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Differential Lead Accumulation in Brassica juncea, Brassica rapa, and Lactuca sativa and Evaluation of Ground Level Barriers to Prevent Lead Contamination of Produce Grown in Raised Beds

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DIFFERENTIAL LEAD ACCUMULATION IN *BRASSICA JUNCEA*, *BRASSICA RAPA*, AND *LACTUCA SATIVA* AND EVALUATION OF GROUND LEVEL BARRIERS TO PREVENT LEAD CONTAMINATION OF PRODUCE GROWN IN RAISED BEDS

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ABSTRACT

High lead (Pb) contamination of soils is a threat to human health. Urban area soils are frequently contaminated with lead from settling of gasoline exhaust, brake dust, and lead paint on homes, old playground equipment, etc. Exposure to soil-lead occurs by ingestion or inhalation and poses an elevated risk for young children. Indirect ingestion can occur through the food chain through activities such as growing certain vegetables with an ability to tolerate and accumulate lead in edible tissues. Many university extension systems recommend growing vegetables in raised beds as a reasonable reduced risk option for avoiding lead accumulation in garden vegetables particularly leafy greens and root vegetables. However, limited research outlines the efficacy of specific raised bed practices in excluding lead from the initially uncontaminated planting space. To address the soil-lead exposure pathway via garden produce, this study evaluates differential uptake of lead in common cool season vegetable crops and supplements gaps in the literature pertaining to raised-bed garden practices in lead contaminated areas. Three species of leafy green vegetables were grown in soil-less media contaminated at 0, 500, 1000, and 2000 ppm Pb to observe plant growth patterns and accumulation in contaminated raised bed conditions. Findings suggest minimal observations of toxic effects on growth and variable lead accumulation above threshold contaminant levels. Simulated raised beds were subsequently constructed to evaluate barriers placed at the interface between contaminated soil and the base of the uncontaminated raised bed soil. The resulting data suggests that neoprene rubber sheeting does not exclude lead from the raised bed.

CHAPTER 1. LITERATURE REVIEW

1.1. Sources, Uses and Dispersal of Environmental Lead

Lead (Pb) is a heavy metal comprising 0.002% (15g/t) of the earth's crust. The metal exists throughout the environment naturally at concentrations below 50 ppm (Pais and Jones, 1997). However, throughout human history, anthropogenic activities have facilitated its relocation, concentration, and subsequent threat to human health.

Records of lead used in human civilizations date back 6000 years and records of lead poisoning, nearly 2500 years. As one of the first metals humans began to manipulate in industry, lead's high density, malleability, and resistance to corrosion made this metal ideal for historic and recent industry products (Riva et al., 2012). For instance, the Romans processed and used lead in dinner ware, cooking tools, wine additives and drinking water pipes for over 400 years (Hernberg, 2000). Throughout the medieval period lead in its inorganic form was groundbreaking as lead-based colors were introduced in paints (Riva et al., 2012). Recent industry lead uses include paints, transportation fuels, plumbing, ammunition, batteries, and orchard pesticides, such as lead arsenate (Riva et al., 2012).

Lead use, and thus environmental lead exposure, in the United States has generally declined since the mid-1970's largely due to the phase-out of lead from gasoline after 1973 and its ban in 1996, the removal of lead from soldered cans and restricted use of lead paints in 1978 (ATSDR, 2019). However, the residual effects of the use of lead in these and other industries have increased the levels of environmental lead to which the average person is exposed. Heavily trafficked areas are at a greater risk for lead deposition. Leaded gasoline combustion results in the release of tetraethyl lead ($Pb(C_2H_5)_4$) and tetramethyl lead ($Pb(CH_3)_4$) into the atmosphere and subsequently significant deposition on to nearby soil where it remains until disturbed and further distributed. A further contributing factor, often faced by urban communities, is that most houses, schools, and government buildings built before 1978 were painted with lead-based paints containing lead carbonate ($PbCO_3$), lead sulfate ($PbSO_4$), lead chromate ($PbCrO_4$), and lead tetraoxide (Pb_3O_4). Deterioration, renovations, demolition, and peeling paints in these buildings can result in suspension and further deposition of lead particles indoors and upon the surrounding soils (Gaitens et al., 2009). Though lead forms various compounds, as a basic element it does not degrade in the environment and binds tightly to soils contributing to its persistence as an environmental human health risk (ATSDR, 2019).

1.2. Quantifying Lead Exposure

To quantify exposure, lead epidemiological studies rely on internal exposure metrics rather than external exposure or ingestion doses. Blood lead levels are the most common metric used, though bone lead levels are more representative of the total body burden. Approximately 94% and 74% of the total lead body burden in adults and children respectively is distributed to bones with the remainder distributed to the blood and soft tissues (Flora et al, 2012). Bone lead is considered an important biomarker for cumulative or long-term exposure studies whereas blood lead levels have been the main criterion for evaluating exposure and body burden for reasons of practicality and expense (ATSDR, 2019).

Based on a growing number of scientific studies that show that even low blood lead levels can cause lifelong health effects, there is no established threshold for the adverse consequences of lead exposure. For most studied endpoints including neurological, renal, cardiovascular, hematological, reproductive, and developmental endpoints, negative effects have been observed at very low blood lead levels (ATSDR, 2019; Lanphear et al., 2005). The reference threshold values cited by the Center for Disease Control (CDC) vary with the age of the individual in question due to differences in fractional absorption of ingested lead. For instance, children breathe more air and consume more food than adults per surface area of the gastrointestinal tract, surface area of the respiratory tract and kilogram of body weight. Additionally, children are still actively growing and developing. The developing bones of children undergo frequent remodeling, continually re-introducing lead into the blood stream. Considering these factors, at certain stages of development, exposure to environmental lead can lead to irreversible developmental damage (Heacock et al., 2018).

Due to observations of serious adverse effects at the lowest blood lead levels studied, minimal risk levels for lead exposure have not been determined. As a zero-risk level of lead exposure for children has not been identified, the CDC adopted a reference value rather than a threshold value for blood lead in children in 2012. This reference value is 5 µg/dL and was based on the 97.5th percentile of blood lead levels in U.S. children aged one to five years taken from the National Health and Nutrition Examination Survey (NHANES) data (CDC, 2013). To assess exposure risk for adults, Adult Blood Lead Epidemiology and Surveillance (ABLES) was created under The National Institute for Occupational Safety and Health (NIOSH). In 2015, NIOSH designated 5 µg/dL as the reference blood lead level for adults (recently lowered from 10 µg/dL) (CDC, 2018).

1.3. Toxic Mechanisms of Lead in Humans

An important biochemical basis for lead toxicity lies in its ability to bind to biologically important molecules, interfering with their function by several mechanisms. Lead's electron sharing capacity results in covalent attachments**.** Oxidative stress occurs via an over-abundance of free radicals exceeding the body's antioxidant threshold to efficiently detoxify the reactive intermediates or to repair the resulting damage (Flora et al., 2007). Metal-mediated free radical generation starts a chain reaction that results in lipid peroxidation, disruption of cell membranes, protein oxidation, and oxidation of nucleic acids with potential to result in oncogenic, or cancerous, mutations.

Lead damage may also occur via ionic mechanisms by the heavy metal's ability to substitute for other bivalent cations such as calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}) and sometimes monovalent cations like sodium (Na^{+}) (Assi et al., 2016). These substitutions affect fundamental cellular processes such as intra- and intercellular signaling, protein folding, ionic transportation, enzyme regulation and neurotransmitter release (Flora et al., 2012). Most notably, this ionic mechanism contributes to neurological deficits. When Pb^{2+} ions replace Ca^{2+}, they gain the ability to cross the blood brain barrier where it can accumulate in and cause damage to neurotransmitter cells of the central nervous system, and interfere with myelin sheath formation (Assi, 2016; Flora et al., 2012; Sanders et al., 2009). Additionally, calcium replacement by lead ions affects key protein kinases regulating long-term neural excitability and memory storage (Vázquez and Peña de Ortiz, 2014). When sodium ion concentration is affected, vital biological activities that rely on action potentials like cell to cell communication and neurotransmitter uptake are also impaired.

1.4. Human Target Tissues and Epidemiological Studies

Lead is known to interfere with a variety of bodily functions including the nervous system, renal function, hematopoietic system, cardiovascular system, and reproductive health.

Lead may affect both the central nervous system (CNS) which consists of brain and spinal cord. and the peripheral nervous system (PNS) which controls the sensory and motor fuction. Young children are most susceptible to CNS effects due to their active development. The developing nervous system absorbs and permits a higher fraction of lead across the blood brain barrier via calcium ion substitution (Sanders et al., 2009). Many epidemiological studies have been published indicating reductions in adult and child cognitive function, neuromotor and sensory functions, and peripheral nerve conduction capacity associated with elevated blood lead levels (ATSDR, 2019). This lack of nerve activity may results in a visual symptom commonly called “wrist drop” where the person’s wrist hangs limp. Children are particularly susceptible to encephalopathy, a progressive degeneration of certain parts of the brain. Major symptoms include lethargy, irritability, reduced attention span, behavioral changes, headache, and memory loss (Rao, 2014; Seo, 2014).

Cognitive performance as a function of lead exposure has been extensively studied. A pooled cohort study of 1,333 children between ages four and six years old was initially conducted in 2005 and re-visited by the U.S. Environmental Protection Agency (EPA) in 2014. The study looked at Full Scale Intelligence Quotient (FSIQ) scores as they relate to blood lead levels adjusting for a variety of familial and living condition factors. The resulting model predicts that when blood lead content increases by 1 µg/dL, child IQ would be expected to reduce by 2.21 points (EPA, 2014 e). Annual estimated direct and indirect medical costs for lead-related neurologic problems including muscular pain, depression, panic disorder and dementia add up to $134,200 according to a 2016 review (Levin, 2016).

In a healthy kidney, amino acids are filtered out of urine and reabsorbed. Alternatively, in the case of acute nephropathy, an impaired tubular transport mechanism inhibits effective urine filtration and can give rise to abnormal excretion of glucose, phosphates, and amino acids, commonly referred to as Fanconi’s syndrome (Spector et al., 2011). These deleterious effects, have been reported mainly in adolescents exposed to lead (ATSDR, 2019). Chronic neuropathy is more severe, leading to irreversible functional and morphological changes characterized by glomerular and tubulointerstitial changes resulting in renal breakdown, hypertension, and hyperuricemia (Spector et al., 2011). In addition, lead in the kidney interferes with activation of vitamin D which plays an important role in calcium metabolism (ATSDR, 2019; Haryanto, 2016). Lead-related renal diseases annually incurs direct and indirect medical costs approximately $167,500 for exposed Americans (Levin, 2016).

Lead directly affects the hematopoietic system through restraining the synthesis of hemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway such as δ-aminolevulinic acid dehydratase (ALAD), aminolevulinic acid synthetase (ALAS), and the mitochondrial enzyme ferrochelatase (ATSDR, 2019). It also reduces the life span of circulating erythrocytes (the most common type of blood cells) by increasing the fragility of cell membranes which results in anemia, a condition where one lacks enough healthy blood cells to carry adequate oxygen to your body’s tissues. Epidemiological studies show altered heme synthesis and a decrease in erythrocytes at blood lead levels of 10 μg/dL and below in children (Wang et al., 2010; Huo, 2019). For painters and battery plant workers, erythrocyte δ-aminolevulinate dehydratase (δ-ALAD) activity and its reactivation index were measured as biomarkers of lead effects showing a negative relationship between δ-ALAD and blood lead levels (Conterato et al., 2013; Queirolo et al., 2010; Ukaejiofo et al., 2009). A 2016 review estimates annual direct and indirect medical costs associated with lead-related anemia add up to $56,000 nationally (Levin, 2016).

Studies evaluating cardiovascular effects related to bone lead levels have found greater systolic and diastolic blood pressures in adults across multiple studies and in children and in women during pregnancy in fewer studies. Other studies positively linked blood lead levels at ≤10 μg/dL to pregnancy-induced hypertension (ATSDR, 2019). In the US, estimated annual direct and indirect medical costs for lead-related hypertension and heart attack are $11,7700 (Levin, 2016).

In men, sufficient evidence suggests that blood lead levels can cause abnormal spermatogenesis affecting motility, abundance, and viability of sperm, as well as chromosomal damage and possible changes in serum reproductive hormone concentrations (ATSDR, 2019). Females are also at risk for serum hormonal changes, and fertility reduction. Even without external lead exposure during pregnancy, some studies show an increased mobility of lead stored in bone during gestation and lactation due to hormonal changes and calcium resorption (Gulson et al., 2003; ATSDR, 2019). In women, lead can result in spontaneous abortion, preterm birth, and decreased age for menopause onset. Lead can also cross from mother to placenta during pregnancy resulting in lead uptake by the fetus which can affect neurological development (Hu, 2006; ATSDR, 2019). Lead-related reproductive complications such as damage to male and female fertility and pre-term births nationally incur estimated annual direct and indirect costs of $297,000 (Levin, 2016).

To place the issue of lead exposure in an even broader economic context, a 2010 report by The PEW Research Center on the States, a division of The PEW Charitable Trust, estimated the average annual economic impact of lead poisoning per birth cohort in the United States. The research group estimated costs related to lead exposure add up to between $192 and $270 billion based on lead related healthcare expenses, IQ loss and lower lifetime salaries, increased special education needs, and behavioral problems and associated crime rates (PEW, 2010).

Altarum, in a separate 2019 report, broke down economic costs by state for the 2019 birth cohort. This assessment is based on costs related to reduced lifetime productivity, increased health care, education, social assistance spending, and premature mortality related to lead exposure. For the 2019 birth cohort in Louisiana, the lifetime economic burden estimate is 1.2 billion. Mitigation of lead exposure has the potential to reduce these long term concerns for human heath and wellbeing and ultimately these related costs. For example, the same alterum report suggests a $1.4 dollar return on every dollar invested in lead hazard abatement in Louisiana such as treating leaded paint, dust, and soil, as well as replacing old windows where necessary in homes built before 1978 (Altarum, 2019).

1.5. Routes of Exposure

Understanding the risks and impacts of lead exposure for humans highlights the need to characterize the remediate potential exposure pathways. Elemental lead and inorganic lead are absorbed most readily by ingestion or inhalation. Ingestion of lead compounds can occur directly or indirectly. Direct ingestion of lead containing dust occurs most commonly in children due to hand-to-mouth behaviors. Indirect ingestion can occur through the food chain via gardening certain vegetables with an ability to tolerate and accumulate lead in their edible tissues (Sikka, 2010). The respiratory route allows exposure to lead containing dust especially in occupational settings and during renovations of older homes. Absorption of lead via the lungs is very efficient especially at particle sizes less than 1 μm. Organic lead compounds such as tetraethyl lead may also be absorbed dermally due to their lipophilic nature (ability to dissolve in lipids or fats) via direct skin contact, though, dermal exposure is not generally regarded as a significant route of exposure. Common points of exposure include lead dust via chipping paint, or contaminated soils, drinking water via lead pipes, and occupational exposure in facilities which process/recycle lead containing products. The primary systemic toxic effects of lead remain the same regardless of the route of exposure.

Surface soils offer a significant medium for lead exposure. Human exposure to lead in soils primarily occurs via two main pathways, (1) direct soil-to-human exposure and (2) indirect plant-to-human exposure. Direct soil exposure involves ingesting soil from hand-to-mouth behavior, from consumption of unwashed produce or breathing contaminated dust. Indirect exposure to soil contaminants via plants occurs when plants accumulate soil lead in their shoots and roots (Defoe, 2017). Exposure via plants is possible by some plants' ability to accumulate lead in different tissues despite lead serving no essential role in plant growth and development.

Current EPA soil lead guidelines cite 400 ppm as the hazard level for bare soil in play areas and 1,200 in non-play areas (EPA, 2013). A 2008 study of New Orleans soils revealed lead levels ranging from 7.72 to 8,550 ppm. Fifteen percent of the 128 tested sites exceeded the 400 ppm EPA guideline (Abel, 2010. A later 2019 study of New Orleans soil lead levels connected observed declining soil lead levels (presumably a result of weather-induced top soil loss throughout the city) and an observable decline in children's blood lead levels (Mielke, 2019). These findings indicate that mitigating exposure to soil lead is an important factor in continuing the decline of childhood lead exposure.

Lead's behavior in the soil and a plant's ability to absorb the element, depend on complex interactions of a variety of physical and biochemical factors including amount of lead and it's form present in the soil, soil particle size, pH, redox conditions, cation-exchange capacity, soil mineralogy, biological and microbial conditions, organic and inorganic ligands, competing cation levels, and plant species involved (Pourrut et al., 2011). If taken up, lead primarily accumulates in root cells because of the blockage by Casparian strips within the endodermis. It is frequently adsorbed by roots usually apoplastically in the space outside of the plasma membrane or via Ca^{2+} permeable channels (Pourrut et al., 2011).

In many cases, the lead, if able to accumulate in plant tissue, will pose minor to severe toxic threats to plants via morphological, physiological, and biochemical mechanisms. Results of previous studies have shown harmful effects including impaired plant growth, impaired root elongation, low seed germination, and seedling death (Pourrut et al., 2011). As in human systems, lead and other heavy metals induce production of reactive oxygen species (ROS) in plant systems. These are short-lived, unstable, and highly reactive molecules with unpaired valence shell electrons. ROS such as $O_2^{\bullet-}$, H_20_2 and $OH^{\bullet}$ are produced naturally as byproducts of energy transfer reactions in chloroplasts and mitochondria. However, when induced by heavy metal stress, ROS can quickly prompt widespread damage via enzyme inhibition (binding to sulfhydryl groups), lipid peroxidation, protein oxidation in addition to, DNA and RNA damage. Lead adsorption has been observed at elevated levels in several food crop species including; mustard (*Brassica juncea*) (Meyers et al., 2008), lettuce (*Lactuca sativa*) (Capelo et al., 2012, Uzu et al., 2009), and others. In these plants and others with certain genotypic traits and physiological characteristics, the plant detoxifies and accumulates lead while experiencing limited toxic effects (Mourato et al., 2015; Ramesar et al., 2014).

To prevent this heavy metal damage, some plants have developed defense mechanisms. Plant defense mechanisms include blocking the entrance of lead into plants, chelation (bonding of ions and molecules to metal ions by organic molecules), binding the heavy metal to the cell wall and sequestration in the vacuole. Largely these defensive mechanisms rely on metabolic mediation for example by glutathione, carotenoids and tocopherols, and enzymatic antioxidant systems such as catalase, superoxide dismutases, peroxidase, glutathione reductase (Ramesar et al., 2014; Shahid et al., 2014; Sungur et al., 2014).

Some of these species with tolerance traits are considered lead hyperaccumulators. Hyperaccumulating species are ideal for remediation efforts, yet they are frequently also food crops such as *Brassica juncea, Lactuca sativa, Triticum aestivum* etc. (Capelo et al., 2012; Liu et al., 2009; Ramesar et al., 2014). These plants and others have exceeded regulatory limits for lead in produce. One regulatory value comes from the National Health and Family Planning Commission (NHFPC, currently the National Health Commission) and the China Food and Drug Administration (CFDA, currently the State Administration of Market Regulation) released in 2017 in a publication outlining standards for maximum levels of contaminants in foods. Under this standard, the maximum lead value allowed for Brassica vegetables and leafy green produce is 0.3 ppm as a concentration of fresh weight. Current regulations regarding allowances of lead in consumables from the EPA and the U.S. Food and Drug Administration (FDA) discuss lead in drinking water (no more than 1.3 ppm in 10% of tested taps) and bottled water (0.005 ppm) (EPA, n.d.; FDA, n.d). Additionally, established USFDA tolerances of lead are published but not regulated for juices at 0.05 ppm, for dried fruits at 0.1 ppm, and for candies at 0.1 ppm.

In one study of *B. juncea* species, 18 of the 30 varieties accumulated over 0.2 ppm Pb when grown in 500 ppm Pb and 26 of the 30 varieties accumulated over 0.2 ppm Pb when grown in 1500 ppm Pb (Liu et al., 2009).

While many studies have shown absorption of lead by plant roots and subsequent accumulation, the results obtained by accumulation and toxicity experiments are difficult to compare and extrapolate due to diverse experimental conditions and contamination methods (Pourrut et al., 2011). Some studies were performed in pot or field conditions in environmentally contaminated soils and others in artificially contaminated soil, media, or hydroponic solution.

This variability in experimental set up highlights the variability in lead behavior in different soil systems.

The soil matrix is a highly dynamic system and heavy metals therefore behave differently based upon the conditions of the system. The bioavailability of lead in a soil system is largely dependent on how it fractions out in the system. Lead mobility and availability decrease in order of acid solubility, reduciblity (associated with Fe and Mn oxides), oxidizable-organic (associated with organic matter and sulfides), and residual fraction (strongly associated with mineral crystalline structure), the first three being mobile fractions (Sungur, 2014). Sorption of heavy metals (i.e. Pb) on to soil components is generally characterized by a biphasic process during which rapid sorption of a large portion of the metal is followed by subsequent slow reactions (Pourrut et al., 2011). One study in a Matapeake soil (pH 5.5) observed 78% sorption within 8 minutes followed by only 1 % sorption in the subsequent 800 hours (Sparks, 2003).

To prevent soil lead exposure and subsequent accumulation in food crops various solutions to contamination remediation have been explored. Preventative efforts to remove sources of soil lead contaminants include sealing in lead paints on older homes. Conventional remediation of heavy metal polluted soil sites involve either onsite phyto- or chemical remediation or excavation and disposal to a landfill site. The latter disposal solution solely relocates the contamination problem to an alternate location while also introducing hazards associated with transportation. Excavation and sealing of contaminated sites are costly and elevate the risk for contamination migration as the soil is disturbed for transport. Remediation via amendments aims to reduce metal mobility and toxicity in soil by transferring soil metals to more geochemically stable phases by sorption to soil constituents or precipitation from solution (Uzu et al., 2009). Lead sorbs frequently to mineral surfaces especially to iron (Fe) and aluminum (Al) oxides in mineral rich soil (Wan et al., 2018). These solid phase interactions contribute to the low occurrences of lead in aqueous environments. Organic and inorganic amendments containing lime and phosphorous (P) have proven effective for reducing lead availability (Paltseva, 2018). At lower pH ranges, lead desorption rates increase, and the metal is less tightly bound to soil making it more mobile (Sparks, 2003, Xiong, 2013). Therefore, raising the soil pH with lime amendments, decreases solubility of lead. Amending with phosphorous through compost or other means can speed up the precipitation of an insoluble lead compound called pyromorphite (Hettiarachchi, 2004). Soil organic matter also plays a role in heavy metal fractionation. Addition of organic matter to contaminated soils can dilute the lead concentration and possibly increase the incidence of lead binding thereby decreasing its availability. In general, organic matter containing substances increases the immobile fraction by encouraging lead to form soil complexes (Sparks, 2003; Xiong, 2013).

Gardening recommendations in lead contaminated areas for to the everyday gardener vary. Numerous Cooperative Extension publications cite the EPA value of 400 ppm is the maximum soil lead concentration considered safe to grow vegetables in the soil, though some recommend adding amendments such as those altering soil pH to limit lead solubility and availability if the gardener choses to grow there. At any concentration above 400 ppm, an expensive remediation solution is excavation and removal of contaminated soils. Alternatively, the University of Connecticut Extension Service recommends growing vegetables in raised beds as a reasonable reduced risk option for avoiding lead accumulation in garden vegetables, particularly when growing leafy greens and root vegetables which are the most likely to accumulate lead in their edible tissues. These recommendations are consistent with those presented by other Extension Services throughout the country, specifically from the University of California, Kentucky State, Oregon State, and the University of Delaware (Brewer, 2016; Craigmill, 2010, Defoe, 2017; Gartley, 2002). No previous studies have evaluated the best barriers for these recommended raised bed gardens to confirm prevention of contaminated soil mobility.

CHAPTER 2. DIFFERENTIAL LEAD (PB) ACCUMULATION IN COLD SEASON VEGETABLES

2.1. Introduction

Lead (Pb) is a heavy metal comprising 0.002% (15 g/t) of the earth's crust. The metal exists sporadically in the environment in concentrations usually below 50 ppm (Pais and Jones, 1997). However, throughout history, anthropomorphic activities have facilitated its relocation, concentration, and subsequent threat to human health.

Lead adsorption has been observed in several food crop species including: mustard *Brassica juncea* (Meyers et al., 2008), lettuce *Lactuca sativa* (Capelo et al., 2012; Uzu et al. 2009). In many cases, this lead accumulation will pose minor to severe toxic threats to plants. Lead may affect plants via morphological, physiological, and biochemical mechanisms. Results of previous studies have shown harmful effects including: impaired plant growth, root elongation, seed germination, seed elongation, seed germination, and seedling death (Pourrut et al., 2011). As in human systems, lead and other heavy metals induce production of reactive oxygen species (ROS) which are short-lived, unstable, and highly reactive molecules with unpaired valence shell electrons.

Different species take up lead differently. Species with tolerance traits are considered lead hyperaccumulators. Hyperaccumulator plants may allocate lead by binding it to the cell wall before it can enter the cell or byproducing antioxidants that help prevent damage by reactive oxygen species caused indirectly by lead. These ROS can otherwise cause lipid membrane damage and ultimately DNA, protein, and carbohydrate damage (Scandalios, 1993). Plants like these, with certain genotypic traits and physiological characteristics, can accumulate lead at elevated rates (Capelo et al., 2012; Liu et al., 2009; Ramesar et al., 2014). While some of these plants are used for remediation efforts and can grow with minimal negative side effects in lead-contaminated conditions, they can pose harm to communities if grown for food. While many studies have shown absorption of lead by plant roots and subsequent accumulation, the results obtained by accumulation and toxicity experiments are difficult to compare and extrapolate due to diverse experimental conditions and contamination methods (Pourrut et al., 2011). Some studies were performed in pot or field conditions in environmentally contaminated soils and others in artificially contaminated soil, media, or hydroponic solution.

The objective of this study was to quantify lead accumulation in a variety of leafy greens that are commonly grown in raised-beds using a raised-bed media mix.

2.2. Materials and Methodology

An accumulation study was designed to quantify differential uptake of lead across common leafy vegetable species. Based on previous lead accumulator research (McBride et al., 2012; Mourato et al., 2015; Liu et al., 2010),, three varieties were chosen of three species of leafy, green, cool season vegetables. The three species selected were mustard (*Brassica juncea*), Chinese cabbage (*Brassica rapa*), and lettuce (*Lactuca sativa*). The mustard varieties selected for this study included ‘Red giant’ mustard, ‘Purple Wave’ mustard, and ‘Tendergreen’ mustard. The Chinese cabbage cultivars selected for this study included ‘Mibuna’, ‘Tatsoi’, and ‘Michihli’ all commonly considered Chinese cabbage. Lettuce cultivars selected for this study included ‘Little Gem’, ‘Great Lakes’ and ‘Arianna’, all butterhead lettuce types. All seeds were purchased from Everwilde Farms, Inc. (Sand Creek, WI). Each cultivar was replicated 10 times across four treatments (0 ppm control, 500 ppm, 1000 ppm, 2000 ppm concentrations of Pb in the medium). These concentrations were evaluated due to the current EPA values of soil lead cautionary levels, using 400 ppm as the hazard level for bare soil in play areas and 1,200 in non-play areas (EPA, 2013).

On January 7, 2019 and November 1, 2019, seeds were planted in 50 count cell trays (T.O. Plastics Clearwater, MN) using a peat based medium (SunGro Metro-Mix 830, Sun Gro Horticulture, Agawam, MA) and grown in a greenhouse with a temperature range between 39°F - 71°F. Transplants were watered daily, using overhead irrigation set twice a day for five minutes each time period. Transplants were fertilized using a liquid fertilizer (Peter's Professional 20-20-20 fertilizer; ICL Specialty Fertilizers, Summerville, SC) at 700 ppm every other week and the seedlings were thinned out to one plant/cell. Seven weeks after seeding, the plants were transplanted into the spiked (untreated control, 500 ppm Pb, 1000 ppm Pb, 2000 ppm Pb) 1 gal (3.7 L) blow molded plastic nursery containers (Nursery Supplies, Forest Hill, LA). The soil media used in this study is similar to standard mixes often used in raised bed conditions. Media used in this study was mixed in large batches using peat moss 4 cu ft (Lambert Peat Moss, Quebec, Canada), 5/8th in screened Pine Bark (Phillips Bark, Brookhaven, MS) 2 cu yd, washed large grain sand (Baer Industries, Port Allen, LA) 4 cu ft, Osmocote 19-5-9 (ICL Specialty Fertilizers, Summerville, SC) 10.5 lb (4767 g), Micromax (ICL Specialty Fertilizers, Summerville, SC) 0.5 lbs. (227 g), dolomitic lime (Lhoist, Port Allen, LA) 8 lbs (3632 g). The media was spiked with a 10,000 ppm Pb standard in a 5% HNO_3 solution (RICCA Chemical Company, Arlington, TX) diluted to desired concentrations using the same irrigation water used throughout the study. All containers were soaked to saturation and 200 ml of either water only for the untreated control or diluted lead solution was applied to each container according to treatments. Inductively coupled plasma-optical emission analytical spectrometry (ICP- OES)

(PerkinElmer, Houston, TX) procedure with an HCl and HNO_3 digestion was used to analyze total lead in soil media to confirm intended initial concentrations (EPA Method 3051A).

All pots were distributed across three prefabricated fiberglass ebb and flow tables (54 in x 191 in). The pots were elevated onto overturned seedling trays to allow drainage (Figure 1). Each table has a drainage hole by which irrigation runoff drained and was collected and contained. The pots were arranged in a split plot design by soil treatments on each table. Pots were distributed randomly within the treatment split plots at each table (Figure 2). All runoff was collected, and contaminated soil media was contained.

Figure 1. Experimental pots placed on top of overturned horticulture seedling trays to facilitate drainage.

The potted plants were hand-watered daily and Bifenthrin (Fertilome, Bonham, TX) was used at labeled rates as needed to control aphids (*Brevicoryne brassicae*), flea beetles (*Phyllotreta striolata*, *Phyllotreta cruciferae*), and cross-striped cabbage worms (*Evergestis rimosalis*). At 42 days of growth in the contaminated pots, all plants were harvested by cutting plant tops at the base of the soil line and weighed for fresh weight (g). All plants were then divided between two dryers (SHEL Lab, Cornelius OR, and VWR Scientific Inc, Suwanee, GA) at an average of 60 °C and dried to a constant weight. An Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) procedure was used to analyze available lead in the soil media and accumulated lead in the above ground plant tissues for the first trial. However, an Inductively Coupled Plasma- Optical Emission Spectrometry (ICP-OES) (PerkinElmer, Houston, TX) procedure with an HCL digestion was used to analyze total lead accumulated in the plant tissues in the second trial due to greater recovery of the heavy metal in tissues via the ICP-OES method (AOAC Method 985.01). Only plant tops were analyzed, roots samples were collected but not analyzed due to insufficient plant material recovery.

Figure 2. Experimental split plot depicted on one of three ebb and flow tables. Left to right, 2000 ppm Pb, 1000 ppm Pb, 500 ppm Pb, control plots.

A respirator and goggles were the personal protective equipment (PPE) used each time lead standards and loose contaminated media were handled. Contaminated materials were disposed of after the conclusion of the experiment by Louisiana State University Hazardous Waste Disposal (LSU Office of Environmental Health and Safety 217 Administrative Support Building, Baton Rouge, LA 70803). Data were analyzed with the statistical program SAS (version 9.2; SAS Institute, Cary, N.C.) Proc GLM with Tukey and Excel for Microsoft 365 (Microsoft Corporation, Redmond, WA).

2.3. Results and Discussion

In trial one, three varieties of three different vegetable crops species were grown across four lead (Pb) treatments in 1-gallon containers. The final biomass of each variety within the three vegetable crops were reported for trial one (Figure 3). Figures 4 through 6 depict accumulation data for the mustards, cabbages, and lettuces respectively. There were no United States accepted health-based standards with which to compare the measured accumulated lead in the vegetable crops trialed in this experiment. For this reason, each of these accumulation figures also features a green line representing the published Chinese standard threshold level for maximum concentration of lead contaminant allowed in brassica and leafy produce (adjusted from 0.3 ppm FW to 3.84 ppm Pb DW). ICP-OES extraction of the media medium in the untreated control pots used in the study was 10.2 ppm Pb.

There were few significant differences measured within cultivars for plant growth (Figure 3). ‘Michihli’ Chinese cabbage growth was stunted in the 1000 ppm Pb and 2000 ppm Pb treatments. For all other plant species there were no statistical differences in final growth biomass across contamination treatments, indicating that lead contamination in media did not affect overall plant growth. ‘Red Giant’ mustard and ‘Mibuna’ Chinese cabbage both displayed downward trends in growth with increasing lead treatment though with no statistical significance. The plants in this first trial experienced elevated insect pressure. A more regular insecticide application schedule was implemented in trial two.

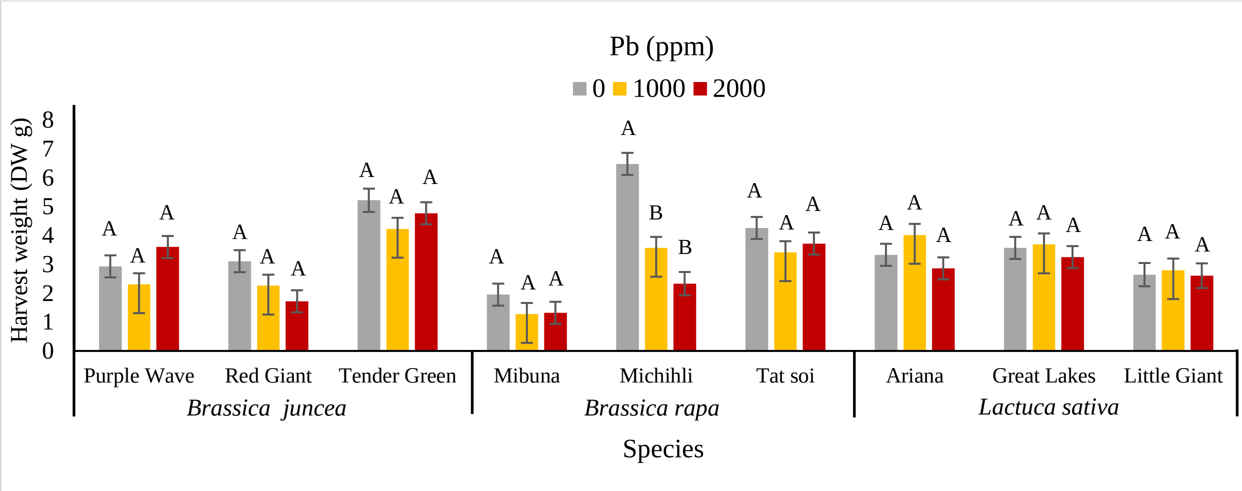

Figure 3. Trial one biomass of edible plant parts of mustard, Chinese cabbage, and butterhead lettuce varieties at time of harvest (42 days) across treatments and species. Letters indicate significant ($P < 0.05$) differences between treatments within each species variety. Bars within a variety that share the same letter are not significant. 28.34 grams = 1 oz.

Lead content in the leafy vegetation of both ‘Purple Wave’ and ‘Red Giant’ mustard varieties had a slight increasing trend when grown in medias with solution concentrations of 1,000 and 2,000 ppm Pb but was not significant. ‘Tendergreen’ mustard showed an increase in lead uptake in the 1000 ppm treatment with no significance. Both ‘Purple Wave’ and ‘Red Giant’ mustard displayed increasing trends of accumulation with increasing lead treatments but was not statistically signifiant. Lead accumulation in all mustard varieties in trial one were below the Chinese standard threshold level and did not significantly differ between the untreated control pots and the pots with 1,000 ppm and 2,000 ppm Pb contamination (Figure 4).

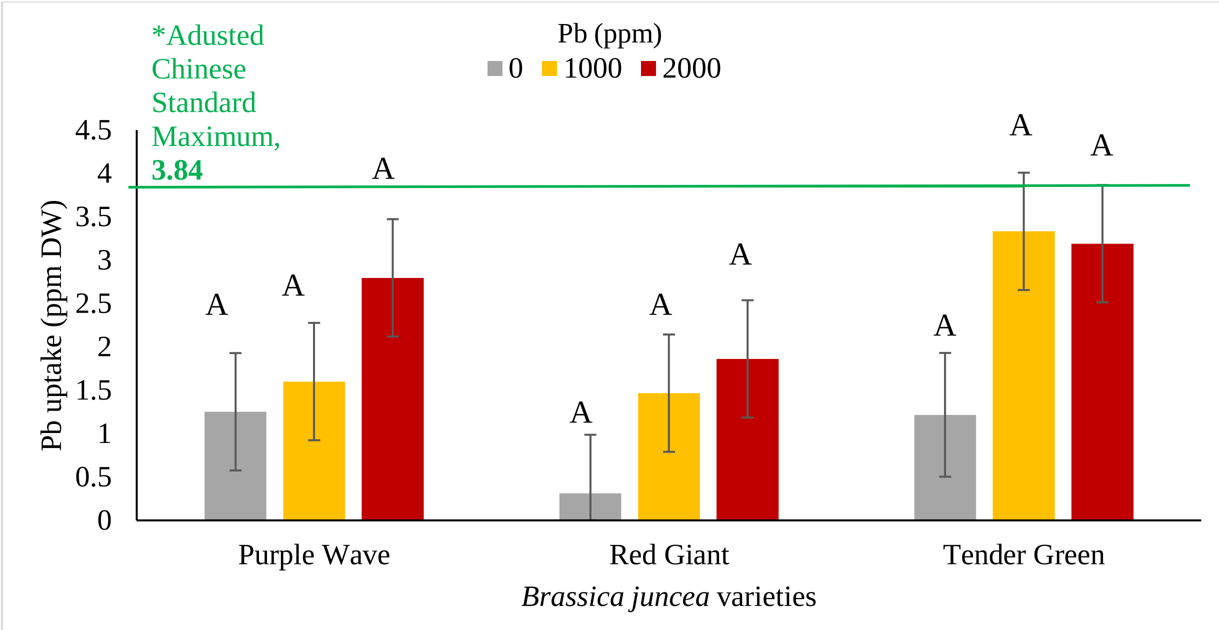

Figure 4. Lead (Pb) uptake in trial one of above ground tissues of mustard varieties grown in three lead concentrations (untreated control, 1000 ppm Pb, 2000 ppm Pb). Concentrations are per a 0.5 gram dry tissue sample. Letters indicate significant ($P < 0.05$) differences between treatments within species varieties. Bars within a variety that share the same letter are not significant. DW = parts per million dry weight. *The 3.84 ppm DW standard displayed has been adjusted for reference from 0.3 ppm FW Chinese Standard for maximum lead contamination (GAIN, 2018) calculated for 92% moisture content of our mustard.

Lead content in the leafy vegetation of ‘Mibuna’ Chinese cabbage was higher in the 2000 ppm Pb treatment as compared to the untreated control (Figure 5). Neither ‘Michihli’ nor ‘Tatsoi’ Chinese cabbage varieties had significant differences between lead treatments. ‘Mibuna’ grown in 2000 ppm Pb treatment accumulated lead at levels above the Chinese standard threshold level. Accumulation in all other cabbage varieties and treatments remained below this threshold.

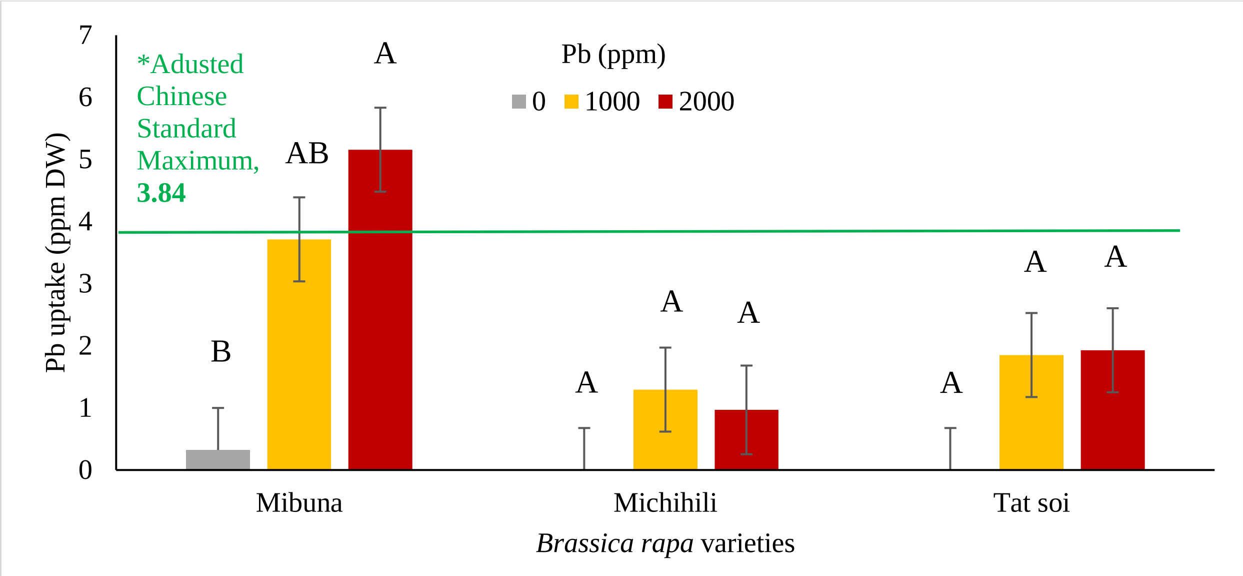

Figure 5. Lead (Pb) uptake in trial one of above ground tissues of Chinese cabbage varieties grown in three lead concentrations (untreated control, 1000 ppm Pb, 2000 ppm Pb). Concentrations are per a 0.5 gram dry tissue sample. Letters indicate significant ($P < 0.05$) differences between treatments within species varieties. Bars within a variety that share the same letter are not significant. PPM DW = parts per million dry weight. *The 3.84 ppm DW standard displayed has been adjusted for reference from 0.3 ppm FW Chinese Standard for maximum lead contamination (GAIN, 2018) calculated for 92% moisture content of Chinese cabbage.

Although there was an increasing trend in lead accumulation in the leafy vegetation of all lettuce varieties from untreated control to 1,000 and 2,000 ppm Pb contaminated pots, none of the accumulation values were significant (Figure 6). Across lettuce varieties, ‘Ariana’ accumulated the highest levels of lead at each treatment level though not to a significant degree. For all treatments, lead accumulation levels in Little Giant lettuce remained below the Chinese standard threshold level. ‘Ariana’ and Great Lakes lettuce both exceeded the threshold in the 2000 ppm Pb treatments.

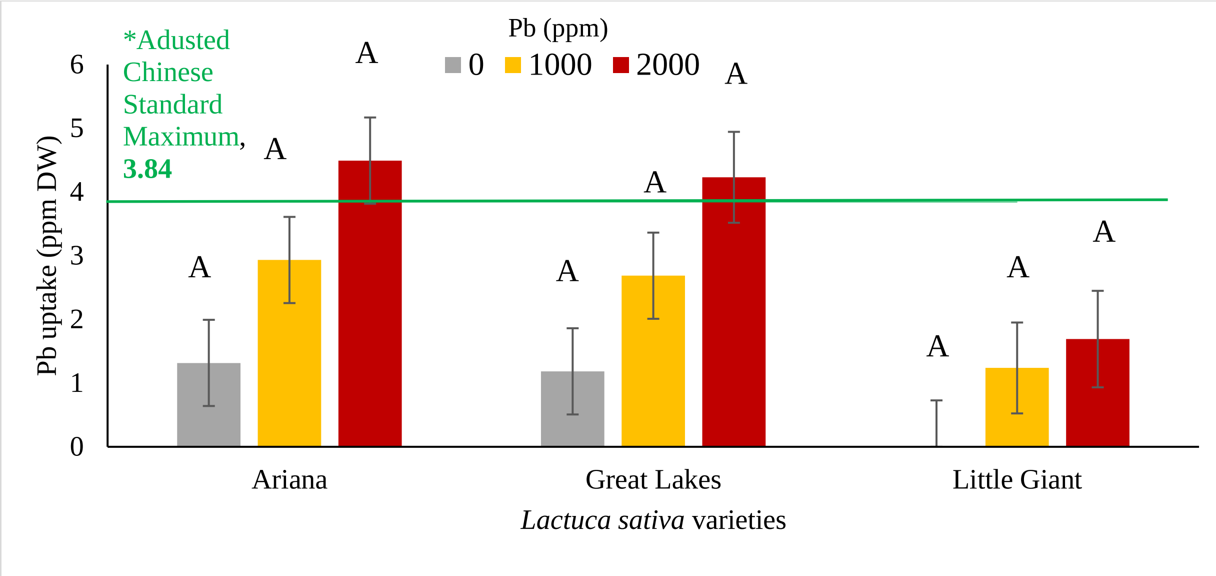

Figure 6. Lead (Pb) uptake in trial one of above ground tissues of butterhead lettuce varieties grown in three lead concentrations (untreated control, 1000 ppm Pb, 2000 ppm Pb). Concentrations are per a 0.5 gram dry tissue sample. Letters indicate significant ($P < 0.05$) differences between treatments within species varieties. Bars within a variety that share the same letter are not significant. ppm DW = parts per million dry weight, *The 3.84 ppm DW standard displayed has been adjusted for reference from 0.3 ppm FW Chinese Standard for maximum lead contamination (GAIN, 2018) calculated for 92% moisture content of lettuce.

Though ICP-MS was used in trial one, the samples in trial two were processed with ICP-OES using a modified extraction method which detected higher levels of lead. This method was located in a more accessible laboratory and reduced our costs overall. A 500 ppm pb treatment was added in the second replication of this trial, to more closely reflect contamination conditions near the EPA yard soil safety recommendation (400 ppm Pb) (EPA, 2013). To remain within a reasonable budget, two plant varieties within each species were selected for continued evaluation in the second trial narrowing down total varieties from nine to six. The varieties used in the second replication included; ‘Red Giant’ and ‘Tendergreen’ mustard, ‘Mibuna’ and ‘Michihli’ Chinese cabbage, and ‘Ariana’ and ‘Little Giant’ lettuce. A rodent ate some of the potted vegetable plants in the second replication. Data points representing plants that were chewed were excluded from analysis.

` The final biomass of each species variety as a factor of treatment is reported for trial two in figure 7. The elevated accumulation values in the control treatments for mustard and cabbage varieties (Figure 8 and 9) were not significantly different from other treatments and could have resulted from background contamination. ICP-OES extraction of the control soil used in the study was 10.2 ppm Pb.

Harvest dry weight of any vegetable crop species was not influenced by any treatment (Figure 7). This finding is consistent with trial one except for ‘Michihli’ Chinese cabbage which, in trial one, displayed a significant decrease in biomass with increasing treatment. However, we speculate the significant insect pressure in the first replication may have impacted overall plant growth.

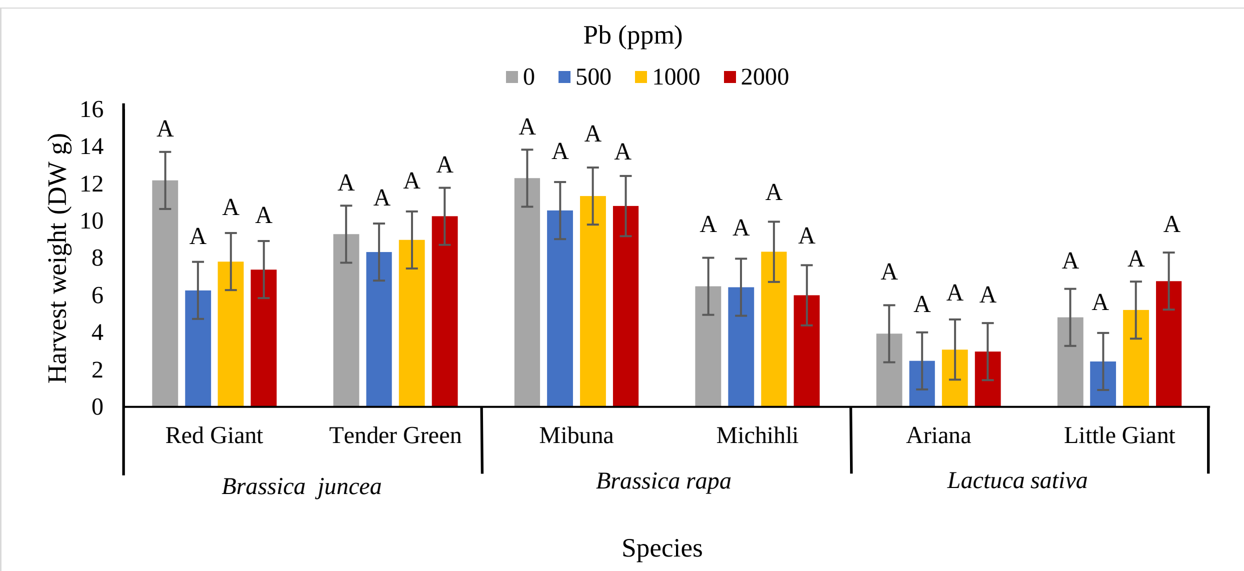

Figure 7. Trial two biomass of edible plant parts of mustard, Chinese cabbage, and butterhead lettuce varieties at time of harvest (42 days) across treatments and species. Letters indicate significant ($P < 0.05$) differences between treatments within species varieties. Bars within a variety that share the same letter are not significant. 28.34 grams = 1 oz.

Mustard, when grown in experimental conditions, showed no significant differences in lead accumulation across treatments in either of the species evaluated (Figure 8). ‘Red Giant’ and ‘Tendergreen’ both exceeded the Chinese standard threshold in control, 1000 ppm, and 2000 ppm Pb treatments.

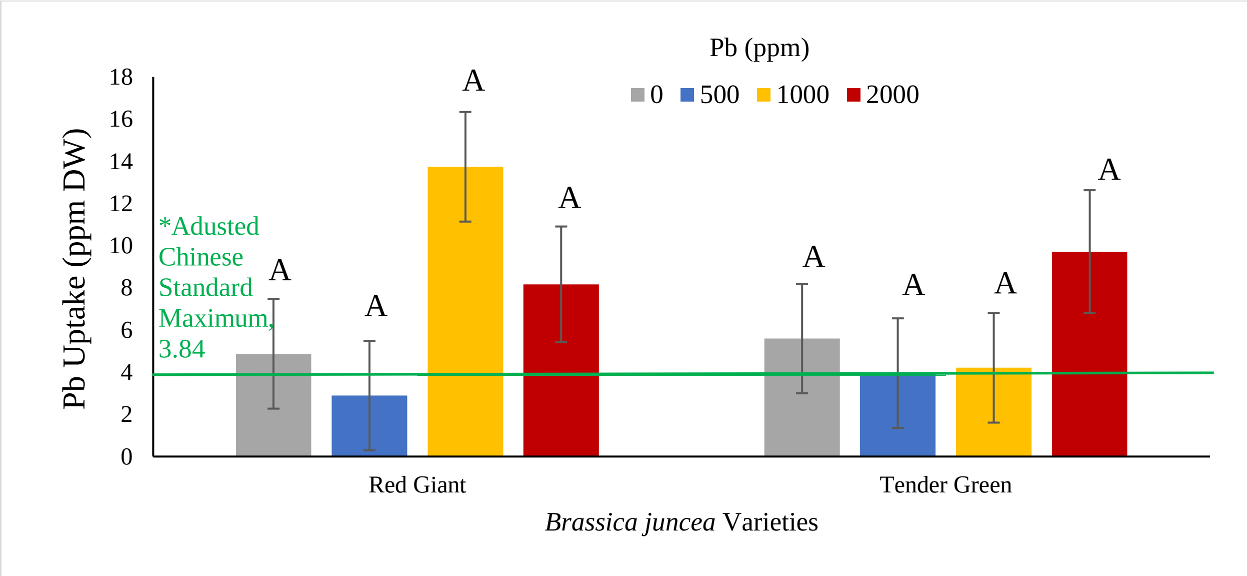

Figure 8. Lead (Pb) uptake in trial two of above ground tissues of mustard varieties grown in four lead concentrations (untreated control, 500 ppm Pb, 1000 ppm Pb, 2000 ppm Pb). Concentrations are per a 0.5 gram dry tissue sample. Letters indicate significant differences between treatments within species varieties. Bars within a variety that share the same letter are not significant. ppm DW = parts per million dry weight. *The 3.84 ppm DW standard displayed has been adjusted for reference from 0.3 ppm FW Chinese Standard for maximum lead contamination (GAIN, 2018) calculated for 92% moisture content of mustard.

The trial two lead content in above ground vegetation of both Chinese cabbage varieties increases with increasing solution concentration of lead from 500 ppm through 2000 ppm Pb though none of the accumulation values were significant (Figure 9). For ‘Mibuna’ Chinese cabbage, the control, 500 ppm, and 1000 ppm treatments were at or below the Chinese standard threshold level where as ‘Mibuna’ accumulations exceeded the threshold in all treatments.

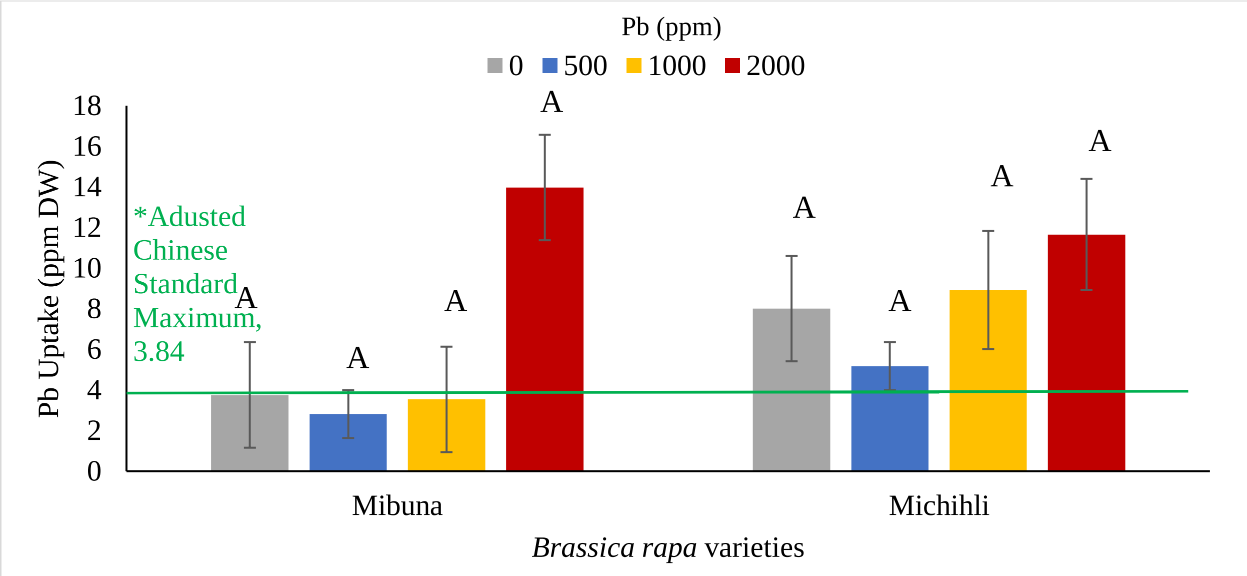

Figure 9. Lead (Pb) uptake in trial two of above ground tissues of Chinese cabbage varieties grown in four lead concentrations (untreated control, 500 ppm Pb, 1000 ppm Pb, 2000 ppm Pb). Concentrations are per a 0.5 gram dry tissue sample. Letters indicate significant ($P < 0.05$) differences between treatments within species varieties. Bars within a variety that share the same letter are not significant. ppm DW = parts per million dry weight. *The 3.84 ppm DW standard displayed has been adjusted for reference from 0.3 ppm FW Chinese Standard for maximum lead contamination (GAIN, 2018) calculated for 92% moisture content of Chinese cabbage.

The uptake and accumulation of lead in the above ground shoots varied depending on the different lead concentrations used (Figure 10). The lead content in above ground vegetation of ‘Ariana’ butterhead lettuce variety increases with increasing solution concentration of lead through 1000 ppm Pb then decreases at 2000 ppm Pb but with no significance from 1000 ppm Pb. The lead content of the ‘Little Giant’ lettuce variety increased with increasing lead treatments though no significance was observed between treatments. Both varieties exceeded the Chinese standard threshold for lead contaminants in all treatments, including control.

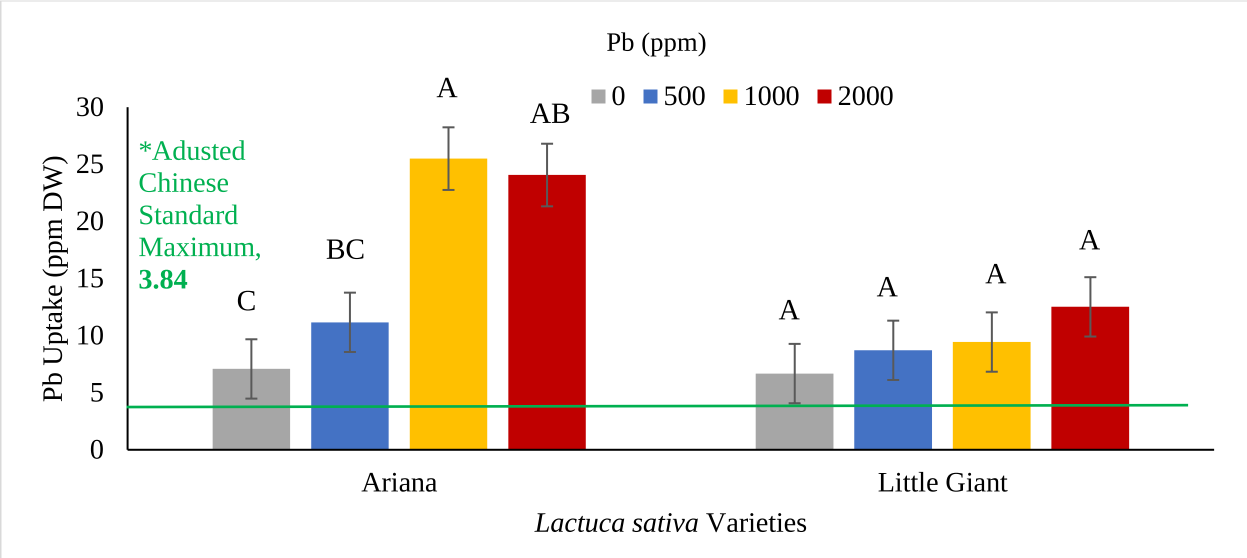

Figure 10. Lead (Pb) uptake in trial two of above ground tissues of Lettuce varieties grown in 1 gal containers filled with media contaminated at four lead concentrations (untreated control, 500 ppm Pb, 1000 ppm Pb, 2000 ppm Pb). Concentrations are per a 0.5 gram dry tissue sample. Letters indicate significant ($P < 0.05$) differences between treatments within species varieties. Bars within a variety that share the same letter are not significant. ppm DW = parts per million dry weight. *The 3.84 ppm DW standard displayed has been adjusted for reference from 0.3 ppm FW Chinese Standard for maximum lead contamination (GAIN, 2018) calculated for 92% moisture content of lettuce.

CHAPTER 3. EVALUATION OF BARRIERS TO PREVENT LEAD CONTAMINATION OF PRODUCE GROWN IN RAISED BEDS

3.1. Introduction

Lead (Pb) is a heavy metal that naturally exists in the environment at concentrations below 50 ppm (Pais and Jones, 1997). However, throughout history, anthropomorphic activities have facilitated its relocation, concentration, and subsequent threat to human health.

Lead use and thus environmental exposure in the United States has generally declined since the mid-1970's largely due to the phase-out of lead in gasoline after 1973 and through its ban in 1996 with the removal of lead from soldered cans and restricted use of lead paints (ATSDR, 2019). However, the residual effects of the lead use in these and other industries have increased the levels of environmental lead to which the average person is exposed. Heavily trafficked areas are at a greater risk for lead deposition. Gasoline combustion results in the release of tetraethyl lead $Pb(C_2H_5)_4$ and tetramethyl lead ($Pb(CH_3)_4$) into the atmosphere and subsequently significant deposition on to nearby soil. Further contributing to the high contamination rates faced by urban communities, most houses, schools, and government buildings built before 1978 were painted with lead-based paints containing lead carbonate ($PbCO_3$), lead sulfate ($PbSO_4$), lead chromate ($PbCrO_4$), lead tetraoxide (Pb_3O_4) and other compounds. Deterioration, renovations, demolition, or any paint peeling in these buildings can result in suspension and further deposition of lead particles indoors and upon the soils surrounding them (Defoe, 2017). Though lead exists in various compounds, as a basic element it does not readily degrade in the environment and binds tightly to soils contributing to its persistence as an environmental human health risk (ATSDR, 2019).

Drinking, eating, and breathing particles containing lead in any concentrations can result in acute or chronic lead poisoning. Many urban area soils are disproportionately contaminated with lead from particulate dispersal via leaded gasoline emissions and lead paint on homes, old playground equipment, etc. Older homes (built before 1978) and neighborhoods adjacent to heavily trafficked roads are often historically associated with marginalized and low-income communities (Meilke, 2019).

Urban gardening is experiencing a resurgence in neighborhoods and schools as an educational tool for food, environmental, and nutrition literacy and as self-sufficiency practices; therefore, an understanding of the state of soil contamination is vital to reduce or eliminate unnecessary food chain transfer of soil lead to urban communities. Unfortunately, various common garden vegetables have heavy-metal tolerance traits and can grow uninhibited by lead and subsequently accumulate the heavy metal in their tissues (Liu et al., 2009; Capelo et al., 2012; Ramesar et al., 2014). In 2018, China released a standard for maximum levels of contaminants in foods. The maximum value allowed for *Brassica* vegetables and leafy greens is 0.3 ppm fresh weight (GAIN, 2018). Many studies have looked at lead uptake and partitioning in plants in contaminated soils, and chelator enhanced removal of lead from soils but few studies have looked at physical barriers to limit or prevent lead movement from contaminated ground into a raised bed environment.

The EPA set maximum bare soil lead concentrations in federally funded project sites. Bare soil play areas and high-contact areas for children are limited to 400 ppm Pb. The rest of the yard is allowed 1,200 ppm Pb (EPA, 2013). Various Cooperative Extension publications cite the EPA value of 400 ppm as the maximum soil lead concentration considered safe to grow vegetables in the soil, though some recommend adding amendments if the gardener choses to grow there. At any concentration above 400 ppm, an effective, yet expensive remediation solution is excavation and removal of contaminated soils. Alternatively, the University of Connecticut Extension Service recommends growing vegetables in raised beds as a reasonable reduced risk option for avoiding lead accumulation in garden vegetables particularly leafy greens and root vegetables (Pettinelli, N.D.). These recommendations are consistent with those presented by other extension services throughout the country, specifically from Kentucky State, Oregon State, UMass Amherst and the University of Delaware (Brewer, 2016; Defoe, 2017; Gartley, 2002). All of these publications recommend growing in raised beds when yard soil contamination is above 400 ppm. While both UMass Amherst and the University of Connecticut recommend using a liner under a raised bed, only the University of Connecticut defines their recommendation as landscape fabric liner, though research based efficacy of lining raised beds was not cited (UMass, 2017).

To address the soil-lead exposure pathway through garden produce, this study will supplement gaps in the literature pertaining to raised-bed garden practices on lead contaminated grounds. The objective of this study aims to evaluate the efficacy of various barriers under raised beds to prevent lead mobility from contaminated ground soil into the uncontaminated raised bed environment.

3.2 Materials and Methodology:

Barrier materials investigated include Premlene™ Neoprene (New Orleans Rubber, Harvey, LA) (0.062 in, non-permeable), landscape fabric (Preen, Lebanon, PA) (0.019 in, permeable), and a no-barrier control. *Brassica rapa* Chinese cabbage 'Mibuna' was selected based on its elevated rates of accumulation in the previous accumulation study (see chapter 2) to be grown out in simulated raised bed conditions.

To simulate raised beds in a contaminated environment, large opaque plastic containers (44 gallons, 133 cm x 52.4 cm x 35.6 cm) (IRIS USA, INC, Surprise, AZ) were modified. Plastic containers were selected because they served as a closed system to allow for full containment of lead contaminated media. Each container had a drainage hole at one end, 1.5 in from the bottom to allow for drainage. This hole was covered with landscape fabric to allow irrigation water to drain without media loss (Figure 12).

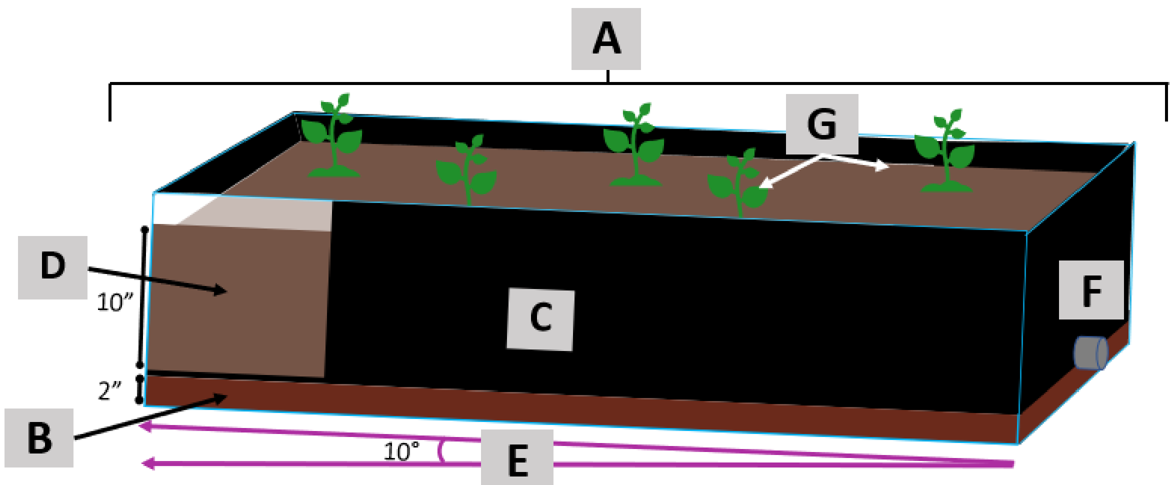

Figure 11. Diagram of raised bed experimental set up. (A) 44 gal plastic container (outlined in blue), (B) Contaminated media (500 ppm Pb), (C) Barrier treatment (cut away to show media profile), (D) Uncontaminated media, (E) Elevation gradient, (F) Drainage hole, (G) *Brassica rapa* ‘Mibuna’ plants.

Each container was elevated at the opposite end at a 10° angle to encourage drainage. The raised bed containers were arranged in two rows. Within each row, containers were placed side by side with an inch of space separating each plot (Figure 13).

These containers were each filled with 5 gallons of contaminated media (500 ppm Pb). The media used in this study is similar to standard mixes often used in raised beds. The media was mixed in large batches using peat moss 4 cu ft (Lambert Peat Moss, Quebec, Canada), 5/8th in screened pine bark (Phillips Bark, Brookhaven, MS), washed large grain sand 4 cu ft (Baer

Industries, Port Allen, LA), Osmocote 19-5-9 (ICL Specialty Fertilizers, Summerville, SC) 10.5 lb (4767 g), Micromax (ICL Specialty Fertilizers, Summerville, SC) 0.5 lbs (227 g), dolomitic lime (Lhoist, Port Allen, LA) 8 lbs (3632 g). The media was spiked with a 10,000 ppm Pb standard in a 5% HNO_3 solution to attain a final concentration of 500 ppm in the media (RICCA Chemical Company, Arlington, TX).

Figure 12. Drainage hole installed in plastic raised bed boxes.

Figure 13. Experimental plot layout. Each plot is elevated at 10° to promote drainage.

Inductively coupled plasma-optical emission analytical spectrometry (ICP- OES) (PerkinElmer, Houston, TX) procedure with an HCl and HNO_3 digestion was used to analyze total lead in media to confirm intended initial concentrations (EPA Method 3051A). The media was then covered and sealed to the edges of the containers using the experimental barriers (Figure 14) and filled with 15 gal of uncontaminated media per container. Each container/barrier combination was replicated three times. All treatment containers were arranged in a complete randomized design (CRD).

Figure 14. Simulated raised bed barriers applied to containers. Contaminated media sealed by selected barriers up to the top of each container's edge (A. neoprene rubber; B. landscape fabric; C. no-barrier control).

On March 11, 2020 and April 11, 2020 seeds of *Brassica rapa* variety 'Mibuna' were planted in 50 cell trays (T.O. Plastics Clearwater, MN) using SunGro Metro-Mix 830 (SunGro Horticulture, Agawam MA) and grown in a greenhouse. Temperatures remained cool through the second trial ranging between 53°F and 78°F. Transplants were watered daily via overhead irrigation twice a day for five minutes. Transplants were fertilized using Peter's Professional™ water soluble 20-20-20 fertilizer (ICL Specialty Fertilizers, Summerville, SC) at 700 ppm every other week and the seedlings were thinned to one plant per cell. Two weeks after seeding, five plants were planted into each raised bed container at 12 inch spacing. The containers were located under a hoop house with open sides where temperatures ranged between 53°F and 78°F. The plants were hand-watered daily and an insecticide Bifenthrin (Fertilome, Bonham, TX) was used as needed at recommended rates to control aphids, flea beetles, and cross-striped cabbageworm. At 30 days of growth in the raised beds all plants were harvested at the base of the stem and the above ground portion of the plant was weighed for fresh weight in grams. All plants were divided between two dryers (SHEL Lab and VWR Scientific Inc.) at an average of 60 °C and dried to a constant weight before grinding through a 1mm sieve. Inductively Coupled Plasma- Optical Emission Spectrometry (ICP-OES) procedure with an HCL digestion was used to analyze available lead in the media and total lead accumulated in the plant tissues (AOAC Method 985.01). The neoprene rubber sheeting was analyzed for lead content using a similar procedure as above. This experiment does not account for raised bed contamination via dust upheaval via wind or activities in the garden area that causes disturbance of contaminated ground (Clark, 2008). It is recommended that in addition to building raised beds, that any exposed contaminated soil be covered with a thick organic or inorganic ground cover.

A respirator and goggles were the personal protective equipment (PPE) used each time lead standards and loose contaminated media were handled. Contaminated materials were disposed of after the conclusion of the experiment by Louisiana State University Hazardous Waste Disposal (LSU Office of Environmental Health and Safety 217 Administrative Support Building, Baton Rouge, LA 70803). Data were analyzed with the statistical program SAS (version 9.2; SAS Institute, Cary, NC) Proc GLM with Tukey and Excel for Microsoft 365 (Microsoft Corporation, Redmond, WA).

3.3. Results and Discussion

Brassica rapa 'Mibuna' was grown in experimental raised bed plots equipped with barrier treatments (no barrier, landscape fabric, and neoprene rubber) dividing contaminated media and uncontaminated media in which the plants grew. The effects of lead on final harvest weight of *Brassica rapa* did not vary significantly across barrier treatments implying that growth was not affected by the treatments applied (Figure 15). Due to lack of significant difference between the final harvest weights in each trial, we are able to compare trial A and trial B. Findings suggest that, counter to expectations, neither barrier sufficiently excluded lead uptake compared to the no barrier control treatment (Figure 16).

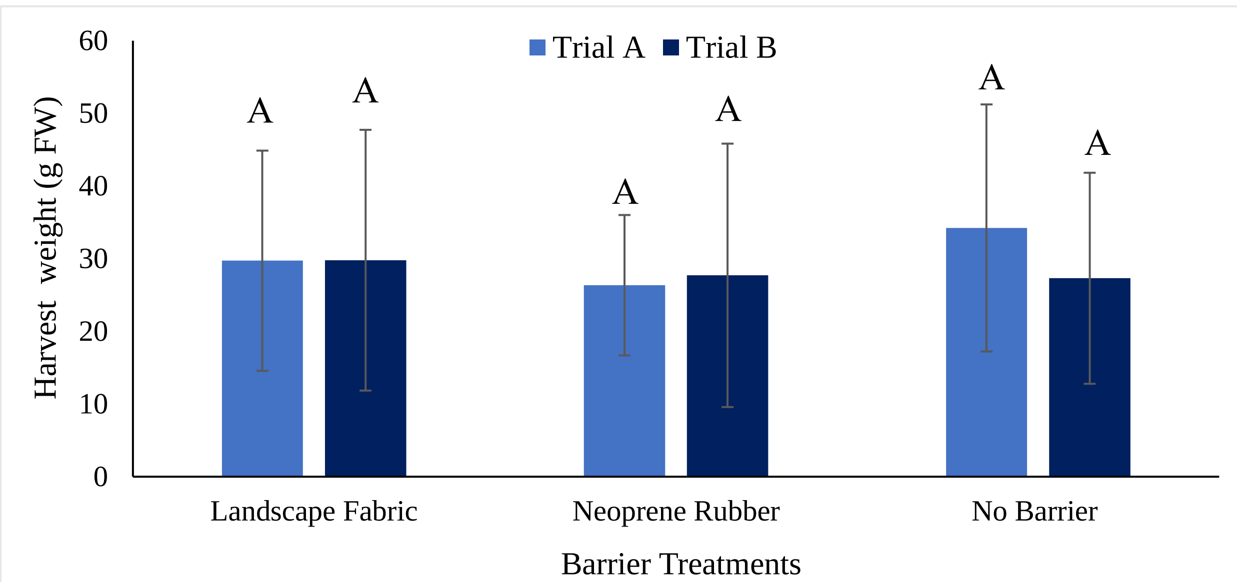

Figure 15. Harvest weight of *Brassica rapa* Chisese cabbage 'Mibuna' in Trials A and B across 3 barrier treatments (no barrier, landscape fabric, and neoprene rubber) over contaminated media (500 ppm). g FW= grams fresh weight. Letters indicate significant ($P < 0.05$) differences between treatments and trials. Bars that share the same letter are not significant. Error bars created using standard errors.

The plants in neoprene and fabric treatments, counter to expectations, exceeded the reference value published in the Global Agriculture Information Network (GAIN) report outlining the Chinese National Food Safety Standard for Maximum Levels of Contaminants in Foods with no significant differences between them (Figure 16).

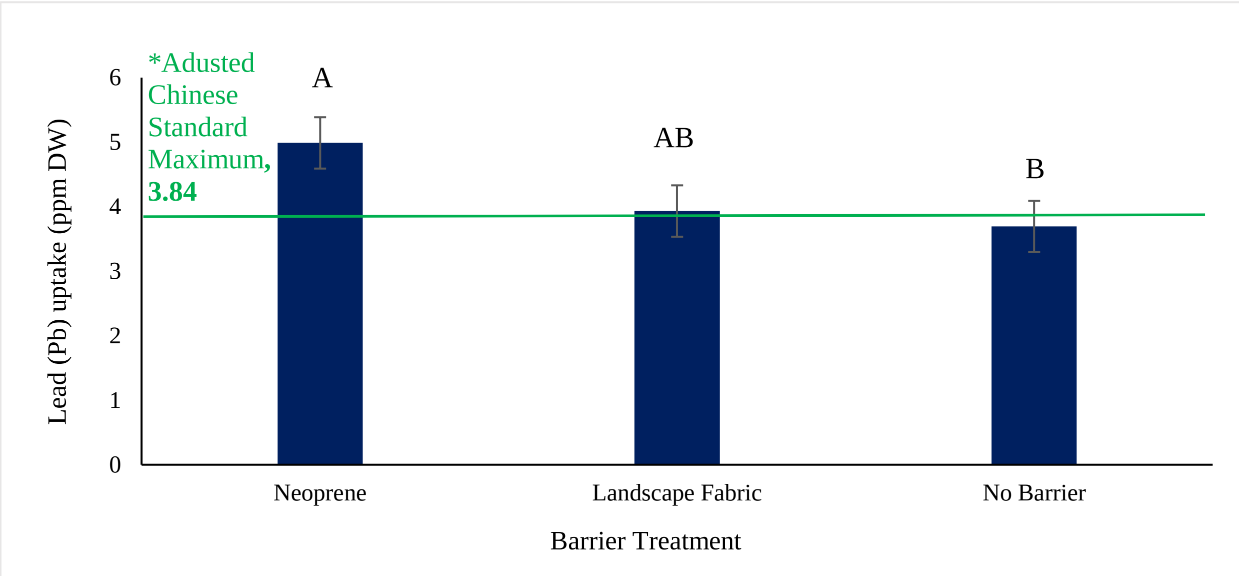

Figure 16. Lead (Pb) accumulation in plants *Brassica rapa* Chinese cabbage 'Mibuna' grown in raised beds with barriers dividing uncontaminated and contaminated media (500 ppm Pb). Displayed data represents two replicated trials. PPM DW = parts per million dry weight. *The 3.84 ppm DW standard displayed has been adjusted for reference from 0.3 ppm FW Chinese Standard for maximum lead contamination (GAIN, 2018) calculated for 92% moisture content of mustard. Letters indicate significant differences at alpha level 0.05. Bars that share the same letter are not significantly different. Error bars were created using standard error.

Not only did the neoprene rubber sheet treatment exceed the threshold discussed above, the accumulation reported is significantly higher than the no barrier treatment. Neither of the two experimental barrier treatments successfully prevented lead uptake in *Brassica rapa* 'Mibuna' to any greater extent than the no barrier treatment as expected. Plausible explanations for this unexpected result could be that the pores in the landscape fabric may have allowed some contaminated media movement, contamination could have occurred via the barrier materials themselves or the plants did not grow deep enough to reach the uncontaminated-contaminated media interface of the simulated raised bed to accumulate substantial lead in the plant tissues. ICP-

OES extractions were carried out on the neoprene rubber revealing the material contained 91 ppm Pb, possibly contributing to the elevated lead accumulation in the Chinese cabbage plants grown in the neoprene barrier treatment. Lead analysis was not performed on the landscape fabric because the accumulation data of the plant tissue did not indicate increased Pb levels in this treatment.

Furthermore, at this point we cannot recommend no barrier. While the no barrier control treatment looked similar to the landscape fabric treatment (Figure 16), consistent with a University of Connecticut Extension publication suggesting landscape fabric lining in raised beds, we (Pettinelli, N.D.). we would recommend further investigations of barrier materials. In this study, the plants were only allowed to grow for 30 days, whereas a home owner may let them grow for 40 or 50 days. We believe that had we allowed these plants to grow for a longer period of time we likely would have seen higher accumulation in the no barrier treatment by allowing more time for the roots to grow in the contaminated zone.

Future projects related to this research should evaluate other impermeable barriers such as other plastics like visqueen, and landscape fabrics of other densities.

CHAPTER 4. CONCLUSIONS

4.1. Harvest weight

Overall, lead treatments did not affect plant growth in any species with the exception of 'Michihli' Chinese cabbage in trial one. In trial one, plant growth at 1000 ppm and 2000 ppm lead treatments was significantly reduced when compared to control treatment implying a possible toxic response to lead in 'Michihli' cabbage, though the effect was not replicated in trial two.

4.2. Lead accumulation

It is evident that different cultivars accumulate lead at different rates. Farmers and gardeners should consider these differences when selecting cultivars if growing in potentially contaminated areas. Trial one was subject to moderate insect pressure in the final week of the study. In this first trial exceedances for the Chinese standard maximum threshold were only found at the 2000 ppm level. In trial two, using ICP-OES extraction, in several cases at all treatment levels. These cultivars which accumulated concerning levels of lead in their edible tissue, also showed few signs of disproportionate stress in the lead contaminated plots. This raises concern for growers who may be growing in contaminated areas without visual symptoms of plant lead related stress.

We used leafy greens in our study as an indicator crop for lead contamination. Extension studies recommend avoiding leafy green and root vegetables in areas contaminated beyond 400 ppm Pb. Following the results of our first study we agree with this suggestion with the caveat that even below 400, certain species can still accumulate lead in edible tissues at concerning levels.

4.3. Barrier Treatments

Neither neoprene rubber nor landscape fabric showed a decrease in lead levels in plants grown in experimental plots compared to the no barrier control contrary to expectations. Further, the neoprene rubber barrier treatment exceeded the no barrier treatment to a significant degree implying that this treatment further contributes to raised bed lead contamination rather than lead mitigation and exclusion. The significant increase in lead uptake observed in the plants grown in the neoprene barrier treatment is possibly due to lead leaching from the barrier material. Subsequent evaluation of the neoprene material used in this simulated raised bed study revealed that this material contained lead (91 ppm Pb). Subsequent investigation of other systems that use neoprene corroborates this conclusion revealing other occurrences of lead leaching from neoprene materials. A study of a water delivery system designed for animals evaluated the water bottle components, finding that the neoprene stoppers used leached lead at concerning levels (Nunamaker et al., 2013).

While the findings displayed in Figure 15 are consistent with observations that no treatment adversely affected plant growth, additional factors to consider for use of any impermeable barrier treatment include ensuring consistent efficient drainage. There were no observable fungal growth issues related to moisture retention, however that would be a long-term consideration to investigate considering this study was conducted in controlled rain shelter conditions which would not be the case for the average urban gardener. If this study were to be replicated, standard procedure would include extending the growing season to give the plants more time to develop a root system into the contaminated layer and the addition of a fourth true control treatment without a contaminated base layer.

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