DIFFERENTIAL LEAD (PB) ACCUMULATION IN COOL SEASON VEGETABLES

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ABSTRACT

Lead (Pb) contamination of urban soils can result in health risks for exposed individuals. Soil-Pb exposure occurs by ingestion or inhalation and poses an elevated risk for young children. Indirect Pb ingestion can occur when vegetables are grown in Pb contaminated soils. The Pb is often found in the edible tissue of vegetables. The objective of this study was to evaluate differential Pb uptake in common cool season vegetable crops. Multiple cultivars of three species of leafy green vegetables (*Brassica juncea, Brassica rapa, & Lactuca sativa)* were grown in Pb treated media (0, 500, 1000, and 2000 ppm Pb) to evaluate Pb accumulation in plant leaves and stems. In both trial 1 and 2, the highest accumulating cultivars at the 2000 ppm level were Chinese cabbage 'Mibuna', and lettuces 'Arianna' and 'Great Lakes'. All three of these cultivars exceeded a food safety standard threshold. In the second trial, all but two cultivars exceeded this threshold in all soil Pb treatments.

Key Words: Hyperaccumulator; Lettuce; Mustard Greens; Chinese Cabbage; Heavy Metal; Soilless media

1. INTRODUCTION

Lead (Pb) is a naturally occurring heavy metal that exists at low concentrations (50 ppm) in the environment (Pais and Jones, 1997). Throughout history, human activities have facilitated its relocation, concentration, and subsequently its increased direct human contact which can threaten public health. Lead particle deposition onto the soil is a major source of exposure and has been corelated to elevated blood lead levels in children (Mielke, 2019). For reference of 'acceptable' soil Pb limits, the U.S. Environmental Protection Agency (EPA) set maximum bare soil Pb 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). Direct exposure to soil Pb may occur by inhalation and ingestion of dust. Indirect exposure may occur by ingestion of contaminated produce grown in contaminated soil.

Lead adsorption and uptake has been observed in several food crops including mustard *Brassica juncea* (Meyers et al., 2008), lettuce *Lactuca sativa* (Capelo et al., 2012; Uzu et al.,2009) and others. In many cases, Pb accumulation will pose minor to severe toxic threats to plant growth and development via morphological, physiological, and biochemical mechanisms. Previous studies have shown harmful effects including impaired plant growth, root elongation, reduced seed germination, and seedling death (Pourrut et al., 2011). One mechanism of Pb toxicity, similarly to human systems, is induction of reactive oxygen species (ROS) which are short-lived, unstable, and highly reactive molecules. ROS in excess have been shown to cause cell damage and death (Scandalios, 1993).

Different species accumulate Pb by various mechanisms while experiencing various degrees of toxicity. Plant species with tolerance traits accumulate large amounts of lead in vegetative tissues and are termed "lead hyperaccumulators". Some traits for tolerating heavy metal damage, include binding the Pb to the cell wall before the it enters the cell and the production of additional antioxidants that help prevent damage by ROS caused indirectly by lead uptake. Plants like these, with particular genotypic traits and physiological characteristics, can accumulate lead at elevated rates without extensive tissue damage (Capelo et al., 2012; Liu et al., 2009; Ramesar et al., 2014). Vegetable crops with these characteristics can grow in contaminated sites with minimal negative side effects and therefore, could benefit remediation efforts. However, using plants with both edible and remediation traits can pose harm to communities if these plants are grown for food. While the United States does not regulate Pb contamination in produce, Chinese regulatory agencies released the National Food Safety Standard for Maximum Levels of Contaminants in Foods in 2017. Per this published standard, the maximum concentration of Pb allowed for Brassica vegetables and leafy greens is 0.3 ppm of total fresh weight (GAIN, 2018).

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). As an example, 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 is to quantify lead accumulation in a variety of leafy greens that are commonly grown in raised-beds using a soilless media generally used by backyard gardeners.

2. MATERIALS AND METHODOLOGY

Based on previous lead accumulator research (McBride et al., 2012; Mourato et al., 2015; Liu et al., 2010), three cultivars 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 cabbage cultivars included 'Mibuna', 'Tatsoi', and 'Michihli', all commonly considered Chinese cabbage. Lettuce cultivars 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 three Pb media treatments in the first trial (0 ppm, 1000 ppm, 2000 ppm) and four Pb treatments in the second trial (0 ppm, 500 ppm, 1000 ppm, 2000 ppm). These concentrations were chosen with consideration of the current EPA values of soil Pb cautionary levels, selecting treatments just above the 400 ppm play area threshold and above and below the 1,200 ppm threshold for Pb contamination 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. The seedlings were watered daily, using overhead irrigation set twice a day for five minutes each time period. The seedlings were thinned to one plant per cell and the transplants were fertilized using a liquid fertilizer (Peter's Professional 20-20-20 fertilizer; ICL Specialty Fertilizers, Summerville, SC) at 400 ppm N every other week. Seven weeks after seeding, the plants were transplanted into the spiked (untreated control, 500 ppm Pb, 1000 ppm Pb, 2000 ppmPb) 1 gallon (3.7 L) blow molded plastic nursery containers (Nursery Supplies, Forest Hill, LA). The media used in this study is similar to standard mixes often purchased for raised beds. The media was mixed in large batches and consisted of 4 ft^3 (0.11 m^3) of peat moss (Lambert Peat Moss, Quebec, Canada), 2 yd^3 (1.53m^3) of 5/8th in (15.8 mm) screened Pine Bark (Phillips Bark, Brookhaven, MS), 4 ft^3 (0.11 m^3) of washed large grain sand (Baer Industries, Port Allen, LA), 10.5 lb (4767 g) of Osmocote 19-5-9 (ICL Specialty Fertilizers, Summerville, SC), 0.5 lbs. (227 g) of Micromax (ICL Specialty Fertilizers, Summerville, SC), 8 lbs (3632 g) of dolomitic lime (Lhoist, Port Allen, LA). 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 municipal water. All containers were soaked to saturation then 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 was used to analyze total Pb in soil media to confirm intended initial concentrations (EPA Method 3051A). T

The 1-gallon nursery containers were positioned across three prefabricated fiberglass ebb and flow tables (54 in x 191 in). The containers were elevated onto overturned web trays to allow drainage from each pot (Figure 1). Each fiberglass table was fitted with a drainage hole to collect irrigation runoff. The treated, planted nursery containers were arranged into a split plot design by soil treatments on each table and distributed randomly within the treatment split plots (Figure 2). All runoff was collected, and contaminated soil media was contained.

The potted plants were hand-watered daily and bifenthrin (Fertilome, Bonham, TX) was applied at labeled rates to control aphids (*Brevicoryne brassicae*), flea beetles (*Phyllotreta striolata, Phyllotreta cruciferae*), and cross-striped cabbage worms (*Evergestis rimosalis*). After 42 days of growth in the contaminated media, all plants were harvested. Plant tops were cut at the soil line and weighed for fresh weight (g). Plant top tissue samples were dried (SHEL Lab, Cornelius OR, and VWR Scientific Inc, Suwanee, GA) at an average of 60°C 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 machinery availability (AOAC

Method 985.01). Because of cost restrictions and an inability to clean roots without spreading Pb contaminated media, only plant tops, the edible portion of the plants were analyzed for Pb content.

A respirator and goggles were the personal protective equipment (PPE) used each time lead standards and loose contaminated soils 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, Baton Rouge, LA 70803). Data were analyzed with the statistical program SAS (version 9.2; SAS Institute, Cary, N.C.) Proc GLM with Tukey.

3. RESULTS AND DISCUSSION

3.1 Trial One

Total biomass (weight) at harvest of each cultivar within the three vegetable crops were reported for trial one (Figure 3). Significant weight differences were found in only one cultivar Pb media combination (Figure 3). 'Michihli' Chinese cabbage biomass was significantly lower in the 2000 ppm Pb treatment compared to the 1000 ppm and control. No other statistical differences in final biomass across media treatments for any cultivars were found, indicating lead contamination in soils did not affect overall plant growth. The plants in this first trial experienced elevated insect pressure so in the second trial a regular insecticide application schedule was implemented and an increase in total biomass weights was observed (Figure 4).

Table 1 depicts accumulation data for the mustards, cabbages, and lettuces. There are 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 tables also features a published Chinese food safety standard threshold level for maximum concentration of lead contaminant allowed in edible tissue of brassica and leafy produce (adjusted from 0.3 ppm fresh weight to 3.84 ppm Pb dry weight level). All accumulation values with an asterisk preceding the value exceeded this threshold. Threshold exceedances for trial one were observed at the 2000 ppm media level in Chinese cabbage 'Mibuna' and lettuce 'Arianna' and 'Great Lakes' cultivars.

Mustard, when grown in experimental conditions, showed no significant differences in lead accumulation across treatments in either of the species evaluated (Table 1). Additionally, no differences were observed within Pb media treatment groups across mustard cultivars. Of the Chinese cabbages, only 'Mibuna' displayed differences between the control treatment and the Pb treated groups. At the 2000 ppm level, 'Mibuna' accumulated significantly more lead than the 'Tatsoi' and 'Michihli' cultivars. Of the lettuce cultivars, Pb accumulation in 'Ariana' and 'Great Lakes' in the 2000 ppm treatments were significantly greater than each of their control groups. At the 2000 ppm level, both 'Arianna' and 'Great Lakes' accumulated lead at significantly greater levels than 'Little Giant' (Table 1).

3.2 Trial Two

Although ICP-MS was used in trial one, the samples in trial two were processed with ICP-OES using a modified extraction method. The ICP-OES method detected higher levels of lead. The machinery used for this method was located in a more accessible laboratory offering reduced costs. A 500 ppm Pb treatment was added in this second trial to reflect contamination conditions near the EPA yard soil safety recommendation (400 ppm Pb) (EPA, 2013). To remain within a reasonable budget, two plant varieties of each edible species were selected for continued evaluation in the second trial narrowing down total number of cultivars from nine to six. The cultivars used in the second replication included 'Red Giant' and 'Tendergreen' mustard, 'Mibuna' and 'Michihli' Chinese cabbage, and 'Arianna' and 'Little Giant' lettuce.

The final biomass of each cultivar by media Pb treatment is reported for trial two in figure 4. The accumulation values in the control treatments for all cultivars were not significantly different from the 500 ppm treatments of each cultivar and could have resulted from background contamination (Table 2). ICP-OES extraction of the control soil medium used in the study was 10 ppm Pb.

There were no significant biomass differences across Pb treatments in any of the studied cultivars in trial 2 (Figure 4). This finding is consistent for all varieties in trial one with the exception of 'Michihli' Chinese cabbage.

'Red Giant' Mustard showed a significant difference in Pb accumulation between the control and 500 ppm groups and the 1000 ppm treatment (Table 2). 'Tendergreen' mustard Pb accumulation at the 2000 ppm level was significantly higher than all other treatments for this cultivar. At the 500, 1000, and 2000 ppm levels, 'Tendergreen' accumulation was significantly higher than 'Red Giant'. 'Tendergreen' mustard exceeded the referenced food safety threshold in all treatment groups. 'Red Giant' also exceeded this value in all treatments except 500 ppm (Table 2).

Neither Chinese cabbage nor lettuce cultivars showed significant differences in Pb accumulation across treatments (Table 2). 'Mibuna' cabbage exceeded the food safety threshold only at the 2000 ppm level treatment whereas 'Michihli' exceeded the food safety threshold value in all treatment groups. In contrast to the first trial, there was not a significant difference in Pb accumulation in 'Arianna' compared to 'Little Giant'; however, both of these lettuce varieties exceeded the food safety threshold accumulation value in all treatment groups (Table 2).

4. CONCLUSIONS

4.1 Harvest weight

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

4.2 Lead accumulation

Leafy green cultivars accumulate lead at different rates; therefore, farmers and gardeners should consider these differences when selecting cultivars especially if they are growing in potentially contaminated areas. Trial one was subject to moderate insect pressure in the final week of the study, which reduced overall above ground plant tissue. First trial exceedances for the Chinese standard maximum threshold were only found at the 2000 ppm level. In trial two, using ICP-OES extraction, exceedances were observed across cultivars at all treatment levels. The cultivars that accumulated concerning levels of lead in their edible tissue did not show signs of disproportionate stress in the lead contaminated plots or reduced biomass. This raises concern for growers who may be growing in contaminated areas. Without visual symptoms of plant lead related stress, it would be hard to tell if their plant had accumulated Pb. Gardeners should choose plants that do not accumulate lead if growing in contaminated areas. In the first trial, 'Arianna' and 'Great Lakes' lettuces were more efficient Pb accumulators than 'Little Giant' though in trial 2, 'Arianna' and 'Little Giant' accumulation values did not vary significantly.

Leafy greens were used in this study as an indicator crop for lead contamination. Extension studies recommend avoiding leafy green and root vegetables in soils contaminated beyond 400 ppm Pb (Pettinelli, N.D.; Brewer et al., 2016; Gartley, 2002; UMass, 2017). Our results agree with this suggestion and add the caveat that even below 400ppm Pb media levels, certain edible species can still accumulate lead at concerning levels.

4. TABLES AND FIGURES

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

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.

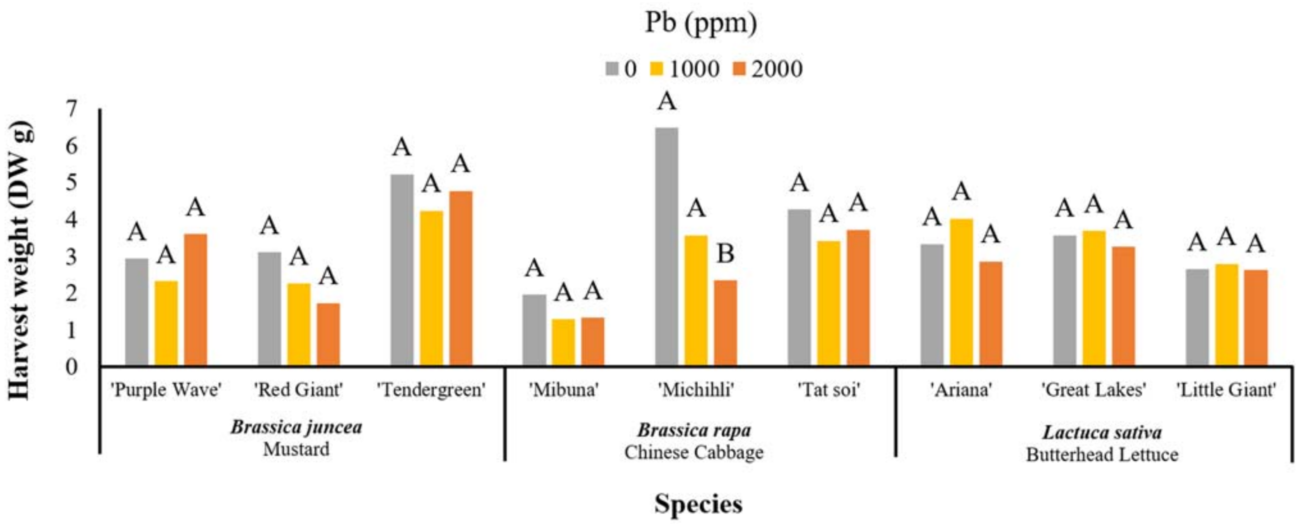

Figure 3. Trial one biomass of edible plant parts of mustard, Chinese cabbage, and butterhead lettuce varieties at time of harvest (42 days after transplant) across treatments and species. Letters indicate significant (*P < 0.05*) differences between treatments within each cultivar, not across cultivars.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

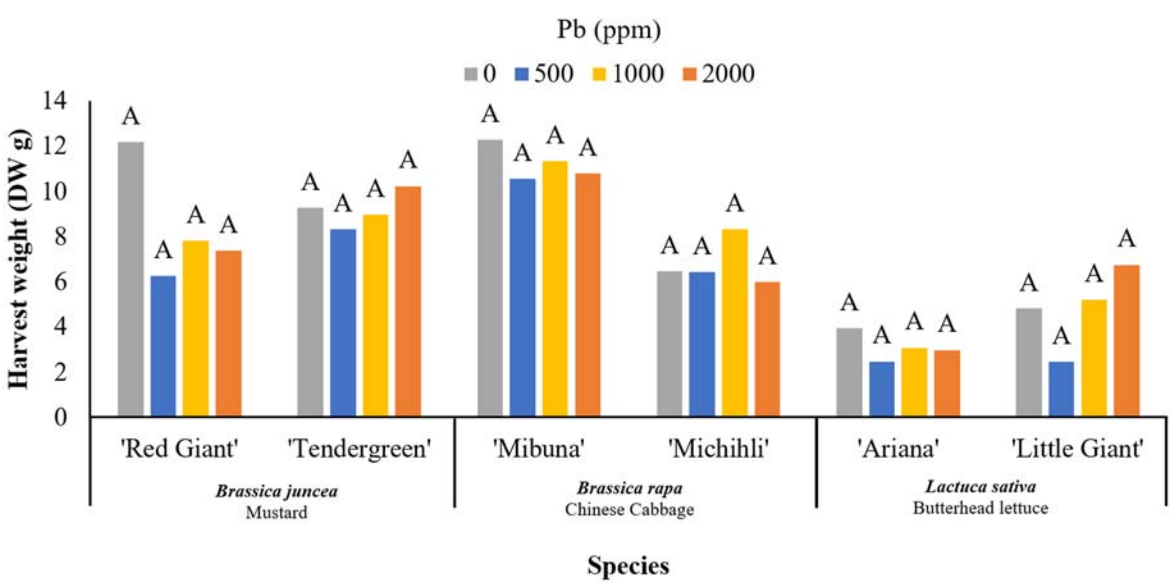

Figure 4. Trial two biomass of edible plant parts of mustard, Chinese cabbage, and butterhead lettuce varieties at time of harvest (42 days after transplant) across treatments and species. Letters indicate significant ($P < 0.05$) differences between treatments within each cultivar but not across cultivars or across vegetable types. Bars within a cultivar that share the same letter are not significant. 28.34 grams = 1 oz.

Table 1. Year one lead (Pb) accumulation in above ground portions of vegetables grown in Pb media treatments.

		Pb Treatments		
Species	**Cultivars**	**0 ppm**	**1000 ppm**	**2000 ppm**
Brassica juncea Mustard	'Purple Wave'	1.25^{def}	1.60^{c-f}	2.80^{a-e}
	'Red Giant'	0.31^{ef}	1.47^{c-f}	1.86^{c-f}
	'Tendergreen'	1.24^{def}	3.33^{a-d}	3.19^{a-d}
Brassica rapa Chinese Cabbage	'Tatsoi'	0.00^{f}	1.85^{b-f}	1.93^{b-f}
	'Mibuna'	0.32^{ef}	3.71^{a-d}	*5.16^{a}
	'Michihili'	0.00^{f}	1.29^{def}	0.97^{def}
Lactuca sativa Butterhead Lettuce	'Arianna'	1.32^{def}	2.93^{a-e}	*4.49^{ab}
	'Great Lakes'	1.18^{def}	2.69^{a-f}	*4.24^{abc}
	'Little Giant'	0.00^{f}	1.30^{def}	1.70^{c-f}

Values with different superscript letters are significant @ p<0.05

Values preceded by an asterisk (*) exceed the referenced food safety threshold for Pb in brassicas and leafy produce (3.84 ppm) (GAIN, 2018).

Table 2. Year two lead (Pb) accumulation in above ground portions of vegetables grown in Pb treatment.

		Pb Treatments			
Species	**Cultivars**	**0 ppm**	**500 ppm**	**1000 ppm**	**2000 ppm**
Brassica juncea Mustard	'Red Giant'	*4.87^{d-g}	2.89^{c-f}	*4.15^{ab}	*8.23^{a}
	'Tendergreen'	*5.60^{g}	*3.95^{g}	*4.21^{g}	*9.31^{bc}
Brassica rapa Chinese Cabbage	'Mibuna'	3.76^{d-g}	2.82^{c-g}	3.54^{c-g}	*13.97^{bcd}
	'Michihli'	*8.01^{c-g}	*5.18^{efg}	*9.22^{c-g}	*11.67^{cde}
Lactuca sativa Butterhead Lettuce	'Arianna'	*7.08^{efg}	*11.16^{g}	*19.73^{fg}	*24.18^{c-g}
	'Little Giant'	*6.68^{efg}	*8.71^{g}	*9.43^{g}	*12.52^{c-g}

Values with different superscript letters are significant @ p<0.05

Values preceded by an asterisk (*) exceed the referenced food safety threshold for Pb in brassicas and leafy produce (3.84 ppm) (GAIN, 2018).

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