2001) has been mentioned that high planting density helps the crop by developing competition for resources with weeds and gaining high yields faster than when using low planting densities. Increased yield in the first growing season as a result of agronomic management practices, such as plant spacing. Where the equal spacing around the plant allowed better growth with success than planting at a higher rate within the row. This spacing gave benefit to make the field under a weed control option; also, *Miscanthus* plant grew up to fill the spaces in-between the rows and became capable of inhibiting soil erosion. (Heaton et al. 2011) indicated that the recommendation in the Midwest is to plant using 30-inch rows with 30-inch spacing between plants. Closer rows resulted in earlier canopy narrower in the second year, which increased yield and reduce weed pressure.

In general, our findings indicated that yields in the first year were relatively higher as the plants established themselves because of soil amendments and tilling; however, overall, the increases were very similar for all three growing seasons. The similar yields may indicate a common factor, most likely water, was limiting growth at the military site. Heaton et al. (2004) reported that stands of *Miscanthus* are not considered mature until after at least three years of growth. So, it is possible that yields may be higher in following years, although in the Midwest yields seem to fall and then stabilize after approximately eight years (Arundale et al., 2014).

The ranges in maximum yield achieved in this study were 7.04 to 13.5, 9.3 to 11, and 7 to 11 Mg ha^{-1} for first, second, and third-year-old stands, respectively (Table 3.4). These ranges were similar to those of Kharytonov et al. (2019) who reported maximum yield of dry above-ground biomass of *Miscanthus* across two years ranged from 4.3 to 6.8 and 8.9 to 9.7 Mg ha^{-1} for first and second-year-old stands, respectively. Dry matter yield of *Miscanthus* in our field was close to those in other reports of young *Miscanthus* grown on a range of soils in the United States. Haines et al. (2015) reported that the maximum yield of *M.×g* across three-year-old stands in the Piedmont and Mountain regions of North Carolina was 24 Mg ha^{-1}, while Davis et al. (2015) indicated that the Maximum yield of *M.×g* across five states (Kentucky, Illinois, Nebraska, New Jersey, and Virginia) ranged from 9 to 17, 11 to 27, and 10 to 31Mg ha^{-1} for first, second, and third-year-old stands, respectively. Gordana et al. (2017) conducted a study in a marginal lands with three different soil types at three locations in the Republic of Serbia for five years and found that the highest yield on Gleysol was 23.12 Mg ha^{-1} in 2014, on Planosol was 10.16 Mg ha^{-1}, and on Technosol was 4.77 Mg ha^{-1} in 2015, noting that the yield of *M.×g* depends on soil amendments and weather conditions. The growth of *Miscanthus* is productive on marginal soils with minimum application of agricultural measures in the year of establishment.

The addition of biosolids caused significant increases ($p < 0.05$) in the growth characteristics of the *Miscanthus* plants in the first growing season (2016). Plots on which biosolids were applied demonstrated significantly higher dry matter yield in 2016 compared to other treatments. Comparatively, the biosolids treatment in our study had the highest overall dry matter yield with 13.5 dry Mg ha^{-1}, most likely due to the high N, P (P is also provided by the TSP treatment), and other nutrients provided by biosolids amendments. Kharytonov et al. (2019) confirmed that energy crops, including *M.×g*, respond well to soil amendments, especially amendments with organic waste. Several studies showed that miscanthus sp. has a substantial yield potential and responded well to mineral fertilization (Saletnik et al., 2018; Ozdemir et al., 2018). The higher biomass yield observed for *Miscanthus* cultivated on biosolids-amended plots could be attributable to the nutrient-providing capacity of the biosolids, which contains significant amounts of macro- and micronutrients (Table 3.1). These observations agree with those stated in other studies on energy crops amended by waste materials, such as Smith and Slater (2010). Examples of biosolids' efficiency in plant establishment on metal contaminated soils are presented by Brown et al. (2003) and Stuczynski et al. (2007). Barriers such as metal toxicity, lack of nutrients, and weak water retention might be erased by the organic treatments when appropriately applied. Biosolids considerably enhance CEC and water holding capacity of the soil and contribute nutrients and microbial inoculum.

Acaroglu and Aksoy (2005) reported that bioenergy crop yields increase progressively until the full establishment of crops optimize land cover by increasing plant density. It is also assumed that the follow-up of plant density and growth rate would further increase the dry biomass yield in the following seasons. From our findings, we found that the *Miscanthus* has potential to grow on contaminated soils with minimum application of agricultural measures only in the year of establishment. Studies such as Pogrzeba et al. (2013) and Nsanganwimana et al. (2016) evaluated the cultivation and growth of different species of *Miscanthus* on heavy metal contaminated soil. Augustsson et al. (2015) indicated that in Europe, mine soil and degraded soils are estimated to cover thousands of hectares. For this purpose, our study may be considered, to a certain point, establishing research because it opens new possibilities for the cultivation of biofuel crops on contaminated soils otherwise unsuitable for agricultural use (Communication from the Commission to the European Parliament, 2014).

Table 3.4 Dry matter yield and height of *Miscanthus* for each harvesting growing season 2016, 2017, and 2018 at the skeet range of the military site.

Year	Treatment	*Miscanthus sp.* biomass yield (Mg ha^{-1})	*Miscanthus sp.* height (cm)
2016	Control	3.6 c†	82 d
	M.x sp. -No-till	7.0 bc	124 c
	M.x sp. -Till	9.6 b	147 b
	M.x sp. -TSP	8.4 b	141 bc
	M.x sp. -BS	13.5 a	168 a
	LSD 0.05	<0.0001	<0.0001
2017	Control	2.0 b	90 b
	M.x sp. -No-till	9.3 a	210 a
	M.x sp. -Till	10.8 a	225 a
	M.x sp. -TSP	9.8 a	233 a
	M.x sp. -BS	9.9 a	229 a
	LSD 0.05	0.0006	<0.0001
2018	Control	4.2 b	117 c
	M.x sp. -No-till	8.0 ab	222 b
	M.x sp. -Till	6.9 ab	223 b
	M.x sp. -TSP	6.9 ab	234 ab
	M.x sp. -BS	10.5 a	251 a
	LSD 0.05	0.0034	<0.0001

† Differences between means within a year followed by different letters are significant at $P < 0.05$.

Lead concentration in miscanthus

The elemental concentration of the above-ground biomass was measured for *Miscanthus* species for the collecting data in 2016, 2017, and 2018 (Table 3.5) but it presented across years because treatments x years was not significant. Average plant concentrations of Pb and P are presented in Table 3.5. Both treatment and years were significant, but their interactions were not significant. Lead concentration was slightly high in existing vegetation on unamended soil compared with other treatments (Table 3.5). All soil amendments significantly reduced the Pb concentration in plant tissues across three years compared to the control. The Pb concentrations in the biosolids plots were lower than the control, and most of the other treatments. This could be attributed to the dilution effect by the *Miscanthus* growth as well as chemical changes. The transformation of soil Pb caused by treatments addition may have resulted in less soluble Pb available for absorption into the *Miscanthus* and translocation of absorbed Pb from rhizomes to aboveground plant tissues. Soil amendments with high P content have been incorporated into pyromorphite, making it less mobile in the soil and the roots of plants (Basta and McGowen, 2004; Hettiarachchi and Pierzynski, 2002). The accumulation of Pb in *Miscanthus* grown on shooting range soils has been mentioned by other researchers. For example, Nsanganwimana et al., (2015) found that the Pb concentrations in *Miscanthus* leaves and stems ranged from 8.2 to14.3 mg kg^{-1} which were in agreement with our results regardless of treatments. In a study conducted by Pidlisnyuk et al. (2016) in the military site during two vegetation seasons, they observed that Pb was less accumulative in both vegetation seasons, and Pb concentration was very small. Overall, plant Pb concentrations in biosolids treated plots were lower than the other treatments.

For P (Table 3.5), the concentration in the biomass was significantly affected by years and treatments but not by their interactions across three growing seasons. The control had significantly greater P concentration compared to the other treatments. Treatments with *Miscanthus* didn’t differ significantly in P concentrations. That can be attributed to the annual accumulation of nutrients in rhizomes, since *Miscanthus* aboveground parts fade at the end of each season. Nsanganwimana et al., (2015) found that the concentrations of P in *M.x* g cultivated in contaminated agricultural plots decreased across the study period.

For the Pb uptake (Table 3.5), the results showed that Pb uptake by the aboveground *Miscanthus* is minimal during the growing seasons. The Pb uptake in the biomass was significantly affected by years only, but not by treatments as well as their interactions across three growing seasons. Kołodziej et al. (2016) reported that Pb uptake by the plant in organic amended soils is generally highest in the first year following application. Kocoń and Jurga (2017) conducted an experiment where *Miscanthus* was cultivated in contaminated soil (Pb – 769 mg kg^{-1}) and reported that *Miscanthus* after the first growing season, accumulated about 40 mg Pb kg^{-1}, which is slightly more than the results presented in our study.

The bioaccumulation factor of Pb in *Miscanthus* across three growing seasons was very low, ranged between 0.007 and 0.013 (Table 3.5). Kocoń and Jurga (2017) also found that the bioaccumulation of Pb in *Miscanthus* was lower than *Sida hermaphrodita* cultivated on loamy soils. In a study conducted by Barbosa et al., (2015) to evaluate tolerance and phytoremediation capacity of giant reed (*Arundo donax*) and *Miscanthus* app. in soils contaminated with heavy metals, they found that the values obtained for Pb bioaccumulation are the lowest of the heavy metals tested in that experiment. These low BF values are a positive feature required for a successful phytostabilization procedure. Overall, *Miscanthus* growing in skeet-range soil was able to tolerate Pb contamination with reduced transfer to its aboveground parts.

Table 3.5 Mean of lead (Pb) and phosphorus (P) concentrations, Pb uptake and bioaccumulation factor (BF) of Pb in biomass. Results are presented across three growing seasons (2016, 2017, and 2018) at the skeet range of the military site.

Treatments	Pb	P	Pb uptake	BF
	------mg kg^{-1}------		mg/m^2	
Control	27 a†	1724 a	9 a	0.019 a
M.xsp. -No-till	15 bc	862 b	12 a	0.012 bc
M.xsp. -Till	16 b	804 b	14 a	0.013 b
M.xsp. -TSP	15 bc	991 b	12 a	0.012 bc
M.xsp. -BS	7 c	1022 b	9 a	0.007 c
HSD†	8.41	304	7.5	0.005
p-value	<.0001	<.0001	0.233	<.0001
Year				
2016	15 ab	762 c	12 a	0.012 b
2017	13 b	1014 b	8 b	0.01 b
2018	20 a	1466 a	14 a	0.02 a

†Tukey's Honest Significant Difference for mean comparisons with an alpha (α) of 0.05

Bioaccessible Pb

A PBET, as described by Ruby et al. (1996) and modified following Medlin (1997), was used to estimate the amount of Pb absorbed via the ingestion of soils. Bioaccessible Pb was expressed as a percentage of total Pb concentration in each plot's soil (Figure 3.3). Lead bioaccessibility of soils at this site was less than 46% over three growing seasons. In general, bioaccessibility of P treated soils were lower compared to the other soils, although the reduction was statistically significant only for the biosolids treated soils. The effect of P amendments on the bioaccessibility of Pb was most likely due to the formation of insoluble Pb phosphates (Hettiarachchi and Pierzynski, 2002; Scheckel et al., 2013). In 2016, bioaccessible Pb in the

Miscanthus -No-till plot soils were significantly higher than the *Miscanthus* -biosolids treated soils (Figure 3.3), indicating that biosolids treatment helped to recover from the short-term increase of bioaccessibility of soil Pb due to *Miscanthus* growth. In 2017 and 2018, there was no increase in PBET-Pb in any *Miscanthus* plots (Figure 3.3). Reductions in bioaccessibility of Pb in biosolids-amended soils were significant compared to the control and some other treatments both in 2017 and 2018. Previous studies have shown that the reduction of Pb bioaccessibility in the amended soils was notable at pH 2.5 (Brown et al., 2004; Ryan et al., 2004). Henry et al., (2015) reported that a minor change in extraction pH has a significant effect on phosphate chemistry and assessments of the ability of phosphates to reduce bioaccessible Pb.

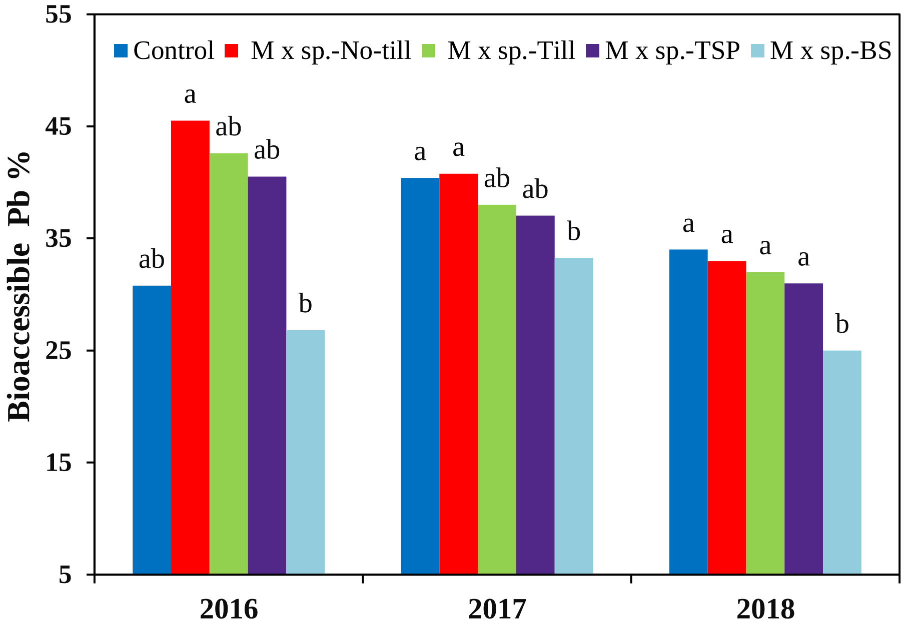

Figure 3.3 The effect of different treatments on bioaccessible Pb (%) in the skeet-range soil over three years (2016, 2017, 2018). Different letters above the bars indicate significant differences between the treatments, according to Tukey's test with $P < 0.05$.

Soil Enzyme Activities

The soil microbial characteristics were evaluated using enzyme activities and phospholipid analysis. In this study, we examined the soil enzymes produced instead of examining microbes themselves. Enzyme activities are significant indicators of soil microbial activity and are useful for understanding how changes in microbial communities are affecting soil processes. The four enzymes examined in this experiment were acid and alkaline phosphatase, arylsulfatase, and Beta-D-glucosidase. These enzymes are primarily generated by bacteria and are essential soil nutrients and vital for C cycling (Aon and Colaneri, 2001). According to Hsiao et al., (2019) found that rhizosphere C had significant support to microbial activity. Results showed there was greater enzyme activity in the biosolids amended plots compared to the non-amended plots except for arylsulfatase (Table 3.6). The scientific literature suggests the use of organic amendments in the cultivation of energy crops increases soil biological activity (Joniec,2018; Ruf et al., 2018; Antonkiewicz et al., 2019). Increased soil enzymatic activity confirms organic amendments make macronutrients available to plants as a result of the mineralization of organic matter (Joniec, 2018).

Ndakidemi (2006) mentioned that the quantity of acid phosphatase exuded by plant roots and microorganisms displays differences between crop species, varieties, and management practices. In this study, biosolids additions caused a significant increase in phosphatase activity across the latter growing seasons (2017, 2018) compared to the control (Table 3.6). Acid phosphatase activity was significantly and positively correlated with TOC, TN, and Mehlich-3 P in the soil and correlated negatively with extractable Pb and pH; however, correlations with pH were not significant (Table 3.7).

The activity for alkaline phosphatase was significantly higher with the biosolids amendment compared to the control (Table 3.6). No significant difference was seen between other treatments. Makoi and Ndakidemi (2008) reported that alkaline phosphatase is part of the breakdown of organic P containing compounds; phosphate groups are cleaved from TOC compounds result in mineralized P and a remainder of the TOC compound. Alkaline phosphatase activity had a significant, positive correlation with pH, TOC, TN, and Mehlich-3 P in the soil and a negative correlation with extractable Pb (Table 3.7), which suggests C, N, and organic matter contents are the main factors affecting alkaline phosphatase activity (Zhu et al., 2012). Martinez and Tabatabai (1997) mentioned that soil pH is another factor influencing the release and stability of this enzyme. Furthermore, Marzadori et al. (1996) stated that Pb significantly decreased phosphatase activity. In this study, a negative correlation was seen between alkaline phosphatase and extractable Pb concentrations. It should be noted that high levels of metal are not always translated into lower enzymatic activities for all soil enzymes due to bioavailability.

The addition of P, which was largely in the organic-P form with the biosolids, was likely responsible for the increase in alkaline phosphatase compared to the other treatments (Table 3.6). The activity of this enzyme is commonly increased in soils with higher soil TOC and decreased in soils with large amounts of mineralized P (i.e., from rock phosphate) (Makoi and Ndakidemi, 2008; Perez-de-Mora et al., 2006). Since P is a vital nutrient, the elevated alkaline phosphatase in the amended soils indicates a greater capability to encourage the growth of plants, more importantly slow release of P might have been important for maintaining reduction of Pb bioaccessibility in this soil. Increases in phosphatase activity were most related to total C, which was increased upon biosolids addition and is vital in ecosystem function. The addition of biosolids significantly increased TOC in 2016 and 2017 compared to the control treatments (Figure 3.4).

Arylsulfatase is defined as an extracellular enzyme, which catalyzes the hydrolysis of organic sulfate esters ($RO\text{-}SO_3^-$), therefore releasing available inorganic sulfate (SO_4). Fitzgerald (1976) reported that its availability depends on its enhanced organic S mineralization or mobilization. The reason for this is that specific proportions of S in different soil profiles are bound into organic compounds and are indirectly available to plants. Amendment additions did not cause a significant increase in arylsulfatase activities in this soil. Furthermore, arylsulfatase activity correlated positively with TOC and TN and correlated negatively with extractable Pb, which indicates suppression of arylsulfatase activity with increasing available metal concentrations (Table 3.7). Some researchers have seen similar results where arylsulfatase activity was repressed somewhat by extractable Pb (Pérez-de-Mora et al., 2005; Pérez-de-Mora et al., 2006; Alvarenga et al., 2008a; Alvarenga et al., 2008b; Zornoza et al., 2012).

Acosta-Martinez et al. (2000) indicated that the β-glucosidase enzyme is sensitive to changes in pH. This enzyme is produced by many microbial groups and is a good indicator of general microbial activity (Claassens, 2006; Makoi and Ndakidemi, 2008). In this study, the activity of beta-D-glucosidase was, in general, high in the plots amended with the biosolids, (Table 3.6). No consistent differences were seen between the other treatments. The increased activity of beta-D-glucosidase in the biosolids plots was likely due to the higher level of OC in the biosolids treated plots. Organic C compounds are the primary substrate of beta-D-glucosidase, and a higher concentration of TOC substrate leads to higher production of this enzyme by microbes (Garcia-Gil et al., 2000; Taylor et al., 2002; Song et al., 2012). β-glucosidase presented a positive correlation with TN, TOC, and Mehlich-3 P and correlated negatively with extractable Pb and pH; however, the correlations with pH were not significant (Table 3.7). Hinojosa et al. (2004) reported that β-glucosidase activity is sensitive to inhibition by the presence of heavy metals. The use of organic amendments is known to increase beta-D-glucosidase activity even in Pb contaminated soil (Perez-de-Mora et al., 2006; Tejada et al., 2007). The increase in enzyme activities, in general, with biosolids amendments was a positive index because the organic amendments enhanced soil function in the Pb-contaminated military soil.

Table 3.6 Effects of soil treatments on soil enzyme activities across 2017and 2018 growing seasons at the skeet range of the military site.

Treatment	Acid Phos†	Alk Phos‡	Aryl§	β-gluco¶
	---------mg p-nitrophenol kg^{-1} dwt h^{-1} --------			
Control	107 b	58 b	26 a	39 ab
M.x sp. -No-till	111 b	60 ab	27 a	40 ab
M.x sp. -Till	104 b	59 ab	26 a	37 b
M.x sp. -TSP	107 b	59 ab	23 a	38 b
M.x sp. -BS	133 a	69 a	27 a	45 a
p-value	0.0050	0.0210	0.1478	0.0148
HSD*	22	10	5	6

† Acid Phos: Acid Phosphatase
‡ Alk Phos: Alkaline Phosphatase
§ Aryl: Arylsulfatase
¶ β-gluco: β-glucosidase
*Tukey’s Honest Significant Difference for mean comparisons with an alpha (α) of 0.05

Table 3.7 Pearson's correlation between pH, TOC, TN, extractable Pb, Mehlich-3 P as%, and enzyme activities at the skeet range of the military site.

	TN	TOC	Ex. Pb	pH	Mehlich3-P	Acid†	Alka‡	Aryl§	ß-glue¶
TN	1	0.86676****	0.217*	-0.020	0.523****	0.771****	0.711****	0.542****	0.630****
TOC	-	1	0.175	-0.129	0.627****	0.504***	0.447***	0.298*	0.414***
Ex. Pb††	-	-	1	0.349	-0.162	-0.489***	-0.358**	-0.326**	-0.236
pH	-	-	-	1	- 0.671****	-0.094	0.082	0.052	-0.102
Mehlich3-P	-	-	-	-	1	0.283*	0.221	0.080	0.353**
Acid	-	-	-	-	-	1	0.820****	0.681****	0.617****
Alka	-	-	-	-	-	-	1	0.591****	0.747****
Aryl	-	-	-	-	-	-	-	1	0.620****
β-glue	-	-	-	-	-	-	-	-	1

†Acid: Acid phosphatase; ‡Alka: alkaline phosphatase; §Aryl: Arylsulfatase; ¶β- glue: β- glucosidase activity
†† Mehlich-3 extractable soil Pb
*Correlation significant at the 0.1 level
**Correlation significant at the 0.05 level
*** Correlation is significant at the 0.01 level
****Correlation is significant at the 0.001 level

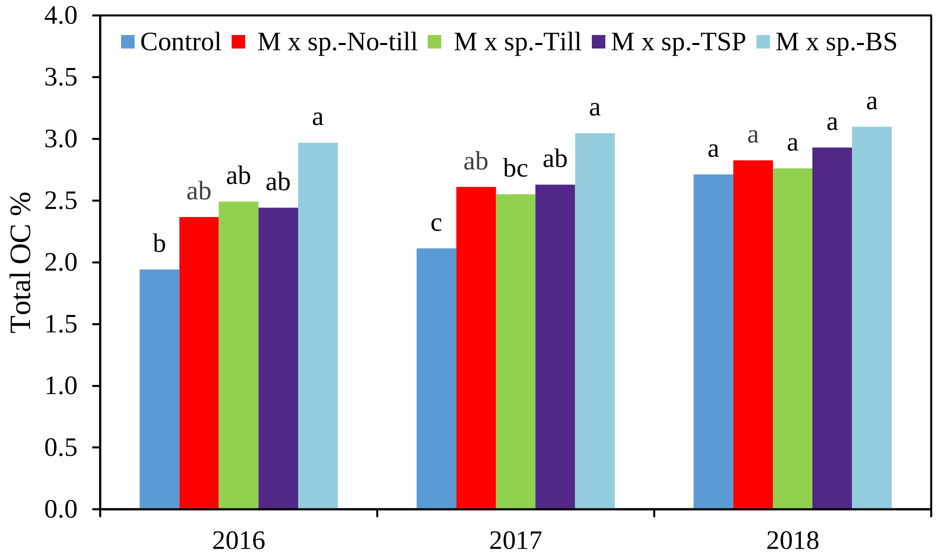

Figure 3.4 Soil organic carbon from 2016 to 2018 in *Miscanthus* plots at the skeet-range site from (2016, 2017 2018). Different superscript letters indicate a significant difference (α=0.05).

Phospholipid Fatty Acids

Results from PLFA analysis indicated that biosolids significantly increased total microbial biomass in this soil (Table 3.8). There was a significant difference between BS and no-tillage plots for fungi and between BS with tillage plots for total microbial biomass. It appears that the BS amendments helped to either maintain or increase the total microbial biomass and microbial communities. Soil amendments did not result in a significant increase in the biomass of Gram- bacteria and Gram+ bacteria, which was unexpected. This could be due to metal contamination or improper substrate to support the growth and function of microbes. Grayston et al. (2004) reported that P had been confirmed to increase the abundance of bacteria relative to fungal PLFAs. In a study conducted by Pidlisnyuk et al. (2018) in a former military site cultivated with *Miscanthus*, they observed that there were positive changes in microbial community structure and state was detected higher ratios of Gram- and Gram+ bacterial and fungal/bacterial PLFA. Zhang et al., (2006) studied the structure of microbial communities during the early stages of the revegetation of barren soils in the region of a Pb/Zn Smelter in Southern China by PLFA analysis. They found that PLFA ratios for fungi consistently increased with time after revegetation. They attributed those changes to increases and improvements in total N, pH, and porosity in the revegetated soils. This explains the increase in fungi in BS plots, which is rich in nutrients. Drenovsky et al. (2004) concluded that OC availability was highly correlated with observed differences in microbial community composition. However, Allison et al. (2007) proposed that the effects of OC on microbial shifts are likely to be more indirect. Conversely, White and Rice (2009) showed that in-field decomposition of grain sorghum residue was noticeable in total, fungal, and Gm+ bacterial PLFAs, suggesting that OC had a direct effect. In this study, biosolids did not appear to shift the microbial population dramatically, but it certainly increased its size. Thus, in terms of increased microbial activity to enhance soil organic matter cycling to support plant growth, biosolids additions to the skeet range soil were successful.

Table 3.8 Gram-negative bacteria, Gram-positive bacteria, fungi, and total microbial biomass in soil samples that were collected at 0-15 cm depth, at the end of 2017 growing season in the skeet range of the military site.

Treatment	Gram -ve	Gram +ve	Fungi	Microbial biomass
	-----------------nmol g^{-1}soil------------------			
Control	11.4 b†	15.2 b	32.3 b	108.9 ab
M.x sp. -No-till	15.4 ab	18.2 ab	33.4 b	102.1 ab
M.x sp. -Till	14.4 ab	16.8 ab	37.2 ab	100.3 b
M.x sp. -TSP	16.0 a	17.8 ab	38.9 ab	108.2 ab
M.x sp. -BS	12.8 ab	19.6 a	47.7 a	116.9 a
	p-value			
	0.042	0.0192	0.0338	0.0424

† Mean in the column with the same letter (s) are not significantly different at P<0.05.

Conclusion

Growth of biofuel crops in the skeet range soil may provide a chance for site rehabilitation. It is essential to choose the right plant capable of survival in both the contaminated environment and climatic conditions. The results of our study show that *Miscanthus* can grow in contaminated skeet-range soil. Tilling and soil amendments increased the dry matter yield. Soil amendments, especially biosolids, significantly reduced the plant Pb concentration. The soil chemical changes induced by *Miscanthus* growth initially affected the bioaccessibility of soil Pb. Organic P source (biosolids) amendments help to keep Pb less bioaccessible even under continual P removal through *Miscanthus* growth.

Further, the application of biosolids was effective in establishing and maintaining vegetative biofuel crops in Pb-contaminated skeet-range soil throughout the three-year study period. Plant biomass establishment was successful from the first growing season, and the biosolids treatments did exhibit a significant increase in biomass production compared to the untreated or contaminated control. The use of biosolids generally improved microbial community composition and enzyme activities, which is indicative of improved soil health. Additional monitoring will be required to see if these applied biosolids levels would be useful in reducing metal concentrations in plant tissue as well as maintaining vegetative growth for longer than three years. In conclusion, the study suggests that the application of biosolids is sufficient in maintaining *Miscanthus* for at least three years. The addition of TSP showed a slight effect. The addition of TSP could be more effective if we raise the molar ratio of TSP for skeet-range soil reclamation efforts.

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Chapter 4 - Effect of Soil Amendments and Biofuel Crop Growth on Lead Uptake and Bioaccessibility in a Skeet Range Soil: A Greenhouse Study

Abstract

Biofuel crop growth and phosphorus (P) amendments can be used in combination for phytostabilization to achieve in situ stabilization of lead (Pb) in contaminated skeet-range soil, but more research is needed to evaluate the effects soil amendments have on plant growth and the potential of soil-plant transfer of Pb in contaminated soil. A greenhouse experiment conducted in spring 2017 to evaluate ability of *Miscanthus* (*Miscanthus sp.*) to grow in Pb contaminated soils; effect of *Miscanthus* growth on bioaccessibility of Pb; and impact of soil amendments on growth of *Miscanthus*, soil-plant transfer of Pb, and bioaccessibility of Pb. The experiment included a contaminated soil containing 1042.5 mg Pb kg^{-1} collected from Fort Riley Military skeet-range and a non-contaminated control containing about 122.6 mg Pb kg^{-1} from the same area, adjacent to the Fort Riley field study. The experimental design was a randomized complete block with three replications. Twelve treatments consisted of two rates; treatments were: non-contaminated control (CT-NC); contaminated control (CT-C) with no amendments; triple superphosphate at 5:3 and 5:6 Pb:P (TSP-1X and TSP-2X); apatite at 5:3 and 5:6 Pb:P (AP-1X, AP-2X); struvite at 5:3 and 5:6 Pb:P (ST-1X, ST-2X); biosolids at 45 and 100 Mg/ha (BS-1X, BS-2X); TSP+AP 1:1 mix at 5:6 Pb:P (TSP+AP-2X), and TSP+ST 1:1 mix at 5:6 Pb:P (TSP+ST-2X). The aboveground biomass of the plants was harvested three times: June 25, 2017, September 15, 2017, and December 16, 2017. Biomass samples and soils collected at the end of the study were analyzed. Soil amendments increased dry matter yield in the first cutting and

decreased in the subsequent cuttings. *Miscanthus* amended with biosolids had lower total Pb uptake, lower Pb concentration in plant tissues, and lower Pb bioaccessibility in soil samples when compared to the *Miscanthus* with no added P across all cuttings. Moreover, the addition of biosolids and TSP-2X to Pb contaminated soil led to a significant decrease in the bioaccessible Pb compared to the contaminated control. Overall, the results suggest that *Miscanthus* can be successfully grown on soils with moderate Pb contamination in combination with suitable P amendments.

Introduction

Over the last decade, military base establishments have increased in number worldwide; military activities on these sites lead to potentially toxic substances (e.g. lead (Pb) and other metal contaminants) residing within shooting range soils. With residential and agricultural areas typically within close proximity to these sites, this potential contamination indicates shooting activities are an increasingly serious problem in industrialized countries. The problem stems from these sites becoming secondary Pb contamination area for the world (Ahmad et al. 2011; Perroy et al. 2014). Elevated concentrations of Pb in these locations along with the adverse health effects linked to Pb exposure has caused shooting range activities to receive more attention in recent decades. Most shooting range soils are contaminated with Pb because it is the primary ingredient in ammunition. On average, each pellet of ammo has approximately 97% metallic Pb (Cao et al., 2003). Martin and Ruby (2004) reported erosion would begin in contaminated land that lacked vegetation because of continued exposure to metal contaminants, and this will restrict the growth of an ecosystem. Revegetation is necessary for contaminated soils to inhibit further contamination of surrounding environments by erosion and runoff. Revegetation can be performed through emerging phytostabilization methods. Phytostabilization uses plants as vegetation on the soil surface to reduce the risk of further environmental degradation because the plants will decrease the amount of metals which get leached into groundwater or spread by wind or water erosion.

Fast growth, high biomass yields, intense and extensive rhizome systems, and tolerance to contamination are all characteristics of biofuel crops that makes them useful plants for phytoremediation (Ali et al., 2013). The second-generation crops, such as *Miscanthus* and Switchgrass, have the potential to be used for phytoremediation or phytostabilization. There are two benefits of this group of biofuel crops. First, they produce high biomass, making them a promising plant for energy, and second, they are a non-food crop that helps to avoid the problems of growing food sources, such as maize or sorghum, in contaminated soils (Kocon and Matyka, 2012). Soil amendments can lower the bioavailability of contaminants and can increase the ability of contaminated materials to become a plant growth media, which is why they are often used alongside phytostabilization. Organic and inorganic soil amendments are used to accelerate the attenuation of metal mobility and toxicity in soils. These amendments convert soil Pb to more geochemically stable phases through precipitation, sorption, and complexation processes (Hashimoto et al., 2009). The most used amendments include zeolites, phosphates, and composts (Wuana and Okieimen, 2011; Zhang et al., 2010). Soil amendments decrease the costs of soil remediation because they are not as expensive as excavation and removal of soils (Scheckel et al., 2013; U.S. EPA, 2011, 2013).Second-generation biofuel crops and phosphate-based soil amendments are a cost-effective and ecologically beneficial method of stabilizing military sites contaminated with Pb (Pidlisnyuk et al., 2014). The form of Pb affects its mobility and bioavailability; speciation is a critical environmental concern in Pb-contaminated soil. Phosphorus amendments to Pb-contaminated soils may induce different Pb-phosphate minerals to form. However, pyromorphites, $Pb_5(PO_4)_3X$ where X = F, Cl, Br, and OH, are the most insoluble and stable form of Pb in soils under a wide range of natural conditions (Nriagu, 1984; Lindsay, 1979; Hettiarachchi and Pierzynski, 2002; Scheckel et al., 2013). These compounds are more insoluble than other Pb forms observed in soils. When Pb and phosphate are in high enough concentration in aqueous solutions, it has been shown that Pb phosphates can rapidly form (Zhang and Ryan, 1999a, 1999b; Ma et al., 1993). Organic and inorganic (e.g. triple superphosphate, struvite, apatite, hydroxyapatite, rock phosphate, phosphoric acid) phosphate materials are used for in situ stabilization of soil Pb (Basta et al., 2001; Brown et al., 2003; Boisson et al., 1999; Cao et al., 2004; Clemente et al., 2005; Hettiarachchi et al., 2000; Hettiarachchi and Pierzynski, 2004). The amount of potentially toxic metals, like Pb and cadmium, available for plant root uptake can be decreased by the active complexes that form between the metals and organic matter in biosolids (Cheng et al., 2011; Brown et al., 2012; Attanayake et al., 2015, 2014; Paltseva et al., 2018). Since organic sources are added in large quantities, these additions can also dilute metal concentrations in soil, thereby further reducing the potential for plant uptake. Various P sources with different solubilities may influence the effectiveness of Pb immobilization. Chemical immobilization success can be determined by the capacity of the chemical to decrease contaminant's potential bioavailability to humans.

The transfer potential of contaminants from soil to plants is assessed using the bioconcentration factor (BCF) or the ratio of contaminant concentration in the plant to the contaminant concentration present in the soil. Smical et al. (2008) reported that the BCF is influenced by many environmental factors: plant species, soil type, the concentration and speciation of contaminant present.

Previously, it was standard procedure to use total soil concentration of soil contaminants to determine soil environmental quality standards. Because, soil physiochemical properties may cause metal bioavailability and speciation to vary significantly, which means predicting soil-to-plant transfer of contaminants based only on total soil contaminant concentrations is not reliable (Wang et al., 2006). McGeer (2004) defined "bioavailability" as the amount of a substance that is available to be absorbed across biological membranes within a specified time and under defined conditions. Although the terms bioavailability and bioaccessibility are used interchangeably, they are different. Bioaccessibility refers to the portion of a contaminant which is liberated from the soil and enters the gastrointestinal (GI) tract, which has the potential to be absorbed or adsorbed (if bioactive upon contact) by the organism (McGeer, 2004).

By decreasing the bioaccessible fraction of the contaminant, human exposure could be limited, and environmental protection would improve. Speciation (i.e., chemical forms) in soils is linked to Pb bioaccessibility (Scheckel and Ryan, 2004). Speciation is influenced by soil pH, organic matter content (Scheckel and Ryan, 2004), and the presence and abundance of P, manganese oxides and iron oxides/oxyhydroxides (Hettiarachchi et al., 2000; Kumpiene et al., 2008).

In situ remediation with soil amendments results in biogeochemical reactions, which convert contaminants to forms with less bioavailability, and this decreases human health and environmental risks. Challenges of using in situ remediation on skeet shooting range soils include the different soil properties and the vast amount of the contaminant. Thus, soil amendments capable of stabilizing the contaminant and efficiently decreasing its mobility in the soil environment are preferred. In Pb-contaminated soils, simultaneous immobilization and stabilization of Pb is beneficial.

Suitable animal model in vivo studies or in vitro gastrointestinal (GI) techniques that correlate well with animal model studies are needed to assess alterations in Pb bioavailability. In this study, a modified physiological based extraction test (PBET) was used to evaluate changes in bioaccessible Pb. The PBET showed a good correlation with Hettiarachchi et al. (2003) animal model study conducted with mine-affected soils and mine waste materials. A sequential extraction procedure (SEP) is another method for determining the potential mobility of Pb in soils. The SEP provides accurate data regarding the manner of occurrence and availability of metals in the soil as well as the possibility for mobilization and transport of metals (Tessier et al., 1979). These methods may be useful in a practical sense, but they could also be useful to ascertain relative variations in metal bioavailability (Pierzynski and Schwab, 1993). The objectives of this greenhouse study were to evaluate the effectiveness of various P sources at two different rates to mitigate the impact of vigorous *Miscanthus* growth on the bioaccessibility and uptake of Pb in contaminated skeet-range soil. Plant uptake measured plant availability, physiologically based extraction procedure measured bioaccessibility, and the sequential extraction procedure was employed to determine Pb distribution in soil.

Materials and Methods

Soil Collection and Processing

The soils used in this study were obtained from a currently unused area of military property in Fort Riley, KS (39°11′30″N 96°35′30″W) which is southwest of Manhattan and just north of Junction City. The base is primarily situated in Riley County, with only a small portion in Geary County. The chosen area for the research field was a leisure “skeet shooting” range with Pb pellets that was used in the 20th century. Approximately 1,000 mg kg^{-1} of Pb over 2m x 3m to a depth of approximately 15 cm. The Pb was heterogeneously distributed at the site, and the Pb concentration does reach 1,500 mg kg^{-1} in some places. Due to existing regulations, the research site is not currently active, but elevated levels of Pb in the topsoil could serve as a contamination source for neighboring areas.

Soils were collected from two different locations within the skeet range. The first was contaminated soil collected from the center of the training area at Fort Riley, while the second was a less-contaminated sample from outside of the training area. Soils were excavated to a depth of 0-15 cm using a shovel from both locations. Visible plant matter was removed from the soil before placing it into containers. The non-contaminated soil was placed into one large bucket while the contaminated soil was placed into 13-gallon polyethylene bags. The contents of the bucket were secured and transported immediately to Kansas State University for further processing. Upon arrival, the soil material was spread out evenly and left to air-dry in the Plant sciences greenhouse complex for about five days at ~25°C before being sieving with a stainless-steel sieve (4-mm opening). After sieving, the soil was thoroughly hand mixed to achieve as much homogenization as possible.

Twelve treatments were selected at two different rates: experiment included two controls treatments (non-contaminated control and contaminated control), first-rate was 76.5 mg P kg^{-1} soil as triple superphosphate (TSP-1X); apatite (AP-1X); struvite (ST-1X) and biosolids 45 Mg ha^{-1} (BS-1X); the second rate was 179.5 mg P kg^{-1} soil as TSP (TSP-2X); AP (AP-2X); ST (ST-2X), biosolids 100 Mg ha^{-1} (BS-2X) and the combination of TSP+AP-2X and TSP+ST-2X. Each treatment was designed based on the total P concentrations in each P source. The treatments were applied w/w basis evenly onto the soil, and the soils and treatments were thoroughly mixed together before being potted. The maximum water holding capacity (MWHC) of soil was determined using the protocol from Jenkinson and Powlson (1976). The nursery pots were lined with a cellophane bag containing only a few small holes to minimize free drainage. In March 2017, the pots were filled with ten kg of control or treated soils (top and bottom pot diameters were 24 and 22 cm, respectively) (March 2017).

Struvite and apatite are slow-release P sources due to their low solubility and compared to soluble phosphates (e.g. triple superphosphate), which cause eutrophication due to runoff, struvite and apatite have less mobility in the environment. In addition, apatite is a low-cost P source, and struvite is a recovered nutrient product from the wastewater treatment process. The origin apatite was rock phosphate and consisted mainly of fluro-apatite, obtained from Occidental Corp. (White Springs, FL) and previously was shown to be effective for immobilizing Pb in soil (Hettiarachchi et al., 2001). The struvite used in this study was obtained from the Greater Chicago Metropolitan Water Reclamation District, U.S.A. Biosolids material was obtained from the Oakland Wastewater Treatment Plant facility in Topeka, KS (Anaerobic digestion belt press). The material is classified as Class B biosolids and is rich in nutrients. The biosolids were stored in 15-gallon plastic containers at ~21°C until it was time to amend the soil.

Experimental Set-up

The duration of this study was nine months; this period was divided into three-month sections as follows: March,25 – June 25, June 25– September 15 and September 15 – December 16. The greenhouse study was conducted under controlled environmental conditions at the Department of Agronomy Greenhouse complex, Kansas State University, Manhattan, KS. The study was conducted using *Miscanthus* (a second-generation biofuel crop of unknown species) and each twelve treatments replicated three times and arranged in a completely randomized design. *Miscanthus* rhizomes were obtained from North Farm, Kansas State University, Manhattan, KS. Rhizomes were harvested from 10-year old miscanthus plots, and then were washed with tap water to remove soil from the roots. The large stand was cut by hand into smaller pieces because of the unequal shape of *Miscanthus* rhizomes. The rhizomes were placed in large plastic bags inside a bucket and stored in cold-storage conditions (<4°C). In early March 2017, the rhizomes were taken to the greenhouse for planting. In each pot, rhizomes weighing 30-35 g/plant were planted by hand at a depth of 10 cm. In order to ensure the rhizomes' center was at the desired death, holes were dug. There were 3-4 rhizomes per pot according to the weight of rhizomes, and pots were watered with deionized water each day (200 mL per pot). A 16-hour daylight and 8-hour dark photoperiod regime was maintained with overhead sodium lights (150 Wm^{-2}). The temperature during the study was within 15 to 21°C. Pots were rotated within block weekly to ensure that all pots received equal amounts of light. Pot servicing was performed by hand weeding each pot to reduce weed pressure to ensure good growth.

A nutritional solution was applied to ensure adequate nutrients to all pots except biosolids treatments were added one time per each growing period. The application rates were 100 mg kg^{-1} nitrogen (as urea), 60 mg kg^{-1} potassium (as K_2SO_4), 7 mg kg^{-1} zinc (as $ZnSO_4.7H_2O$), 7 mg kg^{-1} magnesium (as $MgSO_4.7H_2O$), 3 mg kg^{-1} copper (as $CuSO_4.5H_2O$), 3 mg kg^{-1} manganese (as $MnSO_4.H_2O$), and consequentially 95 mg kg^{-1} sulfur (950 mg S /10 kg) (Mason et al., 2013).

Miscanthus pots received the standard pesticide applications to suppress aphids and other insect pests due to aphid's infestation that began at the end of the first growing period. Aphids' infestations were first started in BS treated pots, where aphids were on the undersides of *Miscanthus* leaves and the stem. Insecticidal soap (potassium K salts of fatty acids) was used to control the aphids. Based on the labeling, it was essential to obtain thorough coverage of all plant parts and make repeated applications three times weekly. The aphids had developed resistance to insecticides and affected plant growth in second- and third-cuttings.

Harvesting and Sample Preparation

The height of the plant was recorded prior to plant harvesting. At the end of each growing period (June 25, September 15 and December 16), after the *Miscanthus* stems had senesced and the plurality of the leaves had dropped down, the plants were harvested. For harvest, all plants were cut at 5 cm above the soil surface using a hand-harvesting knife. All samples were weighed and placed in labeled paper bags and then oven-dried at 65 °C until a constant weight (g pot^{-1}, dry basis) to determine the dry matter by weight. The biomass sample was ground to pass through a 1mm sieve before analysis. The powdered plant material was then stored in labeled polyethylene vials until digested. At the end of the experiment, belowground biomass (rhizomes) was also collected and analyzed (Table B.2 in appendix B). After the last harvest, soil samples were collected from each pot. Soil samples were air-dried and sieved <2.0 mm size for chemical analysis.

Plant Sample Analyses

The ground plant material and homogenized plant samples were digested in triplicate to determine total Pb. For digestion, 0.5 g of ground plant material was weighed on a model SA120 Scientech precision balance (Scientech, Boulder, CO) and placed into 55 mL Teflon tubes (CEM, Mathews, NC). A 10-mL aliquot of trace metal-grade concentrated nitric acid (70%) was carefully added to the Teflon tubes, and then the contents were allowed to sit for 15 minutes in the fume hood to allow venting. Teflon tube lids were tightened, placed on the carousel, and digested in the CEM MARS unit (Matthews, NC). The tubes were ramped up to 200 °C and held for 15 minutes (1600 W at 100%, 15-minute ramp time, 15-minute cooling time). Following digestion, the tubes were removed from the unit and left to cool in the fume hood for an hour or until the contents were at room temperature. Teflon tubes were uncapped slowly, and the solution was filtered through Whatman No. 42 filter papers into screwed plastic scintillation vials under the fume hood. Samples were then stored at 4 °C until chemical analysis.

All plant samples were prepared in triplicate. National Institute of Standards and Technology (NIST) standard reference materials (apple leaves: 1515) were digested and analyzed alongside the plant materials. Plant samples were analyzed on an AA240Z graphite furnace atomic absorption spectrometer (Varian Inc., Australia) with Zeeman background correction.

Soil Chemical Analyses

Soil samples were air-dried and sieved to < 2 mm size for chemical analysis. Soil pH was determined in 1:10 soil: Milli-Q water using an Orion glass electrode (Thermo Orion, model A111). Soil texture was determined using the hydrometer procedure (Bouyoucos, 1962). Available N (NO_3^-N and NH_4^+N) was extracted by shaking with 1M KCl solution (Keeney and

Nelson, 1987), extractable K was determined by 1 M ammonium acetate (Helmke and Sparks, 1996). Extractable P was determined by Mehlich-3 (Mehlich, 1984). Total OC and N in soils were measured using dry combustion method on a LECO CNS-2000 analyzer (LECO Corporation, St. Joseph, MI). Cation exchange capacity (CEC) was measured by ion displacement (Jaynes and Bigham, 1986). Extractable Pb was determined by the Mehlich-3 extraction (Minca et al., 2013), followed by analysis using ICP–OES.

Total Pb and other trace element concentrations in the soils were determined by EPA method SW846-3051A (USEPA, 2007b). Approximately 10 mL of trace metal-grade concentrated HNO_3 was added to 0.5 g of soil and digested in a microwave digestion unit (MARS Xpress, CEM Corporation, Matthews, NC) to determine soil total Pb and other trace element concentrations. In the first step on the CEM MARS unit, the temperature of the soil-acid mixture was increased to 165 °C for 5.5 minutes. The temperature was further increased over a period of 4.5 minutes to 175 °C in the second step, and then the mixture was kept at 175 °C for 5 minutes. After digestion, the tubes were transferred from the unit and left to cool in the fume hood for an hour or until the contents were at room temperature. The soil solution was then filtered through Whatman No. 42 filter papers into screwed plastic scintillation vials under the fume hood. The BCF for plants is typically used to assess the transfer of contaminants from soil to plants (Samsøe-Petersen et al., 2002). Grasses accumulate metal contaminants in different tissues. The ability for metal to be transferred from the soil to the plants was evaluated using three parameters: bioaccumulation factor (BAF), bioconcentration factor BCF, and translocation factor (TF), according to following equations (Malik et al. 2010):

$$\text{BAF} = \frac{\text{metal concentration in aboveground (mg kg}^{-1}\text{)}}{\text{metal concentration in soil (mg kg}^{-1}\text{)}} \qquad \text{Eq. 1}$$

The BAF was calculated as the ratio of dry weight basis of the metal concentration in aboveground biomass and in the soil in which plants were grown.

$$\text{BCF} = \frac{\text{metal concentration in belowground parts (mg kg}^{-1}\text{)}}{\text{metal concentration in soil (mg kg}^{-1}\text{)}} \qquad \text{Eq. 2}$$

The BCF was calculated as the ratio of dry weight basis of the metal concentration in belowground biomass and in the soil in which plants were grown.

$$\text{TF} = \frac{\text{metal concentration in aboveground (mg kg}^{-1}\text{)}}{\text{metal concentration in belowground parts (mg kg}^{-1}\text{)}} \qquad \text{Eq. 3}$$

The TF was used to assess the ability of the plant to translocate the metal to the aboveground plant part from the belowground plant.

The calculations were done using Pb concentrations in biomass (across three cuttings) with total Pb concentration in soil at the end of the experiment.

A blank (trace-metal grade HNO_3 acid) and standard reference materials (NIST 2711a-Montana II) were digested and analyzed beside the soil materials for quality assurance. Samples were then stored at 4 °C until chemical analysis. The total concentrations of Pb and other trace elements were determined using the ICP-OES (Varian 720-ES).

Bioaccessible Pb

Bioaccessible Pb was determined by performing a modified PBET (Ruby et al., 1996) as described by Medlin (1997) on soils. The gastric solution was prepared by adding 1.25 g of pepsin, 0.50 g citrate, 0.50 g malate, 420 µL lactic acid, and 500 µL acetic acid to 1 L of deionized water. The gastric solution was made and acidified to pH 2.5 with trace metal-grade HCl, and then heated to 37°C before it was added to the soil. Next, 100-mL of the gastric solution were combined with the 1 g soil sample (sieved to <250 µm) in a 250-mL wide mouth HDPE bottle. The bottle was then capped tightly. The bottles containing the mixture were shaken for one hour at 150 rpm on a Queue incubator orbital shaker (Parkersburg, WV) preserved at 37 °C. After extraction, the pH of the soil solution was assessed. Different volumes of trace metal-grade concentrated HCl were used to adjust samples with deviations greater than 0.05 log units. The supernatant solution was filtered using 0.45-μm syringe filters into plastic scintillation vials and analyzed for Pb by GF-AAS. A soil standard reference material (Montana II) and blanks were routinely included in the PBET analysis as QA/QC samples.

In this study, extractable Pb was measured using 0.01 mol L^{-1} Sr $(NO_3)_2$. We used Sr $(NO_3)_2$ as it is less likely to be contaminated with trace metals and it also had the advantage of allowing soil pH to remain nearly unaltered during extractions. Here the exchange reactions that occurred closely represent the pH conditions of the bulk soil in the pots (Siebielec et al., 2006). The ratio of soil to 0.01 *M* Sr $(NO3)_2$ solution was 20g:40mL. After shaking the suspension for 1 hour, supernatant was separated and filtered using 0.45-μm syringe filters. The resulting solutions were digested using the EPA SW846-3015A (USEPA, 2007a) method in the microwave digestion unit to reduce the matrix suppression of Pb absorbance in the Graphite Furnace Atomic Absorption Spectrometry and balanced the matrix impacts in the extractions of soil. The solution was analyzed for Pb using graphite furnace atomic absorption spectrometry GF-AAS.

Sequential Extraction Procedure

In general, a sequential extraction procedure (SEP) is an analytical approach that removes elements out of soil samples utilizing a group of chosen extracting solutions to simulate the discharge of elements into solution under various environmental conditions. Sequential extractions are designed for the estimation of the amount of Pb bound to various soil constituents such as Fe/Al oxides, carbonates, and organic matter. The procedure, explained by Zwonitzer et al. (2003) and Scheckel et al. (2003), was applied with small alterations. For the organic matter bound extraction, NaOCl solution was used in place of H_2O_2 because H2O2 has been caused the dissolution of Mn oxides as well as alkaline earth carbonates and phosphates (Anderson, 1963; Jackson, 1956, Lavkulich and Wiens, 1970), while the NaOCl approach allows the efficient elimination of organic carbon which minimizes the dissolution of other minerals, such as crystalline Mn oxides.

Soil Pb was extracted as five operationally-defined fractions: exchangeable, bound to carbonates, bound to Fe and Mn oxides, bound to organic matter, and residual. We used the EPA Method 3051(USEPA, 2007b) (microwave digestion in nitric acid) as an alternative to an HF-$HClO_4$ mixture to extract the residual phase.

The extraction was carried out directly in 50-mL centrifuge tubes to decrease losses of solid material. Before each extraction step, the weight of the residue remaining in the centrifuge tube was recorded and solid losses were minimized. Samples were centrifuged at 2,750 rpm between each successive extraction for 10 minutes; the supernatants were filtered through 0.45-μm, and the tubes are weighed to account for metals remaining in the entrained solution before the addition of the next reagent. In between each step, the solid residue was washed with 8 mL of Milli-Q water and then vigorously shaken by hand. This was followed by 30 minutes in the centrifuge at 2,750 rpm. The supernatant was discarded before continuing with the next extraction step. The filtrate was acidified with two drops of 2% trace metal grade nitric acid and stored at 4 °C in the refrigerator until analysis.

Five steps of SEP (TableA.1 in appendix B) conducted in this study were as follows:

Step 1: Exchangeable (F1). Twenty-five mL of 1 M $MgCl_2$ was added to a 2 g soil sample placed in a 50 mL centrifuge tube which was sealed carefully. This procedure was repeated for each treatment. The tubes were placed on a shaker for 1 hour. The mixture was centrifuged at 2,750 rpm for 30 minutes, and the supernatant collected with 0.45 μm nylon syringe filters. Supernatants were acidified with two drops of 2% trace metal grade nitric acid and stored at 4°C prior to analysis. All extractions were performed in duplicates.

Step 2: Carbonates-bound (F2). Twenty-five mL of 1 M $NaC_2H_3O_2$ (Sodium acetate) adjusted to pH 5.0 with acetic acid (HOAc) was added to each of the residual treatments in step 1, and then sealed in respective tubes. The tubes were shaken for 5 hours, and centrifugation and filtration procedures were followed as in step 1.

Step 3: Iron and Manganese Oxides-bound (F3). Forty mL of 0.04 M $NH_2OH(HCl)$ in 25% (v/v) $HC_2H_3O_2$ (acetic acid) solution was added to each of the residual treatments in step 2 and then sealed in respective tubes. The tubes were heated for 6 hours at 96 °C, and centrifugation and filtration procedures were followed as in step 1.

Step 4: Organic Matter-bound (F4). Twenty mL of 7 M NaOCl (adjusted to pH 8.5 with HCl) solution was added to each of the residual treatments in step 3, and then sealed in respective tubes. The mixture was heated to 90°C for 2 hours with occasional agitation. After centrifuge separation, a second 20-mL aliquot of NaOCl (adjusted to pH 8.5 with HCl) was added, and the sample was heated again to 90 °C for 2 hours. Filtration procedures were followed as in step 1.

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

Statistical Analysis

Data analyses were performed using the PROC MIXED procedure and Tukey's Honestly Significant Difference (HSD) test used for means separation at alpha level 0.05 in SAS for

Windows version 9.4 (SAS Institute Inc., Cary, NC). Outlier data points were removed and considered missing data when the coefficient of Variation (CV) for treatment exceeded 25%.

Results and Discussion

Soil characteristics

The military site soil was classified as a silty clay loam (11.3 % sand, 59.8 % silt, and 28.9 % clay) (Table 4.1). Soil pH was near-neutral, and the electric conductivity was 1.3 mS cm^{-1}. The nutrients required for plant growth were within adequate concentrations in the soil. The CEC was moderate (16.6 cmol$_c$ kg^{-1}), whereas as the percentage of TOC was about 2.6%. The total Pb concentration was found to be 1043 mg kg^{-1}, which is much higher than the residential soil Pb level set by the USEPA, which is 400 mg kg^{-1} of soil (USEPA, 2001). The uncontaminated soil collected adjacent to the former skeet range had a Pb concentration of 122.4 mg kg^{-1}, elevated compared to the background Pb concentration in most surface soils (15-20 mg kg^{-1}).

The basic chosen physical and chemical characteristics of the biosolids used at the site are also presented in Table 4.1. The soil pH was slightly acidic. Total N, P, and Fe of the biosolids were 7.6 %, 13,017, and 11,200 mg kg^{-1}, respectively.

Table 4.1 Chemical and physical properties of the skeet range of the military site (0-15 cm) and the biosolids used in the study.

Soil property	Soil	Biosolids
pH (1:10 soil: water)	6.9	5.5
CEC †, cmol$_c$ kg^{-1}	16.6	-
Sand (%)	14.0	-
Silt (%)	60.0	-
Clay (%)	26.0	-
OC %‡	2.6	-
Total N (%)	0.2	7.6
Mehlich-3 P, mg kg^{-1}	32.4	-
Electrical conductivity (mS cm^{-1})	1.3	-
Ext. K mg kg^{-1}	445.5	-
NH_4^-N mg kg^{-1}	5.2	-
NO_3^-N mg kg^{-1}	12.9	-
Total P mg kg^{-1}	502.0	13,017.0
Pb mg kg^{-1}	1,043.0	43.0
Pb in un-contaminated soil mg kg^{-1}	122.4	-
Ca mg kg^{-1}	2697.8	4,6100.0
Mg mg kg^{-1}	227.5	4,500.0
Na mg kg^{-1}	9.7	1,370.0

†CEC: Cation Exchange Capacity

‡OC: Total organic carbon

Effect of Miscanthus growth and Treatments on Soil pH and Available P

Results of soil pH, Mehlich-3 extractable P, total Pb, extractable Pb by Sr $(NO3)_2$, and Mehlich-3 extractable P are presented in Table 4.2. Soil pH was measured after the third cutting of *Miscanthus*. The soil pH at the beginning of the experiment (6.9) was somewhat higher than the soil pH after 3 harvests. This is likely due to the absorption of cations by plants with the simultaneous release of H^+ causing the soil pH to decrease. The soil pH in all treatments at the end of the study ranged from 6.3 to 6.6 (Table 4.2), which is in the optimal range for *Miscanthus* (Heaton et al., 2010). In general, phosphate treatments combined with *Miscanthus* growth reduced soil pH to approximately 6.3 ± 0.01 and 6.4 ± 0.03 in all pots, except in the biosolids treated pots (Table 4.2). However, no significant differences were found between the treatments. The addition of TSP-1X reduced soil pH to approximately 6.3 ± 0.01, while biosolids showed a slight enhancement of soil pH at both rates. Secretions produced from the *Miscanthus* rhizomes caused the soil pH to fluctuate. Governing molecules in root exudates include different amino acids and organic acids, (e.g. citric, succinic, and propionic acids) which affects rhizosphere pH, desorption/dissolution of mineral nutrients, and the formation of metal complexes in soil (Ryan et al., 2001).

Organic amendments, such as biosolids, reduce the solubility of Pb and increase plant survival and productivity by acting as slow-release fertilizers (Gutser et al., 2005). This will contribute to the long-term success of the phytoremediation strategy. However, in this study, our plants, particularly those amended with biosolids, were compromised by aphid infestations, which damaged production and might have impacted the results. Because of that, unlike in our field study (Chapter 3), the biosolids treatments resulted in a poor growth (Figure 4.1).

In this study, Mehlich-3 (M3) was used to extract P since it is suitable for a wider range of soil pH compared to other soil tests (Ziadi et al., 2009). The highest M3 extractable P concentration was observed in BS-2X-added soils, followed by BS-1X biosolids-added soils (Table 4.2). This could be the reason that these treatments were more effective in reducing Pb availability in general. Soil amendments had increased extractable P levels, and those were well-above those required for average plant growth, which is about 20-30 mg kg^{-1} soil. However, apatite treatments (AP-1X and AP-2X) did not change extractable P levels significantly compared to the control soils, which indicates the relative insolubility of AP compared to the remaining P sources. While excessive levels of plant-available P are not toxic to plants, they are correlated with higher P losses in runoff. Thus, apatite may pose less environmental risk compared to the other P sources.

Total Pb concentrations at the end of the study ranged from 887 to 1075 mg kg^{-1}, and no significant ($P < 0.05$) effects were observed at all treatments. Investigators propose that continuous addition of organic matter in significant quantities dilutes total Pb concentrations in soils and would thus be useful (Attanayake et al., 2015, 2014; Brown et al., 2012; Defoe et al., 2014). Mehlich-3 extractable Pb ranged from 637 to 733 mg kg^{-1}, no significant differences ($P < 0.05$) were observed among the treatments. Soluble Pb as estimated by 0.01M Sr (NO3)2 extraction in the biosolids treatments showed significantly lower Pb concentration (0.1 mg kg^{-1}) compared with AP-1X (0.4 mg kg^{-1}) treatment.

Table 4.2 Selected chemical properties of the shooting range soil at the end of the experiment. CT-C= Contaminated control with no P amendments; TSP-1X= superphosphate-1rate; TSP-2X= superphosphate-2rate; AP-1X= Apatite-1rate; AP-2X=apatite-2rate; ST-1X= Struvite-1rate ; ST-2X=Struvite-2rate; BS-1X=Biosolids-1rate; BS-2X=Biosolids--2rate; TSP+AP-2X=TSP with AP-2rate; TSP+ST-2X=TSP with ST-2rate.

Treatment	pH	Mehlich-3-P	Total Pb	Extractable Pb	
				$Sr (NO_3)_2$	Mehlich-3
		------------------------mg kg^{-1}	--------------------------		
CT-C	6.4 ± 0.03†	22 ± 0.4	1045 ± 9	0.3 ± 0.02	733 ± 26
TSP-1X	6.3 ± 0.03	63 ± 3.3	1011 ± 18	0.3 ± 0.04	679 ± 21
BS-1X	6.6 ± 0.07	101 ± 8.2	957 ± 10	0.1 ± 0.02	637 ± 38
AP-1X	6.5 ± 0.03	25 ± 0.9	1016 ± 25	0.4 ± 0.1	670 ± 43
ST-1X	6.4 ± 0.03	77 ± 11	1035 ± 60	0.3 ± 0.03	650 ± 35
TSP-2X	6.4 ± 0.03	102 ± 1.3	1075 ± 59	0.3 ± 0.03	659 ± 38
BS-2X	6.6 ± 0.03	183 ± 16	887 ± 13	0.1 ± 0.01	584 ± 10
AP-2X	6.5 ± 0.03	28 ± 0.2	1008 ± 25	0.3 ± 0.04	713 ± 26
ST-2X	6.4 ± 0.03	107 ± 11	999 ± 30	0.2 ± 0.02	693 ± 22
TSP+AP-2X	6.4 ± 0.06	66 ± 4	989 ± 30	0.3 ± 0.03	653 ± 14
TSP+ST-2X	6.4 ± 0.03	103 ± 33	973 ± 41	0.2 ± 0.02	707 ± 48
		HSD‡			
	0.2	36.9	172.7	0.18	159.9
		PR>F§			
	<.0001	<.0001	0.0691	0.011	0.1376

† Standard error mean
‡Tukey’s Honest Significant Difference for mean comparisons with an alpha (α) of 0.05
§ P-Value

Biomass Production

The above ground biomass of *Miscanthus* is shown in Figure 4.1. Over three growing periods, overall dry-matter yield was greatest in the inorganic treatments. The inorganic treatments had the highest overall dry-matter yield during the three growing periods. This yield was higher than the control plot, which had a biomass yield of 82.4, 45.7, and 13.0 (g/pot), respectively. However, the increase was not statistically significant. Pots in which BS was added displayed lower dry-matter yield. Though, the biosolids treatment did not have statistically significant effects on plant biomass but it does contain more nutrients. Therefore, biosolids can enhance soil performance overtime by increasing nutrient and water retention capacity. But it should be noted that the biosolids treatments resulted in weak growth, which is illustrated in Figure 4.1. The biosolids treatments may have resulted in differential responses in growth for two potential reasons. First, a few pests' infestations affected the *Miscanthus* biomass starting towards the end of the first growing period. One of these pests was the aphids, which are known to use *Miscanthus* as a host and to be able to cause damage to plants (Bradshaw et al., 2010). Second, it could be that the rate of biosolids utilized was limited to cause a statistical treatment effect. Increasing the rate of biosolids may cause it to have a more significant treatment effect, but this needs to be investigated. Overall, the aboveground biomass exceeded root biomass in all treatments. Bradshaw et al. (2010) reported that in four states of the US corn leaf aphid (*Rhopalosiphum Maidis*) and yellow sugarcane aphid (*Sipha Flava*) were observed to be high enough to begin leaf death in field-grown *Miscanthus.*

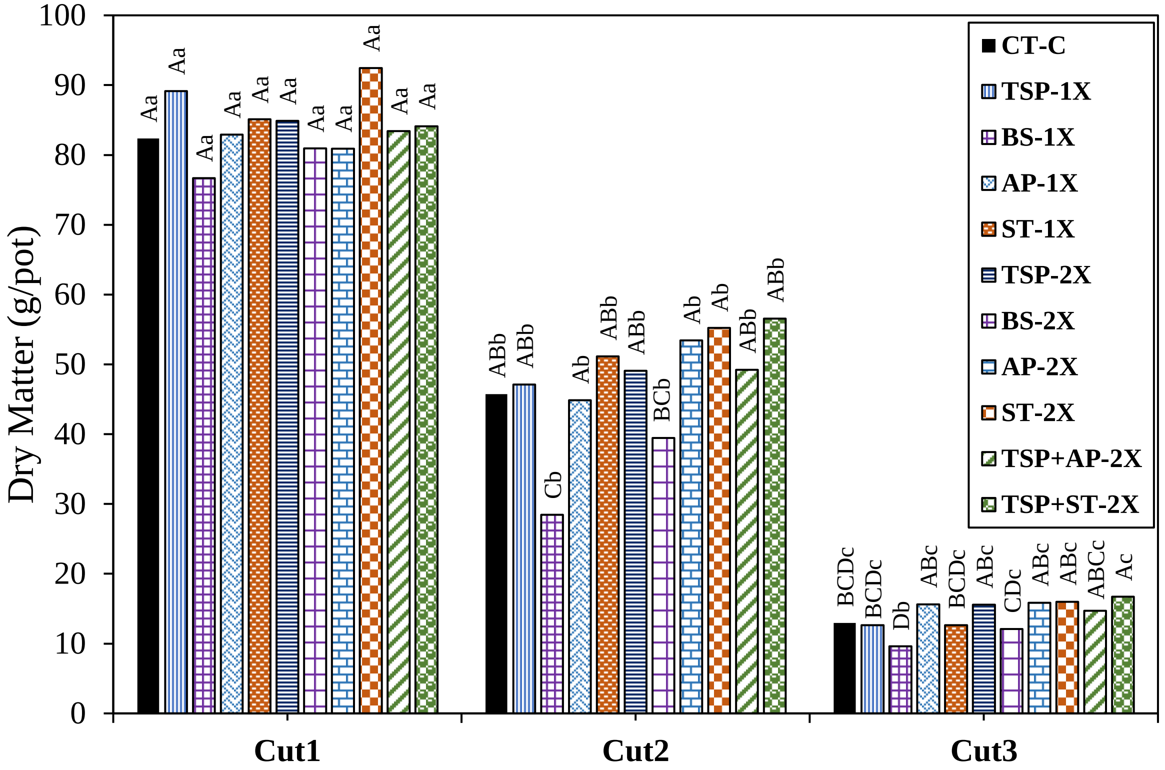

Figure 4.1 Biomass yield of *Miscanthus* harvested over the time periods. Uppercase letters compare treatments within each growing period, and lowercase letters compare each growing period within each treatment. CT-C= Contaminated control with no P amendments; TSP-1X= superphosphate-1rate; TSP-2X= superphosphate-2rate; AP-1X= Apatite-1rate; AP-2X=Apatite-2rate; ST-1X= Struvite-1rate ; ST-2X=Struvite-2rate; BS-1X=Biosolids-1rate; BS-2X=Biosolids--2rate; TSP+AP-2X=TSP with AP-2rate; TSP+ST-2X=TSP with ST-2rate. Letters indicate statistical significance among treatments ($P < 0.05$). Vertical bars represent the mean of three replicates. Different letters within each period indicate a significant difference ($\alpha=0.05$).

Biomass Pb Concentrations

The Pb concentrations in the aboveground of *Miscanthus* are shown in Figure 4.2. In the first, second, and third growing period, aboveground parts of *Miscanthus* plants had differing concentrations of Pb. For all periods of growing biofuels crops, metal concentrations were obtained in the above-ground parts, but differences in concentration were not statistically significant. As shown in Figure 4.2, Pb concentration in plant tissues was not significantly different between the treatments in all three cuttings except for the two biosolids treatments in the third cutting. Although the aboveground biomass was lower, yet two biosolids treatments showed significantly low Pb concentration in the plant in the third cuttings indicating that Pb uptake by *Miscanthus* was significantly lower in the biosolids added plots (Figure 4.2).

Additionally, the interactions were not significant. Plant Pb concentration was decreased in all treatments in the second cutting compared to the first cutting. In the third cutting, the plant Pb concentration was increased again and was similar to the first cutting. The reason for this increased most likely due to the concentration effect due to reduction in biomass production as a result of aphid’s infestation. Lead concentrations were less than 30 mg kg^{-1} in plants grown in biosolids treated soil at all periods while Pb concentrations in plants grown in untreated or the contaminated control pots were approximately 35 mg kg^{-1} in the first period.

In addition to the dilution effect of biosolids on soil Pb concentration, some soil Pb may have been incorporated into pyromorphite, making it less mobile in the soil and the roots of plants (Basta and McGowen, 2004; Hettiarachchi and Pierzynski, 2002; Yang et al., 2001). Biosolids amendment may have caused a transformation of Pb to less available forms of Pb for absorption by *Miscanthus* as well as the translocation from roots to aboveground plant parts. It is not clear why TSP or other P treatments did not show a significant reduction in plant Pb concentration; it could be due to significant acidulation of soil by TSP. It should be noted that Pb concentrations in the aboveground *Miscanthus* in all treatments were below the maximum tolerable dietary limits for animals, which is 100 mg kg^{-1} for cattle (NRC, 2005).

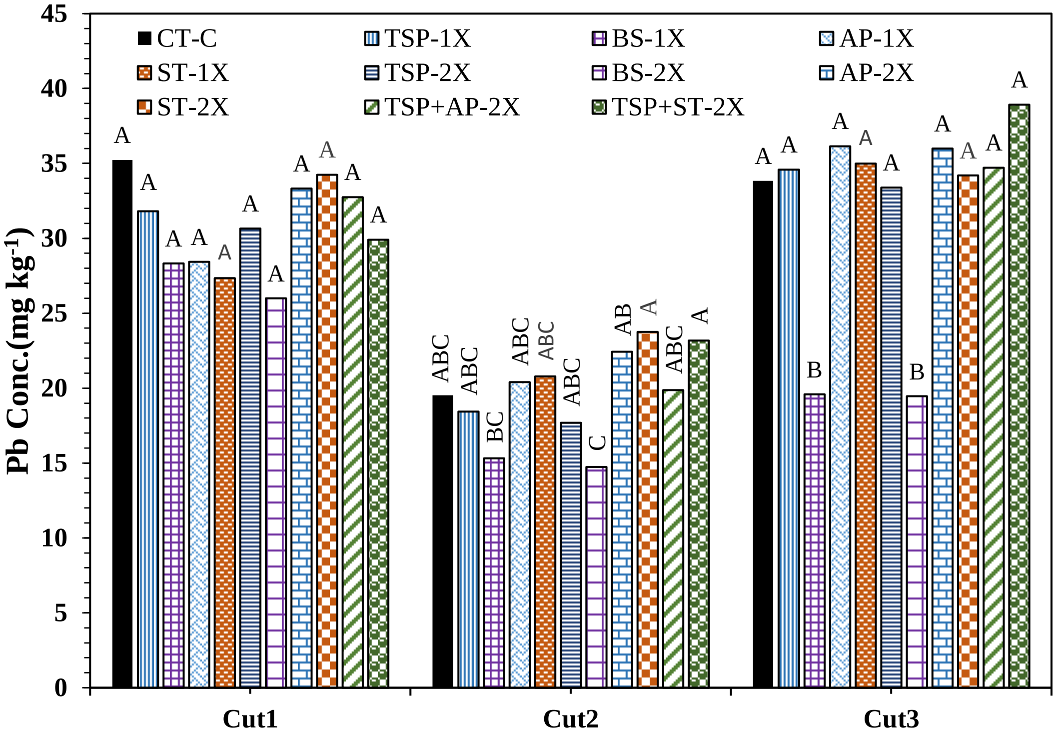

Figure 4.2 Lead concentrations in aboveground parts of *Miscanthus* harvested over the periods. Vertical bars represent the mean of three replicates. CT-C= Contaminated control with no P amendments; TSP-1X= superphosphate-1rate; TSP-2X= superphosphate-2rate; AP-1X= Apatite-1rate; AP-2X=patite-2rate; ST-1X= Struvite-1rate ; ST-2X=Struvite-2rate; BS-1X=Biosolids-1rate; BS-2X=Biosolids--2rate; TSP+AP-2X=TSP with AP-2rate; TSP+ST-2X=TSP with ST-2rate. Different letters within each period indicate a significant difference (α=0.05).

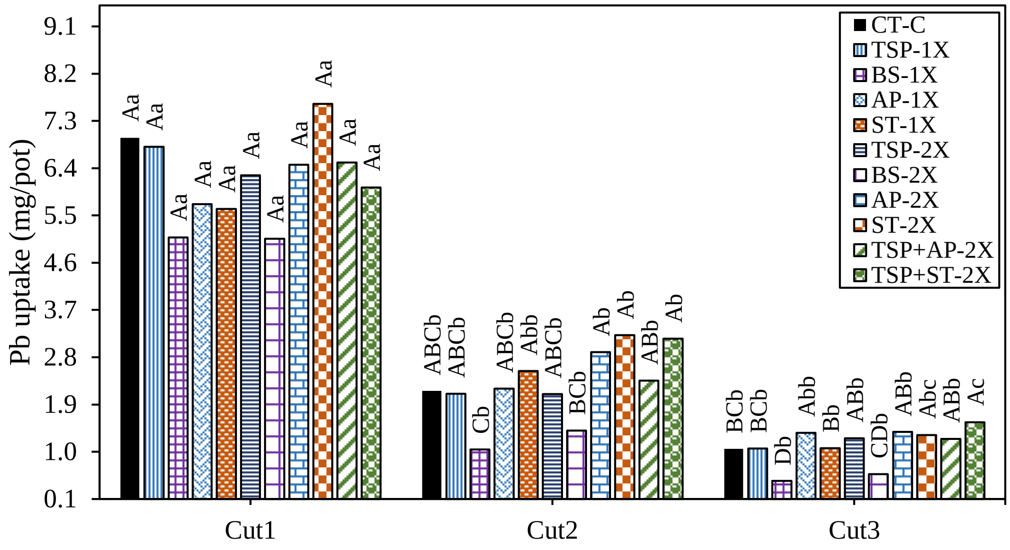

Figure 4.3 Lead uptake by *Miscanthus* harvested over the time periods. Uppercase letters compare treatments within each growing period, and lowercase letters compare each growing period within each treatment. CT-C= Contaminated control with no P amendments; TSP-1X= superphosphate-1rate; TSP-2X= superphosphate-2rate; AP-1X= Apatite-1rate; AP-2X=Apatite-2rate; ST-1X= Struvite-1rate ; ST-2X=Struvite-2rate; BS-1X=Biosolids-1rate; BS-2X=Biosolids--2rate; TSP+AP-2X=TSP with AP-2rate; TSP+ST-2X=TSP with ST-2rate. Letters indicate statistical significance among treatments ($P < 0.05$). Vertical bars represent the mean of three replicates. Different letters within each period indicate a significant difference (α=0.05).

The values of BAF, BCF, and TF are presented in Table 4.3. Increased Pb concentrations in the soil also increased its accumulation in plant rhizome. The rhizome's capability of binding Pb in its xylem is connected to high accumulation of Pb in rhizome (MacFarlane and Burchett, 2000), and this limits translocation to other plant parts (Finster et al., 2004). Both the rates of biosolids amendment reduced Pb concentrations in the *Miscanthus*. This is not surprising, P binds soil Pb and makes it less mobile in the soil (Hettiarachchi et al., 2002; Basta and McGowen, 2004). However, it is not clear why the TSP or the other P treatments did not show this same effect in this soil. The behavior for perennial grasses was mentioned by many authors as reviewed in the works of Nsanganwimana et al. (2014a, 2014b) and Pidlisnyuk et al. (2018).

The bioaccumulation of Pb was low for all treatments with biosolids treatment at both rates being the lowest. In the biosolids treated pots, bioaccumulation factor was 0.02 at 1X and 2X, which was lower than other treatments. This shows plant Pb uptake is not solely dependent upon the soil-metal concentrations. The physicochemical parameters such as soil type, and soil pH may also account for the differences in bioaccumulation of Pb. Alloway (2013) noted that organic matter could bind to certain trace metals, such as Pb, in the soil medium, which would limit uptake into the plant system. In addition to this, Pb solubility increases with decreasing pH (Martinez and Motto, 2000).

The Pb-BCF values were slightly higher (0.3) for BS-1X, ST-2X, and TSP ST-2X, while other treatments were 0.2; however, they did not come close to the critical value of 1 due to low mobility (Andrejic et al., 2019). Lead accumulation in *Miscanthus* above ground plant tissues is low, and the highest concentrations of Pb in *Miscanthus* were found in belowground organs.

Results in this study complement earlier observations of several heavy metals in *M.×g* (Pidlisnyuk et al., 2018; Nurzhanova et al., 2019), as well as Cd in three different *Miscanthus* species planted on contaminated soils (Guo et al., 2016). Our results are also consistent with studies that note the limitation of Pb translocation from below ground parts to the above ground (Kabata-Pendias, 2010). The Pb concentrations in rhizomes were in ranges considered toxic to above ground parts, but this is likely due to a lack of mobility in rhizome cell walls and complexation within cortex vacuoles (Kabata-Pendias, 2010; Andrejic et al., 2019). This accumulation in the rhizome system may determine that roots serve as a barrier to translocation of Pb from below ground plant parts to above ground plant parts (Sharma and Dubey, 2005), indicating that uptake and translocation rates of Pb in plants should be low (Kratovalieva and Cvetanovska, 2001). However, Pb concentrations in belowground parts of *Miscanthus* from contaminated pots are higher than aboveground parts (Kabata-Pendias, 2010).

Table 4.3 Bioaccumulation (BAF), bioconcentration (BCF), and translocation factor (TF) of Pb in contaminated shooting range soils. CT-C= Contaminated control with no P amendments; TSP-1X= superphosphate-1rate; TSP-2X= superphosphate-2rate; AP-1X= Apatite-1rate; AP-2X=patite-2rate; ST-1X= Struvite-1rate ; ST-2X=Struvite-2rate; BS-1X=Biosolids-1rate; BS-2X=Biosolids--2rate; TSP+AP-2X=TSP with AP-2rate; TSP+ST-2X=TSP with ST-2rate.

Treatment	BAF	BCF	TF
		mg kg^{-1}	
CT-C	0.03 ± 0.000†	0.2 ± 0.03	0.1 ± 0.01
TSP-1X	0.03 ± 0.000	0.2 ± 0.03	0.2 ± 0.02
BS-1X	0.02 ± 0.004	0.3 ± 0.007	0.1 ± 0.02
AP-1X	0.03 ± 0.004	0.2 ± 0.02	0.1 ± 0.02
ST-1X	0.03 ± 0.004	0.2 ± 0.01	0.2 ± 0.01
TSP-2X	0.03 ± 0.005	0.2 ± 0.03	0.2 ± 0.02
BS-2X	0.02 ± 0.004	0.2 ± 0.07	0.1 ± 0.00
AB-2X	0.03 ± 0.004	0.2 ± 0.01	0.2 ± 0.01
ST-2X	0.03 ± 0.004	0.3 ±0.01	0.1 ± 0.01
TSP+AP-2X	0.03 ± 0.004	0.2 ± 0.004	0.2 ± 0.01
TSP+ST-2X	0.03 ± 0.005	0.3 ± 0.03	0.1 ± 0.00
		HSD‡	
	0.012368	0.14494	0.0659
		Pr > F§	
	0.1004	0.5755	0.0002

† Standard error mean
‡Tukey’s Honest Significant Difference for mean comparisons with an alpha (α) of 0.05
§ P-Value

Influence of Miscanthus Growth on Soil Pb Bioaccessibility

Bioaccessible Pb was quantified following the Ruby et al. (1996) PBET procedure slightly modified according to Medlin (1997). This method was used to evaluate the amount of bioaccessible Pb and to find the influence of misc *Miscanthus* anthus growth and phosphate additions on decreasing Pb bioaccessibility. Bioaccessible Pb concentrations ranged from 29 to 41% of total Pb in the soil (Figure 4.4). Bioaccessible Pb was not statistically different in soils between treatments, except for the BS-2X and TSP-2X treatments, both of which have significantly lower bioaccessible Pb when compared to control pots. It seems that *Miscanthus* may affect the percentage of bioaccessible Pb.

Results from this study indicate that the differences do not continue after three cuttings of *Miscanthus*. When relatively soluble forms of P are added to soils, there are several potential alterations: plant uptake, microbial immobilization, precipitation with metals (e.g., Ca, Mg, Fe, Al, Pb, Cd, and Zn), and adsorption by inorganic and organic components. `Assuming P and Pb react immediately, these outcomes support plants' ability to take some of the P precipitated by Pb. There is no indication that apatite provided additional P for plant growth. Laperche et al. (1997) experimented with sand culture (a method of growing plants hydroponically, without the use of soil) by using grass to assess the solubility of apatite with or without two apatite sources: artificial apatite or natural phosphate rock. They observed that without either apatite sources, shoot Pb content increased an average of 10 to 100 times depending on the quantity of apatite applied compared to the corresponding treatments with apatite or phosphate rock plus apatite. Their research confirms plants can enhance the dissolution of pyromorphite, which releases Pb that is available for plant uptake.

The addition of biosolids at high rate (BS-2X) significantly decreased bioaccessible Pb in the soil after plant growth when compared to the control. Biosolids amended soils have a higher concentration of organic carbon (C) which may decrease the amount of bioavailable Pb in the soil by making stable complexes with Pb (Brown et al., 2003). Unlike the TSP-1X treatment, the addition of TSP2-X maintained significantly lower bioaccessible soil Pb levels compared to the control. Though the effect was not significant, the TSP-1X treatment did reduce bioaccessible Pb levels in the soil when compared to the control.

A study conducted by Hettiarachchi et al. (2001) in several different locations, evaluated the influence of P and other soil amendments on five different metal-contaminated soils and mine wastes. The results showed increasing the amount of P added from 2,500 to 5,000 mg kg^{-1} resulted in a significantly higher reduction in bioavailable Pb. Brown et al. (1999) reported adding TSP at 10,000 mg of P kg^{-1} of soil was insufficient in decreasing in vitro Pb or Pb uptake by tall fescue (*Festuca Arundinacea*), while TSP at 32,000 mg of P kg^{-1} of soil was the most effective treatment. The authors stated adding TSP at 10,000 mg kg^{-1} however, effect of the lower rate of TSP on soil pH is high enough to make the beneficial effect of TSP on reductions of bioaccessible or plant available Pb. Pelfrêne et al. (2015) studied the effects of reducing bioavailable metal concentrations in contaminated soils under greenhouse conditions in northern France. They studied the effects of long-term using a greenhouse study on the reduction of environmental and human health risk. The results of the study showed misca *Miscanthus* nthus planting could impact the oral bioavailiblty of Cd, Pb, and Zn when used for phytostabilization.

The biosolids treatments had the highest M3 extractable P of all the treatments at the end of the experiment (Table 4.2), and also showed a reduction of Pb bioaccessibility (Figure 4.4). Lead favors binding with Fe oxides and organic C functional groups over phosphate ligands because Pb^{+2} is a soft Lewis acid and therefore favors soft Lewis bases (such as Fe oxides and organic C functional groups over phosphates that are hard Lewis bases). Soils with high amorphous Fe oxide and organic C concentrations that are moderately contaminated with Pb would mostly support Pb solubility by adsorption/desorption reactions.

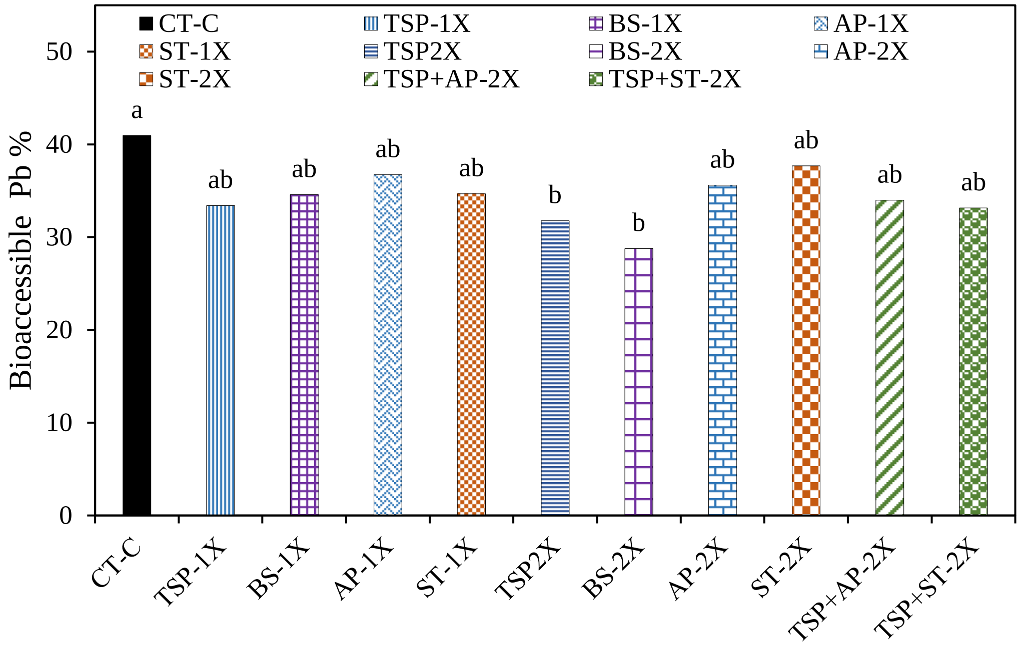

Figure 4.4 Bioaccessible Pb by PBET for the contaminated soil amended with phosphate amendments. CT-C= Contaminated control with no P amendments; TSP-1X= superphosphate-1rate; TSP-2X= superphosphate-2rate; AP-1X= Apatite-1rate; AP-2X=Apatite-2rate; ST-1X= Struvite-1rate ; ST-2X=Struvite-2rate; BS-1X=Biosolids-1rate; BS-2X=Biosolids--2rate; TSP+AP-2X=TSP with AP-2rate; TSP+ST-2X=TSP with ST-2rate. Vertical bars represent the means of three replicates. Different letters indicate a significant difference (α=0.05).

The Effect of Miscanthus and Phosphorus Treatments on the Mobility and Availability of Pb in Soil

The Pb sorption/desorption reactions between the solution and solid phases determines Pb concentrations in soil solution and the availability of Pb to plants (Yang et al., 2006; Lindsay, 1991). The Pb distribution in soil fractions is affiliated with soil pH, organic matter, free iron oxide, cation exchange capacity, soil minerals, and co-existing ions (Hu et al., 2012). Operationally-defined Pb fractions in soils were determined by following the Tessier et al. sequential extraction procedure, SEP (Tessier et al., 1979); results are presented in Table 4.4. Lead concentrations in the soil fractions depended on the different phosphate treatments. In the original (untreated) soil, the order for the different Pb fractions was as follows: Carbonate-bound > Fe-Mn oxide-bound > exchangeable > residual > organic-bound.

The exchangeable Pb fraction was lower than the carbonate-bound and ranged from 199.0 to 317.0 mg kg^{-1}. It makes up to 21 to 32 % of total Pb in the soil. The BS-1X and 2X had significantly lower exchangeable when compared to other treatment groups (Table 4.4). This is something we saw repeatedly with few different extractions- all indicating that this treatment is making Pb less plant available and less bioavailable to humans. The carbonate-bound Pb varied between soils (Table 4.4), and the values ranged from 281.0 to 346.0 mg kg^{-1} comprising up to 28 and 36% of total Pb in the soil. There were no significant differences in carbonate-bound values for biosolids and inorganic phosphates treatments except ST-1X, TSP-2X, TSP+AP-2X, and TSP+ST-2X, which had significantly lower values compared to the control. In control soil, there was an increased amount of carbonate bound Pb, most likely due to the root-bound effect. It appears that some P treatments were successful in overcoming that root bound effect to some extent. Carbonate-bound Pb was the highest fraction in this soil. The iron-manganese oxide-bound Pb fraction ranged from 248.0 to 309.0 mg kg^{-1}, forms about 24 to 30% of the total Pb in soil. The iron-manganese oxide-bound Pb and organic-bound Pb fractions results had no significant differences in all treatments. The content of organic-bound Pb fraction in soils (Table 4.4) had a range from 19.7 to 27.0 mg kg^{-1} with 2 to 3% of total Pb in the soil. There were no significant differences between treatments in this fraction. Organic-bound Pb fraction was lower in the soils primarily due to lower soil organic matter contents, and a high decomposition rate of organic matter due to the elevated temperature of the region (Błońska and Lasota, 2017). Investigation of the data further showed that organic-bound Pb in soil was less than all fractions. Residual-Pb ranging from 167.7 to 243.3 mg kg^{-1} comprising of 16 to 25% of total Pb in the soil. There were no significant differences in residual-Pb for all treatments except TSP+ST-2X, which had significantly higher values compared to the control.

The exchangeable, bound-to-carbonate, and bound-to-Fe and Mn oxide soil fractions contained the majority of the extractable Pb in the skeet-range soil. The organic-bound and residual soil fractions contained relatively little extractable Pb when compared to the bound-to-carbonate, bound-to Fe and Mn oxide, and exchangeable soil fractions. Carbonate-bound and exchangeable metals are able to become bioavailable more quickly compared to other soil fractions because they are weakly bound to soil components and may be considered more mobile (Pardo et al., 1990). The second fraction (carbonate fraction) contained the highest percentage of Pb (36%) found in the study. This is likely caused by the processes Pb ammo shots undergo for weathering such as the oxidation of metallic Pb and carbonation of oxidized Pb (PbO) (Cao et al., 2003). The second most concentrated fraction was the exchangeable containing about 32% of Pb, which posed a medium environmental risk. This fraction was followed by Fe and Mn oxide bound fraction which had 30% of Pb.

The exchangeable fraction is the most mobile and readily available for biological uptake in the environment, and electrostatic adsorption holds the Pb in this fraction (Singh et al., 2005). In the present study, the geochemical fractionation results are consistent with the high affinity of Pb for bound-to-Fe and Mn oxides minerals also. It is not surprising that the Fe and Mn oxide-bound fraction holds more extractable Pb, because Pb is an easily hydrolyzed metal, which binds to (hydr)oxide soil minerals at ideal soil pH's at a high rate (Mckenzie, 1980). The Fe and Mn (hydr)oxide form a significant sink for Pb in the aquatic system. The Pb absorption resulted in moderately higher concentrations of Pb associated with the Fe-Mn colloids in this fraction (Purushothaman and Chakrapani, 2007). The surface area and surface site density are comparatively larger for oxide minerals than for carbonate (Bilinski et al., 1991). According to Bordas and Bourg (2001), absorption and co-precipitation cause Pb to aggregate from the aqueous system in this phase. According to Sharmin et al. (2010) oxide (both amorphous and crystalline) minerals are capable of being released into the environment when bound to Pb in the presence of a low pH. It is evident from the results of sequential extraction that the Pb in the shooting range soil is bound to different fractions with various strengths.

Table 4.4 Lead fractions as percentage in the shooting range soil samples collected at the end of the greenhouse study as influenced by phosphate treatments. CT-C= Contaminated control with no P amendments; TSP-1X= superphosphate-1rate; TSP-2X= superphosphate-2rate; AP-1X= Apatite-1rate; AP-2X=Apatite-2rate; ST-1X= Struvite-1rate ; ST-2X=Struvite-2rate; BS-1X=Biosolids-1rate; BS-2X=Biosolids--2rate; TSP+AP-2X=TSP with AP-2rate; TSP+ST-2X=TSP with ST-2rate.. Each column represents the mean of three replicates and different letters indicate a significant difference (α=0.05).

Treatments	Fractions %				
	Exchangeable	Carbonate - bound	Fe and Mn oxide bound	Organic-bound	Residual
CT-C	29 ± 0.3† a	40 ± 0.3 a	30 ± 0.3 a	2 ± 0.0 a	16 ± 0.0 b
TSP-1X	30 ± 0.9 a	35 ± 2.0 ab	27 ± 2.0 a	3 ± 0.3 a	19 ± 0.0 ab
BS-1X	21 ± 1.0 b	36 ± 2.0 ab	29 ± 0.7 a	3 ± 0.0 a	24 ± 1.0 ab
AP-1X	32 ± 1.0 a	35 ± 2.0 ab	27 ± 2.0 a	2 ± 0.3 a	20 ± 1.0 ab
ST-1X	31 ± 2.0 a	33 ± 2.0 b	24 ± 2.0 a	3 ± 0.3 a	20 ± 1.0 ab
TSP-2X	30 ± 0.9 b	31 ± 0.6 ab	26 ± 4.0 a	2 ± 0.3 a	22 ± 2.0 ab
BS-2X	20 ± 0.9 a	36 ± 0.9 ab	30 ± 1.0 a	3 ± 0.0 a	25 ± 1.0 ab
AP-2X	29 ± 2.0 a	34 ± 0.6 ab	26 ± 2.0 a	2 ± 0.0 a	20 ± 3.0 ab
ST-2X	32 ± 2.0 a	34 ± 2.0 ab	25 ± 1.0 a	2 ± 0.0 a	19 ± 1.0 ab
TSP+AP-2X	30 ± 0.3 a	28 ± 2.0 b	26 ± 0.9 a	2 ± 0.0 a	22 ± 3.0 ab
TSP+ST-2X	28 ± 1.0 a	31 ± 1.0 b	27 ± 2.0 a	3 ± 0.3 a	25 ± 6.0 a
Total	310	374	297	27	233

† Standard error mean

Tukey’s Honest Significant Difference for mean comparisons with an alpha (α) of 0.05

Conclusion

The present study showed that *Miscanthus* could grow in contaminated skeet-range soil. *Miscanthus* with biosolids treatments showed a low Pb uptake. Lead bioaccessibility decreased as the P rate increased for both biosolids and TSP treatments. The biosolids treatments were equal to or more effective in reducing Pb bioaccessibility in this soil compared to inorganic P treatments. The increase of available P in soil by the biosolids treatment helped reduce the transfer of soil Pb to plants and potential transfer to humans. Continuous addition of biosolids would help to maintain a significantly lower total Pb concentration in soils, reducing the potential risk of soil Pb being transferred to *Miscanthus* by decreasing plant Pb, and to humans by reducing bioaccessible Pb concentrations. The sequential fractionation of Pb in soils showed that Pb was bound to different fractions with various strengths. The distribution of Pb in different fractions proves their variation in bioaccessibility and mobility. Since the bioaccessibility of the contaminant is affected by physical, biological, and chemical properties and processes, field studies should be conducted evaluating the ability of these amendments to be practically useful.

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Chapter 5 - Evaluation of In Situ Soil Amendments on Speciation and Bioaccessibility of Pb in Contaminated Military Soil: An Incubation Study

Abstract

Phosphorus (P) is known to bind metals such as lead (Pb) in contaminated soils effectively. The stabilization of Pb using additions of P to the highly contaminated soils of military sites through precipitation as Pb phosphates have been well documented. A six-month-long laboratory incubation study was conducted using a soil with 1,092 mg kg^{-1} total Pb concentration with a neutral pH, using a randomized complete block design with three replications. The main objectives of the study were to evaluate the effectiveness of multiple sources of P treatments at different rates on reducing soil Pb bioaccessibility over time. Fifteen treatments consisted of three rates; treatments were: non-amended (control); triple superphosphates, apatite and struvite at 5:3, 5:6 and 5:15 Pb:P; Biosolids at 45,100 and 225 Mg ha^{-1}; TSP+apatite and TSP+struvite 1:1 mix at 5:15 Pb:P. Changes in bioaccessible Pb over time were measured using a modified physiological based extraction test (PBET). Chemical changes in Pb were evaluated using XAS. Although all treatments had a little acidulating effect, the pH of biosolids amendments was consistently lower than other treatments throughout the study. Percent of bioaccessible Pb in biosolids amendments were significantly (α=0.05) lower than the other amendments despite the more acidic pH. High biosolids treatments (5X) was the most effective in controlling the bioaccessibility of soil Pb, and the reductions ranged from 63 to 76% compared to the control. Pyromorphite minerals dominated irrespective of the treatments, Pb speciation in

this soil. This study suggests that in this skeet-range soil contaminated with Pb, organic P source was most effective in reducing environmental risks.

Introduction

Lead (Pb) is a natural component of all soils, and is ubiquitous throughout the environment ((Adriano, 2001; Mielke et al., 2010). Different anthropogenic activities in past years have increased the amount of Pb in the environment through processes such as mining, sewage sludge application, fossil fuel combustion, and manufacturing industries (Basta et al., 2005). Another, often overlooked contributor, is outdoor firing ranges. Activities on shooting ranges, such as training, testing, and the deployment of weapons, have resulted in both severe and chronic troubles in the environmental system at military sites and surrounding areas. Firing ranges have negative impacts on the environment. A primary concern is the transport, and ultimate fate, of potentially toxic metal contaminants from ammunition that accumulate in the soil. Of these, Pb is the dominant contaminant in ammunition. The Agency for Toxic Substances and Disease Registry (ATSDR) listed Pb as the second most hazardous substance in the National Priority List (NPL) sites in the US (ATSDR, 2017). Because the form of Pb defines its mobility and bioavailability, speciation becomes an important crucial environmental issue in Pb contaminated soils.

This research centers on forming Pb phosphates through phosphate amendments in Pb-contaminated skeet-range soil, particularly pyromorphite, which is the most insoluble form of Pb in soils under a wide range of environmental conditions (Nriagu, 1984). Laboratory evidence confirms the hypothesis that Pb phosphates can form quickly when sufficient Pb and phosphate are existing in aqueous systems (Zhang and Ryan, 1999a, 1999b). Pyromorphites, $Pb_5(PO_4)_3X$ where X = F, Cl, Br, and OH, are the most insoluble and stable form of Pb in soils under a wide range of natural conditions (Nriagu, 1984; Lindsay, 1979; Hettiarachchi and Pierzynski, 2002; Scheckel et al., 2013). These compounds are more insoluble than other Pb forms observed in soils. Many reports have shown immobilizing Pb as pyromorphite in soils is beneficial (Chappell and Scheckel, 2007; Chen et al., 2006; Hashimoto et al., 2009; Hettiarachchi and Pierzynski, 2004; Hettiarachchi et al., 2004; Yoon et al., 2007). Butkus and Johnson (2011) studied the potentialities of inadequate immobilization or dissolution of pyromorphite after P treatment. Investigations have successfully applied P amendments to control Pb mobility and bioavailability in shooting range soils for many years (Ogawa et al., 2015; Seshadri et al., 2017). For example, in a study conducted in shooting range soil by Sanderson et al. (2015), they found that phosphate amendments caused a decrease in Pb bioaccessibility by up to 55%. Siebielec et at., (2012) observed the effectiveness of biosolids compost as organic phosphate in the rapid stabilization of Pb at military range contaminated soils. This approach is more economical and less disruptive than the standard remediation option of soil removal.

A number of inorganic (e.g. triple superphosphate, apatite, hydroxyapatite, rock phosphate, phosphoric acid) phosphate materials are used for in situ stabilization of soil Pb (Chappell and Scheckel, 2007; Clemente et al., 2005; Basta et al., 2001; Cao et al., 2004; Hettiarachchi et al., 2000; 2001; Hettiarachchi and Pierzynski, 2004: Yang et al., 2001). Recent studies have also assessed the ability of biosolids as a suitable amendment to reduce soil Pb bioaccessibility (Attanayake et al., 2015; Brown et al., 2003 and 2005; Defoe et al., 2014). In addition to traditional organic amendments applied for nutrient cycling, it is also possible to use recovered products of the wastewater industry, such as struvite. Struvite is a crystalline phosphate mineral that frequently accumulates on equipment surfaces during anaerobic digestion as well as post-digestion processes (Siciliano, 2016). Struvite is known as an alternative way to recover P from wastewater and can be applied as a P source for agricultural soils (Plaza et al., 2007; Antonini et al., 2012). The solubility of struvite is low in the water; Naidu et al. (2012) reported that struvite dissolves slowly over time in soil environments.

Amendment impacts could differ across soils due to the chemical heterogeneity of soil environments. Furthermore, it is not well known how the newly formed Pb phosphates might be affected by the appropriate amounts of P source or by the distance in the soil from P-containing materials, which has effects for the amount of P that is required to be added and the level of mixing that is needed (Hettiarachchi et al., 2001). Understanding the influence of treatment additions in soil that can transform Pb to less bioavailable forms, especially pyromorphites, needs direct speciation investigations using techniques such as x-ray based advanced spectroscopic methods. Scheckel and Ryan (2004) reported that the purpose of the Pb stabilization study is to determine the quantity of soil Pb that is transformed into less soluble phosphate phases; however, this is uncertain in heterogeneous, often non-equilibrated systems such as soils. Speciation of metals in complex environments has been investigated by applying spatially-resolved synchrotron-based techniques, such as x-ray absorption near-edge spectroscopy (XANES), coupled with statistical analysis via linear combination fitting (LC F) (Baker et al., 2014; Hashimoto et al., 2009; Scheckel and Ryan, 2004; Smith et al., 2011). Here, we try to understand Pb speciation after subjecting the soil to an in vitro bioaccessibility procedure through a direct speciation technique, x-ray absorption near-edge spectroscopy (XANES). There are numerous results emphasizing that synchrotron-based x-ray spectroscopic techniques enable identification of biogeochemical transformations of contaminants upon stabilization. The identification of metal sorption and surface precipitation on soil components is also improved by XANES (Sparks, 2003). Newville, (2004) reported that the EXAFS spectra include information on bond distances, coordination number, and species of the neighbors of the target atom and provide more detailed insight into the local structure around the target atom (Arcon et al.,2005). When the chemical change is verified, the next challenge is evaluating the reduction associated with stabilized soil Pb.

Both in-vivo (animal model) and in-vitro (gastrointestinal technique) methods are used to investigate changes in Pb bioavailability. An animal model study conducted with mine-affected soils and mine waste materials showed a good correlation (Hettiarachchi et al., 2003; Li et al., 2015; Schroder et al., 2004; Smith et al., 2011). However, it is difficult to conduct animal feeding studies due to the cost, and the unfavorable public opinions connected with animal studies. Consequently, the use of in-vitro assays proven to be well correlated with animal model studies to measure treatment effectiveness is far more common. In this study, a modified physiological based extraction test (PBET) was applied to evaluate alterations in bioaccessible Pb over time.

The objective of this laboratory-based study was to 1) evaluate the effectiveness of P source, P rate and time on reducing soil Pb bioaccessibility, and 2) elucidate the mechanisms of P-induced reductions in bioaccessibility of Pb in a contaminated skeet shooting range soil.

Materials and Methods

Soil Collection and Processing

The soil used in this study was obtained from a currently unused area of military property in Fort Riley, KS (39°11′30″N 96°35′30″W) which is southwest of Manhattan and just north of Junction City. The base is primarily situated in Riley County, with only a small portion in Geary County. The selected area for the research field was a leisure skeet shooting range with Pb pellets from the 20th century. The Pb was heterogeneously distributed at the site, and the Pb concentration does reach 1,500 mg kg^{-1} in some places. Due to existing regulations, the research site is not currently active, but elevated levels of Pb in the topsoil could serve as a contamination source for neighboring areas.

Soils were excavated to a depth of 0-15 cm using a shovel. Visible plant matter was removed from the soil before placing it into 13-gallon container. The container was secured and transported immediately to Kansas State University for further processing. Upon arrival, the soil material was spread out evenly and left to air-dry for about five days at ~25°C before being subjected to sieving with a stainless-steel sieve (2-mm opening). After sieving, the soil was thoroughly hand mixed to achieve as much homogenization as possible.

Experimental Set-up and Treatments

The experiment included fifteen treatments: no P added (control); first-rate of 89.8 mg P kg^{-1} soil as triple superphosphate (TSP-1X), apatite (AP-1X), struvite (ST-1X) and biosolids at 45 Mg ha^{-1} (BS-1X); the second rate of 179.5 mg Pkg^{-1} soil as triple superphosphate (TSP-2X), apatite (AP-2X), struvite (ST-2X) and biosolids at 100 Mg ha^{-1} (BS-2X); the third rate of 448.8 mg Pkg^{-1} soil as triple superphosphate (TSP-5X); apatite (AP-5X); struvite (ST-5X), biosolids at 225 Mg ha^{-1} (BS-5X), and the combination 1:1 of TSP+AP-5X, and TSP+ST-5X. Struvite is a recovered nutrient product from the wastewater treatment process and obtained from the Greater Chicago Metropolitan Water Reclamation District, USA. Biosolids material was obtained from the Oakland Wastewater Treatment Plant facility in Topeka, KS (Anaerobic digestion belt press). The quantity of P needed for each treatment was calculated based on the total P content of the P source and applied w/w basis evenly onto the soil, and the soils and treatments were thoroughly mixed together. The biosolids treatments provided approximately 586, 1302 and 2929 kg of total P/ha, respectively, with 1X, 2X and 5X treatments. The experimental design was a completely randomized design with three replications. Two hundred grams of soil were used for each replicate and treatment were added on w/w basis. The soil pH was near neutral, so no pH adjustment or lime was added although pH was monitored throughout to understand the effect of treatments on soil pH. The treated soils were placed in 250 mL high-density polyethylene containers, added with deionized water to bring the moisture content to 60% of its maximum water holding capacity (MWHC). Soils were again mixed thoroughly with a glass rod before covering with a clear plastic wrap (minimize water loss) and secured with a rubber band. Individual batches of treatments were prepared and incubated for 7, 30, 90, and 180 days (total of 15*3*4 = 180 treatment bottles) at 25 °C in a precision low-temperature incubator. The containers were weighed weekly, and if it was below the 60% MWHC, moisture values were adjusted (using deionized water). All bottles were thoroughly mixed with a glass rod before returning to the incubator. Thorough mixing also helped to keep proper aeration during the incubation period. At the end of each period, the corresponding group of treatments were removed from the incubator, soils were air-dried at 40°C and dried soils analyzed for pH, electrical conductivity (EC), PBET-Pb, Mehlich-3 extractable P (M3-P), and Mehlich-3 extractable Pb (M3-Ex. Pb). Sub samples of soils were stored in plastic scintillation vials for speciation work.

Chemical Analysis

Prior to the initiation of the study, the soil was characterized for select physico-chemical parameters (Table 5.1). Available P was extracted by Mehlich-3 (M3-P) (Mehlich, 1984); soil pH was measured using a 1:10 soil: deionized water mixture and a pH meter with Thermo Scientific Orion glass electrode (Beverly, MA). Electrical conductivity (soil: deionized water; 1:5) was determined using an Inlab 731 electrode (Mettler-Toledo Inc.). Extractable Pb (Ex. Pb) was determined by using the Mehlich-3 P solution (Minca et al., 2013), and analyzed using ICP–OES. Total organic C in soils was measured using a dry combustion method on a LECO CNS-2000 analyzer (LECO Corporation, St. Joseph, MI). Available nitrogen (N) was determined by 2 M KCl extraction (Keeney and Nelson, 1982), extractable K was measured using1 M ammonium acetate (Helmke and Sparks, 1996). Cation exchange capacity (CEC) was determined by ion displacement (Jaynes and Bigham, 1986).

Total Pb and other trace element concentrations in soils were determined by EPA method SW846-3051A (USEPA, 2007). Briefly, 10 mL of trace metal-grade concentrated HNO_3 was added to 0.5 g of soil and digested in a microwave digestion unit (MARS Xpress, CEM Corporation, Matthews, NC) to determine soil total Pb and other trace element concentrations. The soil digestate was then filtered through Whatman No. 42 filter papers into plastic scintillation vials under the fume hood and capped. Total concentrations of Pb and other trace elements were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Varian Inc., Australia). A standard reference soil (NIST 2711a-Montana II) was digested and analyzed as a quality assurance/quality control (QA/QC) sample to assess digestion and analytical methods.

Physiologically Based Extraction Test

Bioaccessible Pb was determined by a modified PBET (Ruby et al., 1996) procedure as described by Medlin (1997) on soils. The gastric solution was prepared by adding 1.25 g of pepsin, 0.50 g citrate, 0.50 g malate, 420 µL lactic acid, and 500 µL acetic acid to 1 L of deionized water. The prepared gastric solution was acidified to pH 2.5 with trace metal-grade HCl, and then heated to 37°C before it was added to the soil. Next, 100-mL of the warm gastric solution was combined with the 1 g soil sample (sieved to <250 µm) in a 250-mL wide mouth HDPE bottle. The bottle was then capped tightly. The bottles containing the mixture were shaken for one hour at 150 rpm on a Queue incubator orbital shaker (Parkersburg, WV) and preserved at 37 °C. After extraction, the pH of the soil solution was assessed. Different volumes of trace metal-grade concentrated HCl were used to adjust samples with deviations greater than 0.05 pH units. The supernatant solution was filtered using 0.45-µm syringe filters into plastic scintillation vials and analyzed for Pb by GF-AAS. A soil standard reference material (Montana II) and blanks were routinely included in the PBET analysis for QA/QC.

Synchrotron-based X-ray Analysis

Air-dried composite samples from the first and fourth incubation periods were chosen for this study. Soils were prepared by mixing equivalent quantities of soil from each replicate of the treatments (i.e., composite sample representing all replicates equally). The triplicate samples were ground to a fine powder with an agate mortar and pestle to ensure maximum homogenization. Composite samples were packed in sample holders with 2 mm thick Plexiglas slits. Both sides of the sample holders were sealed with Kapton® tape and labeled prior to mounting on the sample stage. The slit allows the X-ray beam to pass through the sample that was sandwiched in between the two pieces of Kapton.

Pb X-ray absorption near-edge spectroscopy (XANES) spectra were collected at the beamline 5 BM-D (DuPont-Northwestern-Dow Collaborative Access Team, DND-CAT) at the Advanced Photon Source (APS), Argonne National Laboratory, in Argonne, IL. Data were collected at Pb L_{III}-edge (13035eV) in fluorescence mode with the Canberra Inc.13-element Ge solid-state detector. The selected beamline has an energy range between 4.5-25 keV and a-Si (III) double crystal monochromator with a focused beam size of 15000 x 500µm and an unfocused beam size of 1.2 x 12 mm. We used 1.2x 12 mm beam size.

Two Al foil layers were installed on the detector to decrease background fluorescence emissions due to Fe in soil samples. With respect to the sample surface, the angle of the incident X-ray beam was at 45°. The energy was calibrated using standard Pb-metal foil. With every sample, the XAFS of the Pb-metal foil standard was collected simultaneously. This standard spectrum was applied to distinguish any energy drifts induced by the monochromator drifts during the sample run. Twenty Pb standards were used for data analysis, including pyromorphite(Py), hydrocerussite (Hce), cerussite, Plumboferrite (Pf), Pb-adsorbed-hydroxyapatite (HA), Hydroxypyromorphite (HPy), Pb-hloropyromorphite (Cpy),Pb sorbed (SPb), Pb bound to humic acid (Pb-HA), Pb bound to fulvic acid (Pb-FA), anglesite (Ag), galena (Ga), leadhillite, magnetoplumbite, plumboferrite, hinsdallite, plumbogummite, vauquelinite and plumboyarosite. The Pb standards spectra were collected in transmission mode. The number of spectra collected per sample was 3 to 6 (mostly 6), and 2 scans were collected for each standard.

Data processing and analysis were done using Athena software version 0.8.061 (Ravel, 2005). In the process of data analysis, first, the spectra were merged, then normalized, and the background was removed. Then, the sample and standard spectra were aligned for any energy drifts using the first derivative spectra. The Liner Combination Fitting (LCF) was used to obtain combination fits using all of the standard Pb compounds mentioned above. Although a small region was used for fitting, the results can be used to describe and compare treatment differences.

Statistical Analysis

Statistical analyses were performed using PROC MIXED in SAS for Windows Version 9.4 (SAS Institute Inc., 2017). Treatment differences were identified using Tukey's Honestly Significant Difference (HSD) test for means separation (α = 0.05). Changes in bioaccessible Pb were evaluated as a function of time and treatment. The effects of P additions on bioaccessible Pb and percent of bioaccessible Pb $(i.e., [\text{bioaccessible Pb mg kg}^{-1}/\text{total soil Pb mg kg}^{-1}]\ x\ 100)$ were analyzed using PROC MIXED. Regression analysis was completed using Microsoft Excel 2016's statistics program. The variables in the regression models were selected by forward elimination of other predictors using SAS Version 9.4.

Results and Discussion

Soil Characterization

Selected physical and chemical properties of the soil are presented in Table 5.1. The soil is characterized as a silty clay loam (14% sand, 60% silt, and 26% clay). Soil pH was near-neutral, and the electric conductivity was 1.3 mS cm^{-1}. The CEC was moderate (16.6 $cmol_c$ kg^{-1}), whereas as the percentage of TOC was about 2.6%. The nutrients required for plant growth were within adequate concentrations in the soil. The concentration of soil Pb was found to be 1092.2 mg kg^{-1}, which is much higher than the residential soil Pb level set by the USEPA, which is 400 mg kg^{-1} of soil (USEPA, 2001). The majority of available N was in NO_3^- (12.9 mg kg^{-1}); whereas NH_4^+ was (5.2 mg kg^{-1}) the major available source of N in the soil.

The basic physical and chemical properties of the biosolids used in this study are presented in Table 5.1. The pH was slightly acidic. Total N, P, and Fe were 7.6 %, 13,017, and 11,200 mg kg^{-1}, respectively. The biosolids contained low levels of cadmium (Cd) and antimony (Sb), whereas it did contain measurable levels of Pb.

Table 5.1 Selected properties of the skeet range of the military site (0-15 cm) and the biosolids used in the study.

Soil property	Soil	Biosolids
pH (1:10 soil: water)	6.9	5.5
CEC †, cmol$_c$ kg^{-1}	16.6	-
Sand (%)	14.0	-
Silt (%)	60.0	-
Clay (%)	26.0	-
OC %‡	2.6	-
Total N (%)	0.2	7.6
Mehlich-3 P, mg kg^{-1}	32.4	-
Electrical conductivity (**mS** cm^{-1})	1.3	-
Ext. K mg kg^{-1}	445.5	-
NH_4^-N mg kg^{-1}	5.2	-
NO_3^-N mg kg^{-1}	12.9	-
Total P mg kg^{-1}	502.0	13,017.0
Pb mg kg^{-1}	1,092.0	43.0
Cd mg kg^{-1}	0.4	1.0
Zn mg kg^{-1}	49.3	266.0
Mn mg kg^{-1}	311.0	272.0
Cu mg kg^{-1}	9.7	91.0

†CEC: Cation Exchange Capacity
‡OC: Total organic carbon

Effects of Treatments on Soil pH

There was a significant decrease in pH caused by some P amendment additions (Figure 5.1). The acidulation effect of the high rate of biosolids addition was the highest and the pH of high biosolids treatment was significantly lower than all other treatments including the control. Measured pH in the control treatment stayed constant during first month incubation, then decreased to about 6.6 to 6.4 by the end of the study. The pH in most inorganic P treated soils continued to decrease slightly after one month and reduced to ~ 6.3 and 6.2 by the end of the study. The pH of BS 2X treatment decreased significantly by 90 (6.8) and 180 days (5.8) by the end of the study compared to the other amendments. The pH of BS 5X treatment was significantly ($p=0.05$) lower than all other treatments (~6.1, 6.1, 5.8 and 5.5) throughout the study period. Also, in the high ST treatment (5X) pH significantly ($p=0.05$) decreased to ~6.2, 5.8 after 30 and 180 days compared to the control and other amendments. Sullivan et al. (2015) reported that biosolids processing could influence the effect on soil pH following soil application. Mineralization of organic N to NH_4^+, followed by nitrification to NO_3^- produced protons and resulted in a decrease in pH. Another reason could be that the soluble salts in the biosolids potentially could displace protons bound to clay or organic matter and bring them into the soil solution.

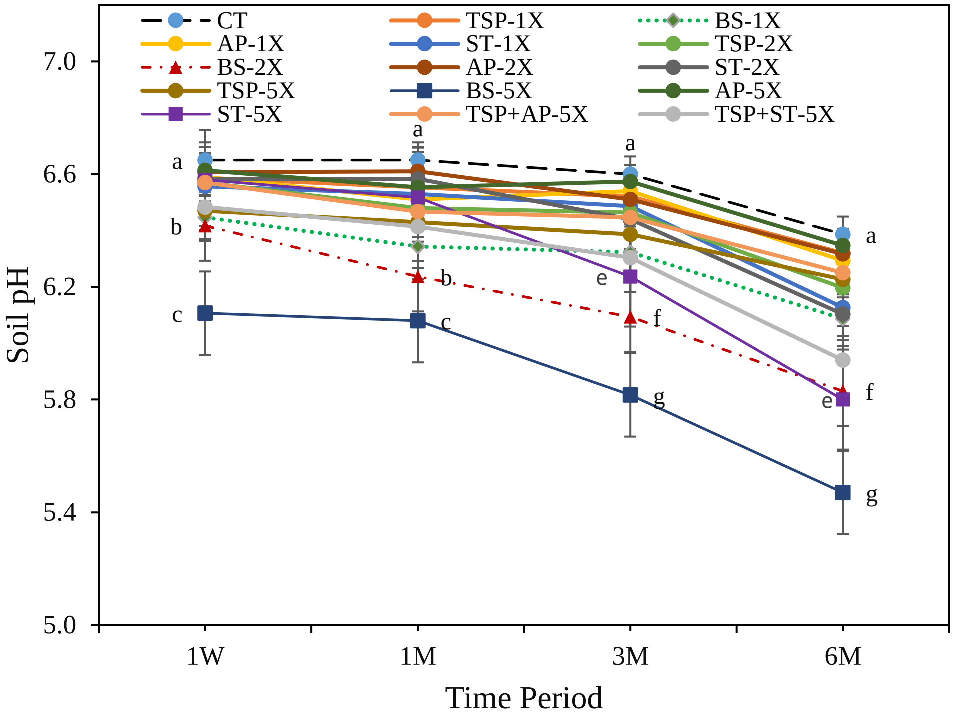

Figure 5.1 Soil pH of treatments during incubation study. CT= control; TSP-1X, TSP-2X, and TSP-5X = triple superphosphate ; AP-1X, AP-2X, and AP-5X= apatite; ST-1X, ST-2X, and ST-5X = Struvite ; BS-1X, BS-2X, and BS-5X = Biosolids ; TSP+AP-5X = triple superphosphate+ apatite , and TSP+ST-5X = triple superphosphate+Struvite. Different letters within each treatment indicate a significant difference (α=0.05).

Effect of Amendments on the Soil Chemical Properties

Soil amendment additions changed some of the chemical properties of the soil; however, all of these properties were significantly different from the control, specially biosolids treatments over time of periods (Table 5.2). Total Pb concentrations across four periods ranged from 1093 to 996 mg kg^{-1}, and there were a significant ($P < 0.05$) effects observed between biosolids 2X and 5x compared to control. The data showed that Pb concentrations were slightly reduced in soils where biosolids were added to the soil. The initial total Pb concentration in the soil was 1092 mg kg-1(prior to biosolids treatments), and at the end of the experiment, Pb concentration in biosolids added soil was 996 mg kg-1(8.8% reduction) in 5X (225Mg ha-1). This is due to the dilution effect as observed by other researchers using organic-based soil amendment addition to field plots at high rates (Brown et al., 2012; Attanayake et al. 2014; Defoe et al., 2014).

The EC was significantly ($P < 0.05$) higher in the biosolids treated soils (1X,2X, and 5X) compared to other treatments, although the EC values are well below the levels that can be found in saline soils (>4 mS cm^{-1}). Increases in soil EC after biosolids additions were due to large amounts of soluble salts existing in applied- biosolids. In addition, a slight overall increase in EC was observed for all treatments.

The M3-P confirmed that biosolids introduced additional amounts of inorganic soluble P into the soil as confirmed by M3-P concentration in the skeet-range soil (Table 5.2). Soluble inorganic P, more specifically, orthophosphate ions such as HPO_4^{-2} and $H_2PO_4^-$, can react with Pb to develop stable Pb phosphate minerals, such as pyromorphite (Scheckel et al., 2013). The M3-P concentrations were higher in the biosolids treatments than other P treatments. The highest M3-P concentration was observed in BS-5X-added soils, followed by BS-2X biosolids-added soils. Further, the BS-5X treatment was significantly ($P < 0.05$) different compared to the BS 1X, BS 2X treatments, and other phosphates treatments. The higher level of extractable P in biosolids -treated soil compared to the other treatments throughout the study period suggests that biosolids, especially at the high rate is capable of maintaining a high level of available P for a long time. As expected, TSP treatment showed the second highest M3-P, and this was followed by struvite and apatite treatments.

Soil amendments had a significant effect on extractable Pb concentrations ($P < 0.05$) (Table 5.2). Extractable Pb was significantly lower in the BS-5X treatment (46-51%) throughout the study compared to the low rates of biosolids (BS-1X and 2X) treatments as well as other treatments. Most inorganic phosphate amendments decreased extractable Pb percentages throughout the study, but these reductions were not significant compared to control and varied from one compound to another. Unlike for other treatments, when biosolids were added to the soil, the extractable soil Pb concentration was lowered due to two reasons, one is due to dilution effect upon addition of a large amount of biosolids and the second reason could be due to the chemical changes occurred due to P and organic C compound in biosolids. The biosolids amendments have been known to have a dilution effect on soil Pb concentrations. Farfel et al. (2005) recorded a 12 to 20% decrease in mean soil Pb concentrations of urban yards (800 to 2,003 mg kg-1) when they were amended with biosolids compost (110 to 180 dry t/ha).

Table 5.2 Selected soil properties as Mehlich-3P (M3-P), electrical conductivity (Ec), and Mehlich-3 extractable Pb, % (Ex. Pb) in the shooting range soil at day 7, 30, 90 and 180 of the incubation study as determined at pH 2.5. CT= control; TSP-1X, TSP-2X, and TSP-5X = triple superphosphate ; AP-1X, AP-2X, and AP-5X= apatite; ST-1X, ST-2X, and ST-5X = Struvite ; BS-1X, BS-2X, and BS-5X = Biosolids ; TSP+AP-5X = triple superphosphate+apatite , and TSP+ST-5X = triple superphosphate+Struvite. Means within a P treatment, significant different at P = 0.05 using Tukey's hone significance.

Treatment	Total Pb, mg kg^{-1}	Mehlich-3P, mg kg^{-1}				Electrical conductivity, mS cm^{-1}				Mehlich-3 extractable Pb, %			
		7d	30d	90d	180d	7d	30d	90d	180d	7d	30d	90d	180d
CT	1093±17	42.0	44.8	48.7	45.0	0.09	0.08	0.13	0.20	68	72	70	68
TSP-1X	1061±17	103.0	97.0	100.3	108.3	0.09	0.09	0.15	0.20	60	68	72	67
BS-1X	1024±10	124.3	134.7	132.3	142.0	0.21	0.20	0.29	0.38	58	67	70	64
AP-1X	1091±13	44.2	46.4	54.3	50.4	0.08	0.09	0.13	0.21	65	68	73	76
ST-1X	1076±18	44.2	137.9	53.9	55.8	0.09	0.09	0.17	0.24	60	68	74	70
TSP-2X	1058±19	140.0	142.7	145.3	162.7	0.09	0.10	0.15	0.22	60	68	71	69
BS-2X	1017±13	212.0	214.7	253.0	239.7	0.35	0.39	0.46	0.65	57	62	62	57
AP-2X	1070±12	46.7	51.1	57.0	50.3	0.08	0.09	0.15	0.20	73	72	73	71
ST-2X	1044±17	133.4	47.1	55.6	67.6	0.09	0.09	0.19	0.31	69	69	76	72
TSP-5X	1027±17	291.3	289.7	307.7	327.0	0.12	0.14	0.17	0.25	71	71	68	73
BS-5X	996±16	405.0	405.3	462.7	491.7	0.62	0.58	0.82	1.08	54	57	56	49
AP-5X	1024±9	49.4	57.9	69.1	55.9	0.09	0.09	0.14	0.20	69	71	73	70
ST-5X	1046±17	48.5	89.5	148.3	103.5	0.11	0.11	0.26	0.47	68	71	71	69
TSP+AP-5X	1029±12	176.0	173.0	197.3	181.7	0.16	0.11	0.15	0.18	63	68	70	68
TSP+ST-5X	1082±11	292.7	196.3	193.7	228.7	0.13	0.11	0.23	0.35	71	68	73	65
HSD†	71.1	161.0	130.0	69.0	33.0	0.04	0.04	0.07	0.04	16.0	4.8	11.0	10.0
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0013	<.0001	<.0001	<.0001

† Tukey's Honest Significant Differences for mean comparison with an alpha (α) of 0.0

Time and Treatment Effects on Pb Bioaccessibility

An in vitro bioaccessibility procedure was used to assess Pb bioaccessibility and ascertain the influence of treatment additions on reducing bioaccessible Pb at pH 2.5. Bioaccessible Pb concentrations ranged from 278 (BS-5X) to 484 (control) mg kg^{-1} (25 to 44% of total Pb in soil) (Figure 5.2 and 5.3). The PBET extraction showed a significant reduction in bioaccessibility ($p<0.05$), with all phosphate amendments ranging between 37%-44% after one week, 29%-36% after one month, 26%-35% after three months, and 25-34% after six months, with an overall mean of 41%, 34%, 32% and 31% for each period respectively. The BS-5X caused the most considerable reductions in bioaccessible Pb (reductions 63%, 71, 73, and 76%, respectively), followed by BS-2X, then BS-1X. Due to similar trends perceived between PBET-Pb and percent bioaccessibility and the dilution effect, our discussion will concentrate on percent Pb bioaccessibility (Figure 5.3). The values of percent bioaccessibility are "normalized" for variations in total Pb concentrations caused by the biosolids treatments. Our results show that percent of bioaccessible Pb was influenced by treatment and time. We noted insignificant fluctuations in percent bioaccessibility for the non-amended control over the incubation period. This fluctuation indicates that a small percent of Pb in this soil may be weakly bound, and thus consequently exchangeable, depending on soil pH. Slight changes were also seen in the soils amended with all phosphate amendments. This is most likely due to the slight acidification effect that was seen with increasing incubation time, but we do not assume that soil pH alone was sufficient for the increased percent bioaccessible Pb, though acidic conditions favor Pb solubility (Hettiarachchi et al., 2001). On the other hand, available P in the soil through biosolids addition is likely to have an active role.

Percent of bioaccessible Pb reduced with incubation time for most of the phosphate treatments. On average, in BS-1X, -2X, and -5X amended soils, relative reductions in percent bioaccessible Pb ranged between 60 and 75%, compared to the non-amended control, though there was variability among samples. The biosolids are known to increase a plant cover, dilute total Pb concentrations, and decrease bioaccessible Pb when applied to Pb-contaminated soils in mining wastes, contaminated soils. Previous research shows that biosolids and other sources of organic P can reduce total soil Pb and Pb accessibility (Attanayake et al., 2014; Defoe et al., 2014). Sauvé et al. (2000) reported that iron oxides have a high ability to adsorb Pb and concomitantly support a low free Pb^{2+} activity in solution. The partitioning of Pb in our soil could vary significantly based on soil properties. Earlier studies reported that Pb is connected with carbonate and organic fractions of the soil (Fayiga et al., 2011), exchangeable and organic fractions (Bruell et al., 1999), and residual Pb in firing range soils (Cao et al., 2003). It may be that biosolids are mainly responsible for adsorbing the Pb in solution, and thus reducing Pb's bioaccessibility. It is believable that the biosolids would be most effective since it contains both high amounts of P and Fe; these compounds have been reported to reduce Pb bioaccessibility significantly. Brown et al. (2005) noted a 37% decrease in Pb bioaccessibility upon the addition of high compost to a Pb-contaminated soil. Studies that examine bioaccessible Pb (determined at pH 2.5) in contaminated shooting-range soils are limited. The Pb bioavailability in soils contaminated with waste materials ranged from 10 to 28%, whereas it was 30 to 89% for contaminated shooting range soils as determined by an in the Vivo mouse model (Smith et al. 2011).

Under acidic soil pH conditions, the metal sorption capacity of soils is lower, due to the protonation of functional groups in inorganic and organic sorptive surfaces. Despite the strongly acidic conditions imposed during the PBET extraction (pH of 2.5), persistence of sorbed Pb in the solid phase indirectly suggests strong inner-sphere Pb adsorption or precipitation of more or less insoluble Pb solids. The soil Pb bioaccessibility observed in this study could be a result of the specific sorption of Pb onto Fe oxides and organic surfaces. To develop precipitates of Pb compounds, the concentration of Pb^{+2} in the soil solution should be high and exceed the sorption ability of the sorptive soils in the soil such as Fe-oxides, clay minerals, and organic molecules. These sorptive soils have a high affinity to Pb^{+2} ion, which is a soft Lewis acid with large atomic size. Essington (2004) reported that the soft Lewis acids favor binding with soft Lewis bases like those occurring in Fe-oxides and organic molecules over hard ligands such as PO_4^{-3}, CO_3^{-2}.

Several possible factors contribute to the variability of Pb bioaccessibility. Brown et al. (2003) and Attanayake et al. (2015), among others, have documented how soil amendments can help decrease the mobility of Pb in soil. In general, our results are compatible with previous studies that observed a significant decrease in bioavailable Pb through the addition of phosphate amendments (Melamed et al., 2003; Defoe et al., 2014; Henry et al., 2015; Hettiarachchi et al., 2003). The reduction occurs as a result of phosphate reacting with Pb to form more stable forms of minerals such as pyromorphite (Hettiarachchi et al., 2001; Scheckel et al., 2013). Many studies have determined that Pb sorbed onto clay minerals and oxides, combines with phosphate to form pyromorphite (Hettiarachchi et al., 2003; Scheckel et al., 2013; Defoe et al., 2014). In a study conducted in shooting range soil, Sanderson et al. (2016) reported that the bioavailability of Pb in phosphate stabilized soils can be reduced by 20–40% through the conversion of Pb into the pyromorphite complex. As noted by Porter et al. (2004), the ideal molar ratio of Pb:P in pyromorphite structure is 5:3; however, higher concentrations of P are likely needed in soils since other mineral surfaces can react with P and limit its availability by converting metal from more soluble species to the less soluble metal phosphate minerals (Nriagu, 1984).

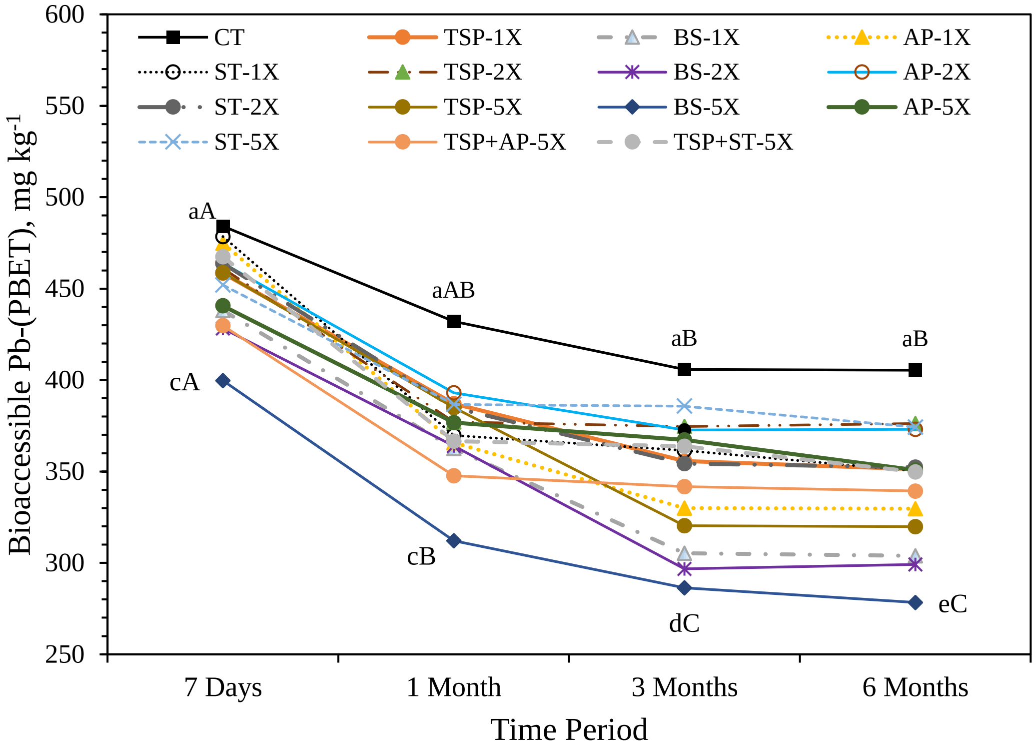

Figure 5.2 Bioaccessible Pb (PBET) concentrations of soils during the incubation study. The different Uppercase letters represent significant differences (P< 0.05) when comparing treatments within each period. The different lowercase letters compare each period within each treatment and represent significant differences (P< 0.05). Tukey’s HSD for mean comparison with an alpha (α) of 0.05.

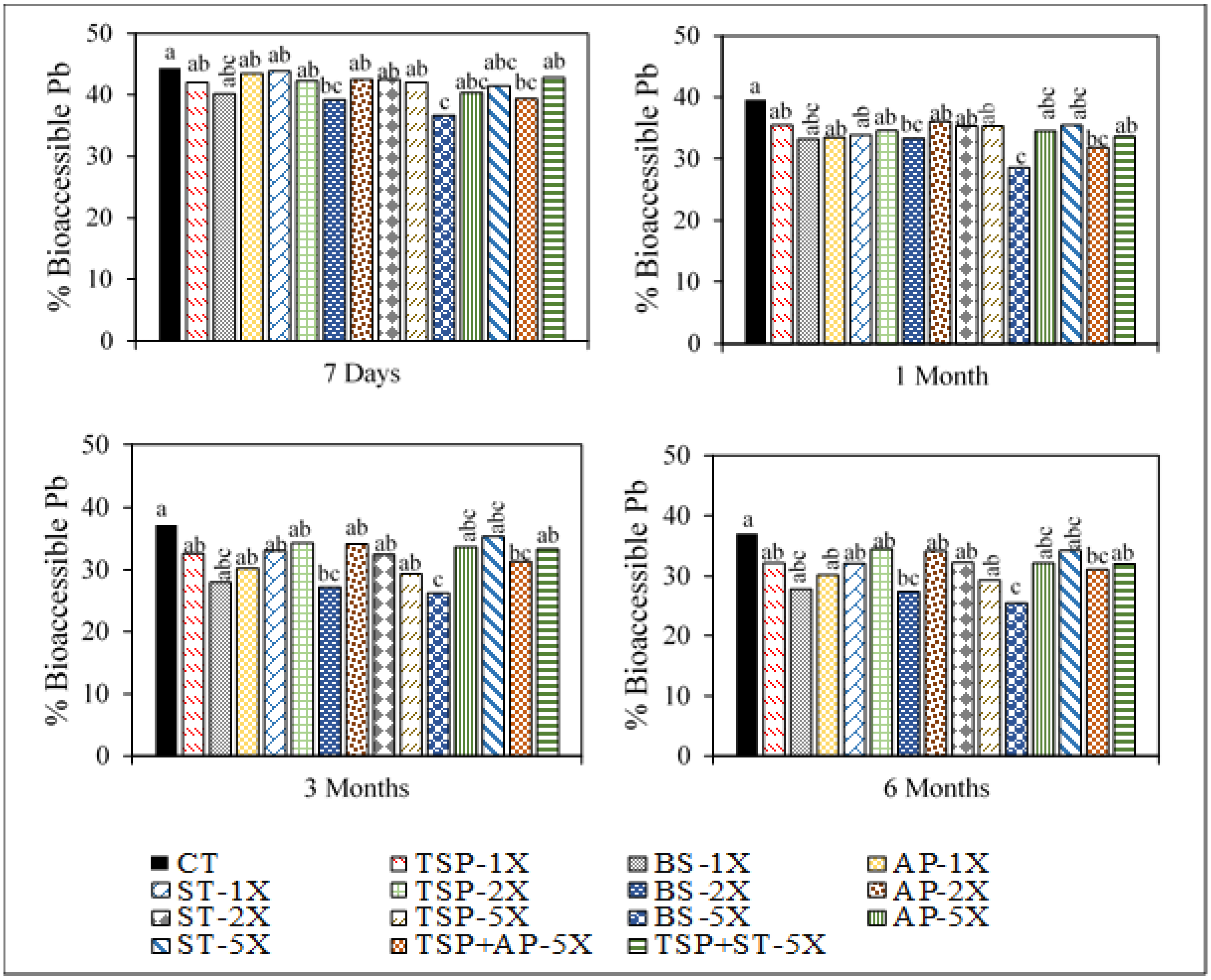

Figure 5.3 Percent bioaccessible Pb at day 7, 30, 90, and 180 of the incubation study. CT= control; TSP-1X, TSP-2X, and TSP-5X = triple superphosphate ; AP-1X, AP-2X, and AP-5X= apatite; ST-1X, ST-2X, and ST-5X = Struvite ; BS-1X, BS-2X, and BS-5X = Biosolids ; TSP + AP-5X = triple superphosphate + apatite , and TSP + ST-5X = triple superphosphate + Struvite. Values above bars represent the exact calculated percentages. Different letters within a treatment indicate a significant difference (α=0.05).

Effect of Treatment on Pb Speciation

At the end of the incubation study, Pb X-ray absorption near-edge spectroscopy (XANES) studies were performed on eleven select treatments. The treatments chosen for XANES studies were the non-amended control, TSP-1X, TSP-5X, AP-1X, AP-5X, ST-1X, ST-5X, BS-1X, BS-5X, and the combination of TSP+AP-5X, and TSP+ST-5X from the first period and last incubation period. In this experiment, Py represents the sum of chloro- and hydroxyl-pyromorphites, Pf represents Plumboferrite, Hce represents hydrocerussite, Pb-Fh represents Pb sorbed to Fe oxy(hydr)oxide, Pb-OC represents the Pb sorbed to SOC and Hap represents Pb-adsorbed-hydroxyapatite. The LCF analysis of the Pb XANES spectra for different P amendments indicated the presence of pyromorphite minerals in soils (Figure 5.4, Table 5.3). According to the results of the Pb XANES study, the dominant Pb species presented in non-amended soils were a mix of few Pb minerals or solids, included Py (44.5%), Pf (30.1%), Hce (15.3%), and Pb-OC (10.1%) at 7-days after incubation. While after 180 days, the non-amended soils Pb speciation was changed to Py (34.2%), Pf (30.3%), and Pb-OC (35.5%) (Table 5.3). No other Pb minerals were identified in the control samples. The Pb-OC fraction was only about ~10% in control. Schroth et al. (2008) observed that Pb sorbed to SOC may redistribute to other phases such as Fe oxy(hydr)oxide and birnessite, upon decomposition of SOC. Moreno et al. (2006) mentioned that the Pb-sorbed by iron oxides might form inner-sphere complexes limiting Pb solubility. Most of the treatments contain close proportions of pyromorphite, Pf, and Pb-OC at both periods. In addition to Py, Pf, and Pb-OC, some treatments contained various proportions of ferrihydrite sorbed Pb (TSP1X 24.4%, BS1X 22.6%, and the compensations of TSP+AP, TSP+ST 26.1% and 58.6%, respectively, after 7 days of incubation. Increased Fe-bound Pb upon P addition, resulted in evidence for the possibility of forming ternary complexes (Weesner and

Bleam, 1998). Seven days of incubation showed pyromorphite (63.7%) was the dominant Pb species. After 180 days, the same trend still existed (56.2%). This indicated that all P amendments were successful at changing Pb from more soluble species to the less soluble Pb phosphate minerals, particularly pyromorphite. This could partly be attributed to the temporary acidity produced during the dissolution of some P treatments that could potentially release Pb for the reaction. However, consequences differ by P amendment and incubation time. The efficiency of LCF relies on the data goodness and how well the reference spectra match the experimental samples (Roberts et al., 2002). Baker et al. (2014) stated that the best-fit compositions might not give true composition. The inclusion of other possible Pb standards spectra could further fine-tune our results.

Lindsay (1979), Ryan et al. (2001), and many others identified pyromorphite as the most stable form of Pb phosphates in soils. Hashimoto et al. (2009) reported that the increased amounts of chloropyromorphite when comparing a P amended, Pb contaminated soil incubated for 380 days compared to 7 days. Cotter-Howells and Thornton (1991), observed the formation of pyromorphite in garden soils with Pb concentration ranging from 2,400 to 22,800 mg kg^{-1}.

Pyromorphite mineral has low solubility, and therefore, the results obtained support the Pb bioaccessible results obtained from the PBET-Pb analyses. The difference in the bioaccessibility of Pb at each period (Figure 5.3) reflects the differing makeup of Pb species in the soil (Table 5.3). The source of contamination was due to weathering of ammunition, resulting in the accumulation of Pb in shooting range soils. Weathering products of Pb ammunitions include cerussite ($PbCO_3$), hydrocerussite [$Pb (CO_3)_2(OH)_2$], and small quantities of anglesite ($PbSO_4$) (Sever, 1993). Soluble minerals under neutral soil conditions, such as anglesite, may have been transformed into more stable Pb phosphate-like minerals over time.

Amendments of biosolids have shown a redistribution in Pb species, resulting in an apparent decrease in pyromorphite-like Pb species and increase of Pb-OC bound like species (Figure 5.4, Table 5.3). Acidic conditions and high P and Pb availabilities favor pyromorphite formation (Hettiarachchi et al., 2001; Scheckel and Ryan, 2004). Scheckel and Ryan (2004) confirmed that under normal environmental conditions, Pb or P availability could be a rate-limiting component for pyromorphite formation, which could guide the formation of other Pb phosphate minerals.

Table 5.3 Relative proportions of Pb species in the skeet range of the military site as estimated by linear combination fitting on XANES spectra.

Time	Treatment	Py†	Pf	Hce	Pb-Fh	Pb-OC	Hap	R-factor‡
		%						
7 days	CT	44.5	30.1	15.3	-	10.1	-	0.0010
	TSP 1X	-	12.5	-	24.4	58.1	-	0.0002
	BS 1X	-	21.7	-	22.6	55.8	-	0.0002
	AP 1X	63.7	29.5	-	-	6.8	-	0.0002
	ST 1X	56.9	14.9	-	-	28.3	-	0.0006
	TSP 5X	44.2	33.6	-	-	22.2	-	0.0002
	BS 5X	50.0	28.9	-	-	21.1	-	0.0002
	AP 5X	35.9	29.3	-	-	34.8	-	0.0002
	ST 5X	35.7	29.6	-	-	34.8	-	0.0002
	TSP+AP-5X	-	12.2	-	26.1	61.8	-	0.0001
	TSP+ST-5X	23.6	5.1	-	58.6	-	12.7	0.0005
180 days	CT	34.2	30.3	-	-	35.5	-	0.0002
	TSP 1X	35.9	41.4	-	-	22.7	-	0.0007
	BS 1X	23.7	31.3	-	-	45.0	-	0.0002
	AP 1X	50.3	30.5	-	-	19.3	-	0.0002
	ST 1X	51.1	31.2	-	-	17.8	-	0.0002
	TSP 5X	39.0	25.5	-	-	35.6	-	0.0002
	BS 5X	30.0	27.3	-	-	36.6	6.1	0.0002
	AP 5X	56.2	26.3	-	-	21.7	-	0.0002
	ST 5X	45.1	34.8	-	-	10.1	10.0	0.0003
	TSP + AP 5X	38.0	28.0	-	-	34.0	-	0.0002
	TSP + ST 5X	-	49.3	-	-	43.9	30.4	0.0015

† Py: sum of chloro and hydroxyl pyromorphites, Pf: Plumboferrite, Hce: Hydrocerussite, Pb-Fh: ferrihydrite sorbed Pb, Pb-OC: Pb adsorbed to SOC, Hap: Pb-adsorbed-hydroxyapatite.
‡ R-factor: normalized sum of the squared residuals of the linear combination fit.

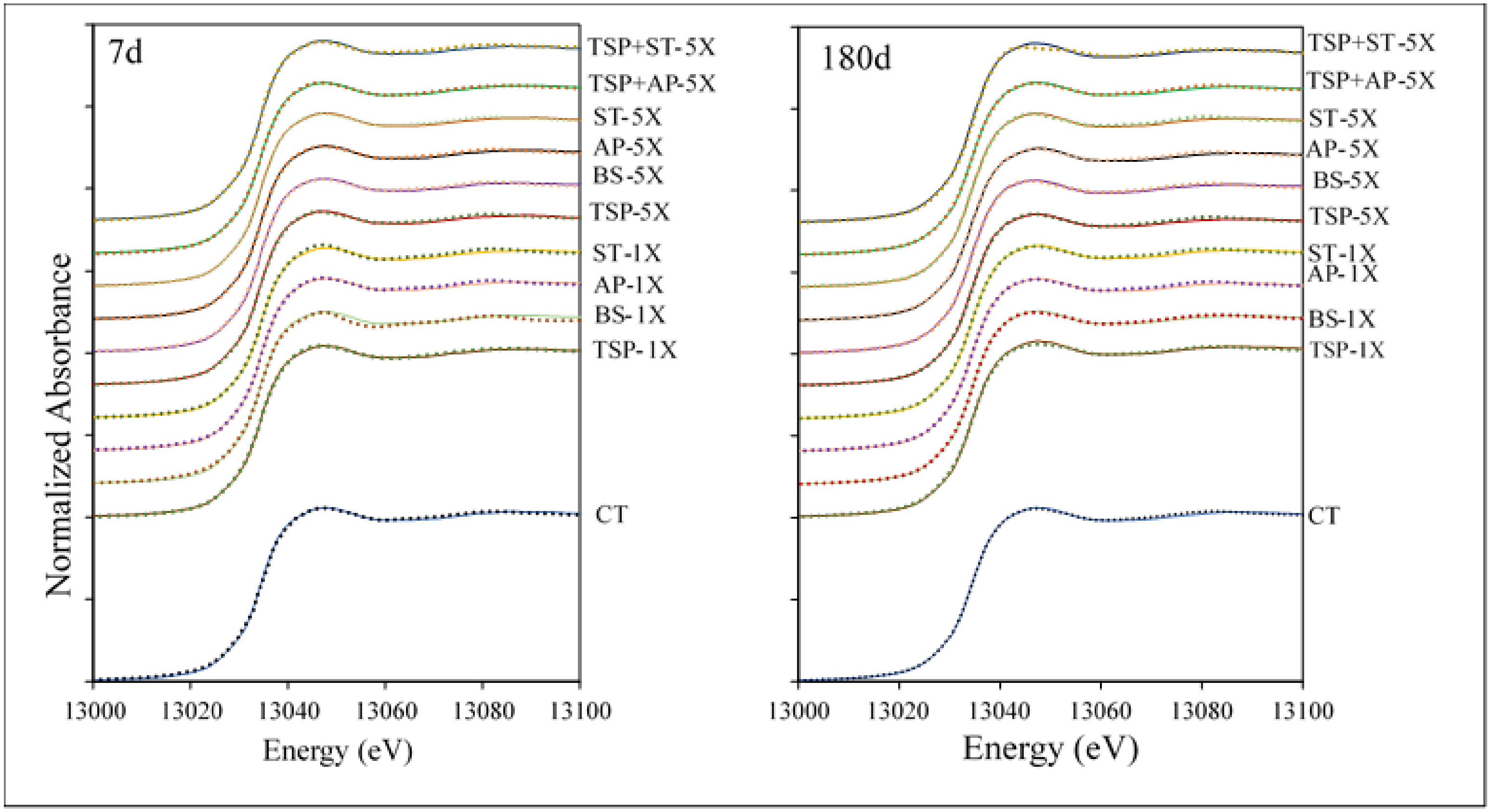

Figure 5.4 Normalized X-ray absorption fine structure (XANES) Pb absorbance spectra of the selected rate of treatments after 7 and 180 days. The dotted line overlying the XAFS spectra of the treatments is the result of a linear least-squares fit using Pb standard spectra as components. CT= control; TSP-1X and TSP-5X = triple superphosphate ; AP-1X and AP-5X= apatite; ST-1X and ST-5X = Struvite ; BS-1X and BS-5X = Biosolids ; TSP+AP-5X = triple superphosphate+apatite , and TSP+ST-5X = triple superphosphate+Struvite.

Conclusion

Bioaccessible Pb in the skeet-range soil was reduced when amended, most likely due to P addition and the inherently high sorption capacity of the soil due to high concentrations of active Fe species. Additionally, the percent of bioaccessible Pb in the treatments was reduced when incubation time increased, indicating changing mechanisms of Pb sorption over time (i.e., initial outer-sphere to inner-sphere or initially more soluble Pb-solid species to stable solid species). The most effective treatment in decreasing bioaccessible Pb was the BS-5X amendment. In general, the application of BS-5X was more effective than BS-1X and BS-2X in reducing extractable Pb concentrations. One of the predominant Pb species in the untreated soil was pyromorphite, and more pyromorphite was formed upon P treatments and over time. It may be essential to neutralize soil pH if these amendments at high rates were used in a field setting. From a risk evaluation view, it is essential to consider P-based amendments when remediating soils contaminated with Pb.

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Chapter 6 - Conclusion

Unfortunately, Lead contamination is common in shooting range soils since Pb is the primary material used in shot. Interestingly, several soil investigators consider the problem of the metal to be "resolved" in that we understand how (theoretically) to improve or avoid contaminated soils. In actuality, however, soil metal contamination falls in a gap between basic science and real anthropogenic activities in the environment. People do not correct their soils properly; they are uninformed of how extensive contamination is and are unaware of the cause of exposure. It is unclear if the training areas have the potential to sort through these different options and hazards on their own. Thus, we are faced with the dilemma that a useful activity such as military training may mean raised risk on the environment and human health from metals. This dissertation is dedicated to identifying the extent of Pb levels in military shooting range soil and investigating the potential for mildly contaminated military soils to be used for biofuel crop production safely. A series of field, greenhouse, and laboratory studies were performed on shooting range soils to determine an improved understanding of the fundamental mechanisms responsible for the change induced by *Miscanthus* growth and the decreased bioaccessibility of Pb through treatment additions. Effectiveness of in situ soil treatments utilizing organic and inorganic phosphate amendments was evaluated using in vitro bioaccessibility test known as Physiologically Based Extraction test and x-ray absorption spectroscopy for understanding underlying mechanisms of reducing Pb bioavailability, thereby minimizing its exposure risk to the environment.

The field research site is a United States military base located in Fort Riley, KS, considered as an entertainment “skeet shooting” range with Pb pellets from the 20th century. The distribution of Pb concentrations in shooting range soils showed high Pb concentrations ranging from 900 to 1,500 mg kg^{-1}, demanding attention, and remediation practices to limit contamination of the environment and human exposure.

Field study

A 3-yr long field study was conducted to determine the feasibility of using *Miscanthus* sp. as biofuel crop, for the phytostabilization of the contaminated military site and also to evaluate the effect of soil amendments on *Miscanthus* growth, soil-plant Pb transfer, bioaccessibility of soil Pb, and soil health. Five treatments were selected to evaluate how different establishment methods and soil amendments affect soil and plant lead content and stability. Soil treatments were triple superphosphate and class B biosolids.

Findings:

Soil amendments, especially biosolids, significantly reduced the plant Pb concentration. The soil chemical changes induced by *Miscanthus* growth initially affected the bioaccessibility of soil Pb. In general, *Miscanthus* yields in the biosolids-amended plots were significantly higher than non-amended control soils. The plant Pb concentration and the bioaccessible Pb in soils were lower in the biosolids amended plots than the non-amended soils

Greenhouse and Incubation Studies

In our field study, we were not able to test different sources or rates of P amendments due to limited land area available for our research. To overcome this weakness, we designed a greenhouse and laboratory incubation studies to test additional P sources at different rates.

Findings:

The greenhouse study revealed that biofuel crops might affect Pb bioaccessibility in treated soils. Additions of phosphate amendments were effective in minimizing the exposure risks in Pb-contaminated soils. Percent bioaccessibility values help to separate the dilution effect from the chemical effect of in situ soil treatments. Generally, percent bioaccessible Pb in amended soils with biosolids decreased with incubation time, indicating that these reductions were not only due to dilution of total Pb in soils due to biosolids addition. Repeated applications of phosphate amendments in combination may be successful in further reductions of Pb bioaccessibilities where it is of great concern. X-ray absorption near edge structure spectroscopy (XANES) confirmed the soil treatment-induced Pb speciation changes.

Appendix A - Chapter 3

Information about Miscanthus that was used in this research

As we mentioned in the literature that the *Miscanthus* genus is one of the biofuel crops and non-food crop, woody, rhizomatous, that originates from tropical and subtropical areas of southeast Asia. There are three varieties, in particular, *M. sacchariflorus (M.x sacc), M.x sin, and triploid M.x g*, are of essential importance as bioenergy crops with triploid *M.x g* being the most commonly cultivated form. *Miscanthus* that used in this research was originally brought it in 2007 from *Bluemel* nursery in Maryland, USA. Although called *Giganteus*, it has been classified as a tetraploid *M.x sacc*. *Miscanthus* obtained from the same nursery by the University of Minnesota in the 1980s and labeled as *Giganteus* is different and appears to be *M.x g* (unpublished observations). Definitive DNA analysis of both accessions is in progress.

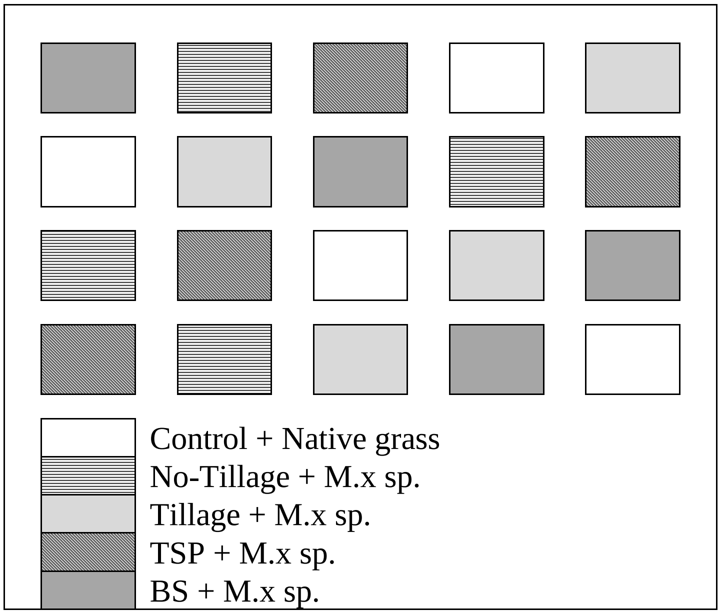

Figure A.1 Schematic representation of the field site. Plants within each plot were spaced 0.46 m. The different shadings represent the five treatments in the study. Each plot was 1.83m x 1.83m in size.

Table A.1 Percentage N in Miscanthus harvested at the skeet range of the military site after 1st harvest 2016. The column represents the mean of four replicates. Different letters within a treatment indicate a significant difference (α=0.05).

Treatment	N%
Control	1.1 ± 0.04†a
M x sp.-No-till	0.4 ± 0.08 b
M x sp.-Till	0.3± 0.03 b
M x sp.-TSP	0.3 ± 0.02 b
M x sp.-BS	0.5 ± 0.05 b
HSD‡	0.23
p-value	<.0001

† Standard error mean.

‡Tukey's Honest Significant Difference for mean comparisons with an alpha (α) of 0.05.

Table A.2 Total metal concentrations in the skeet range of the military site, from 2016 to 2018. Mean within a column and followed by the same letter are not significantly different.

Year	Treatment	Total						
		Pb	P	Mn	Zn	Cu	Cd*ns	As*ns
		(mg kg^{-1})						
2016	Control	1093 ± 103†b	374 ± 27b	340 ± 3a	32 ± 5b	9 ± 0.3b	0.3 ± 0.02	10 ± 2.5
	Mx sp.-No-till	1290 ± 34ab	392 ± 8b	333 ± 9a	28 ± 1b	10 ± 1b	0.4 ± 0.09	9 ± 0.6
	Mx sp.-Till	1396 ± 29a	391 ± 18b	324 ± 8a	46 ± 2b	8 ± 0.5b	0.4 ± 0.06	7 ± 0.9
	Mx sp.-TSP	1457 ± 42a	537 ± 30b	332 ± 3a	46 ± 1b	8 ± 0.2b	0.5 ± 0.03	8 ± 0.4
	Mx sp.-BS	1103 ± 50b	1255 ± 120a	328 ± 5a	76 ± 4	17 ± 2a	0.5 ± 0.02	7 ± 0.5
	HSD‡	262	238	27	12	4	0.2	5
	p-value§	0.002	<.0001	0.4007	<.0001	<.0001	0.0887	0.5297
2017	Control	1496 ± 86a	430 ± 35b	335 ± 3a	47 ± 5c	8 ± 0.4b	0.4 ± 0.04	10 ± 1.2
	Mx sp.-No-till	1305 ± 37ab	457 ± 8b	340 ± 2a	44 ± 3c	8 ± 0.4b	0.3 ± 0.02	8 ± 0.8
	Mx sp.-Till	1190 ± 51ab	436 ± 16b	342 ± 3a	44 ± 2b	8 ± 0.2b	0.4 ± 0.03	8 ± 0.6
	Mx sp.-TSP	1432 ± 110a	546 ± 24b	343 ± 12a	46 ± 3	8 ± 0.6b	0.4 ± 0.01	9 ± 1
	Mx sp.-BS	1082 ± 68b	1477 ± 170a	345 ± 5a	73 ± 5	17 ± 1a	0.4 ± 0.06	7 ± 1.2
	HSD	339	356	28	18	3	0.2	4
	p-value	0.0122	<.0001	0.7968	0.0007	<.0001	0.327	0.3382
2018	Control	1468 ± 93a	502 ± 26bc	297 ± 9a	45 ± 7a	8 ± 0.6b	0.4 ± 0.02	12 ± 1.9
	Mx sp.-No-till	1465 ± 34a	456 ± 36bc	300 ± 11a	51 ± 15a	8 ± 0.5b	0.4 ± 0.05	19 ± 5.4
	Mx sp.-Till	1362 ± 84a	429 ± 23c	297± 1a	38 ± 1a	8 ± 0.3b	0.4 ± 0.00	10 ± 1.1
	Mx sp.-TSP	1376 ± 143a	536 ± 22b	300 ± 11a	37 ± 2	8 ± 0.4b	0.4 ± 0.04	10 ± 0.9
	Mx sp.-BS	1222 ± 136a	1509 ± 56a	303 ± 6a	68 ± 2	17 ± 0.7a	0.5 ± 0.04	10 ± 1.1
	HSD	394	96	36	34	2	0.16	12
	p-value	0.31179	<.0001	0.9787	0.0664	<.0001	0.2453	0.0965

† Standard error mean

‡Tukey’s Honest Significant Difference for mean comparisons with an alpha (α) of 0.05

§ P-Value

*NS = no significant difference in treatments

Appendix B - Chapter 4

Table B.1 Summary of the SEP modified (Tessier 1979) by (Zwonitzer et at., 2003)

Fraction	Reagent	conditions
Exchangeable	25 ml 1M $MgCl_2$ (pH 7)	Shaken 1 h†, centrifuged‡, room temperature, rinsed with 8 mL of Milli-Q water, filtered§
Carbonates	25 ml 1M NaOAc (Sodium acetate) (pH 5)	Shaken 5 h, centrifuged, room temperature, rinsed with 8 mL of Milli-Q water, filtered†
Fe and Mn oxides	40 ml 0.04M NH_2OH/HCl in 25% (v/v) HOAc	Heated 6 h# at 96°C with occasional agitation, centrifuged, rinsed with 8 mL of Milli-Q water, filtered
Organic matter	20 + 20 ml of 7 M NaOCl (pH 8.5 adjusted with HCl)	After adding 20 ml, the mixture was heated to 2 h at 90°C with occasional agitation, centrifuged, then adding 20 ml again, samples were heated again to 2 h at 90°C with intermittent agitation, rinsed with 8 mL of Milli-Q water, filtered
Residual	20 mL of 4 M HNO_3	Heated 2 h at 80°C, centrifuged, rinsed with 8 mL of Milli-Q water, filtered

† on a reciprocal shaker at 130 rounds per minute

‡ centrifuged for 30 minutes at 2750 rpm

§filtered by 0.45 µm before analyzing on ICP-OES

¶ Heated in a water bath, set to the temperature at 96°C for six hours

Table B.2 Biomass yield, Pb concentrations and Pb uptake of belowground organs miscanthus harvested after third cutting. CT-C= Contaminated control with no P amendments; TSP-1X= superphosphate-1rate; TSP-2X= superphosphate-2rate; AP-1X= Apatite-1rate; AP-2X=Apatite-2rate; ST-1X= Struvite-1rate ; ST-2X=Struvite-2rate; BS-1X=Biosolids-1rate; BS-2X=Biosolids--2rate; TSP+AP-2X=TSP with AP-2rate; TSP+ST-2X=TSP with ST-2rate.Each column represents the mean of three replicates and different letters indicate a significant difference (α=0.05).

Treatment	Belowground organs DM (g/pot)	Pb concentration (mg kg^{-1})	Belowground organs Pb uptake (mg/pot)
CT-C	73.0 ab	255 a	16.0 abc
TSP-1X	72.0 ab	231a	17.0 abc
BS-1X	42.0 c	263 a	11.0 c
AP-1X	55.0 bc	225 a	14.4 bc
ST-1X	79.4ab	216 a	17.1abc
TSP-2X	86.0ab	229 a	15.4 abc
BS-2X	86.0 ab	250 a	22.5 ab
AP-2X	82.0 ab	226 a	18.4abc
ST-2X	76.1 ab	252 a	19.2 ab
TSP+AP-2X	76.0 ab	190 a	14.3 bc
TSP+ST-2X	87.0 a	265 a	23.1 a

Appendix C - Chapter 5

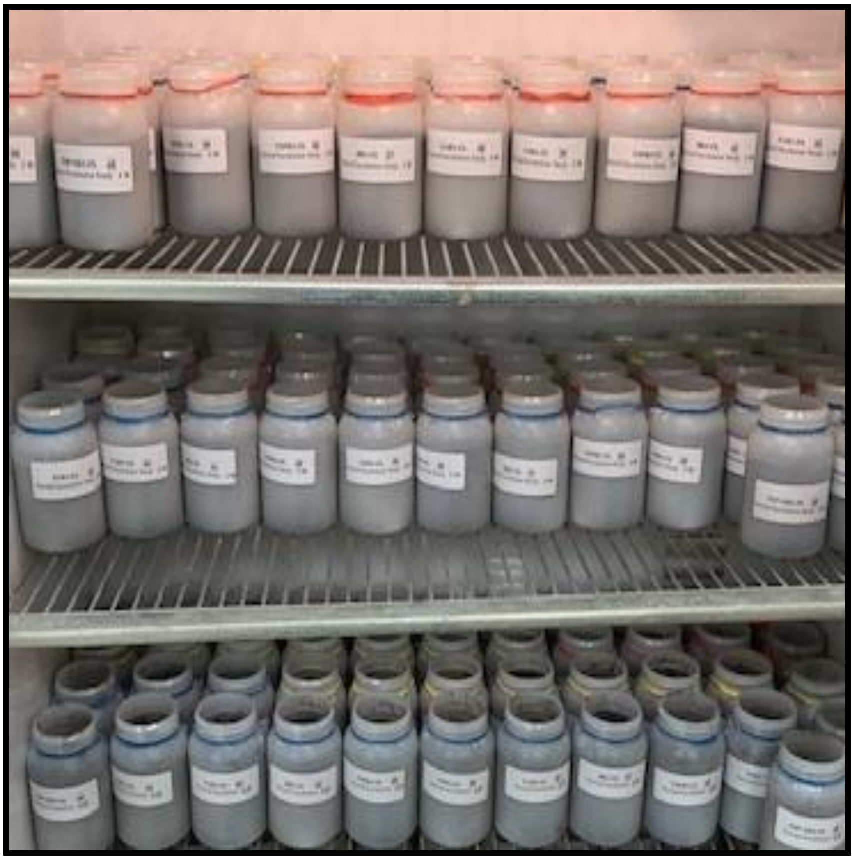

Figure C.1 Incubation study samples incubated at a controlled temperature incubator maintained at 25°C for six months