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Lead Absorption by Radish Is Affected by Soil Texture and Cultivar

Tracey Emmerick Takeuchi[1], Arthur James Downer[2*]

[1]Plant Science Department, California Polytechnic University, Pomona, CA, USA
[2]Division of Agriculture and Natural Resources, University of California, CA, USA
Email: *ajdowner@ucanr.edu, traceytakeuchicpp@gmail.com

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Abstract

Lead (Pb) was detected in potential urban garden soil in Claremont California and was absorbed by radish cultivars and giant red mustard in a bioassay of the contaminated soil. Radish cultivars grown in two soils with two lead salts developed the same lead tissue concentrations. In a subsequent study, Cultivar "Rudolf" accumulated less lead nitrate than cultivars "French Breakfast" and "White Beauty". Cultivar "Rudolf" grew the least biomass likely accounting for its reduced Pb accumulation. All radish cultivars accumulated more lead when grown in clay vs. sand soil and radish Pb accumulation increased when the concentration of lead was increased in either soil type. The solubility of the lead ion used was not associated with Pb uptake by radish.

Keywords

Lead, Pb, Radish, Mustard, Soil, Clay Loam, Sandy Loam, Plant Growth Bioassay

1. Introduction

Lead is a naturally occurring metal that is used in many industrial processes, some of which lead to contamination of urban environments. Lead deposition in urban areas is often atmospheric, originating from manufacturing, especially battery production, as well as in gasoline, and paint residues [1]. Lead toxicities in people result in developmental, cognitive, motor, behavioral and physical injury, and for this reason, there is no acceptable lead level in the human system [2] [3].

In urban environments, lead may enter soils through atmospheric deposition, from dust near roadways, and from paint chips near buildings. Food grown in lead contaminated soils may be ingested, entering the consumer either directly as transported particles containing lead or by absorption of lead salts in the crop consumed. Lead is persistent in soil [4]. Since food insecurity is highest in large cities [5], recent efforts to produce food locally and in community gardens has increased, and lead ingestion must be examined carefully in urban food production systems. Attempts to alleviate food insecurity of impoverished citizens in cities by "guerrilla farming" activists may be putting consumers of such foods at risk, especially when farming on "Brownfield" [6] sites previously contaminated with lead [4]. Thus, those attempting food production in cities may be at risk for lead consumption and poisoning [7].

Radish (*Raphanus sativus* L.), a root vegetable, commonly grown worldwide, is consumed raw, as well as in a variety of preparations [8]. Because radish grows rapidly from seed, it is preferred in school gardens where children can observe its rapid growth and development. All parts of radish are consumed, including foliage, the fleshy tap root, seed oils, and the immature seed pods [9]. Ten species of radish are described in literature [10]. The ability of globe radish (*Raphanus sativus* "radicola") to absorb lead is known, and some varieties are "hyper-accumulators" [11]. The variability of lead uptake in radish cultivars is not known.

Lead salts occur in soil as oxides of metallic Pb or as salts that result from chemical reactions that may occur in soil. The solubility of lead salts in water is quite variable, from highly soluble lead nitrate (367 g/L) to practically insoluble lead sulfate (0.032 g/L). Lead is a divalent cation and is easily adsorbed onto clay colloids, or other cation exchange sites including organic matter, when it is in a soluble ionic state. Since lead reacts with oxygen and many other elements, there are likely many possible lead compounds and intermediates which may be present in contaminated soils.

In this study we present lead uptake data by five cultivars of radish growing in soils of two different textures, using two different lead salts as the lead sources.

2. Materials and Methods

2.1. Lead Bioassay from a Contaminated Urban Site

Soil was collected from 211 West Foothill Blvd, Claremont, CA (34˚06'31.9"N 117˚43'06.1"W), USA, which was a site known to be contaminated with lead. Multiple samples were collected along three linear transects of the site equidistant within a plot measuring 45 × 45 m. combined and homogenized. Samples were collected from the upper 20 cm of soil in spring of 2013. The site soil was dried, ground and sieved at the University of California Agriculture and Natural Resources lab (UCANR lab) and analyzed for lead content using a nitric acid digest/extraction and detection by inductively coupled atomic plasma emission spectrometry (ICP AES) following procedures of Sah and Miller [12] & Meyer and Keliher [13].

Soil was passed through a 2 mm screen to remove rock and other large particles and blended with washed sand in a 3:1 (soil:sand) ratio to improve bulk density for container culture of radish seedlings. Urea (46% N) was added at a rate of 1.25 g per 5” container containing 1.54 kg of blended sand and site soil to provide nitrogen for seedling growth. Five varieties of radish and one of mustard were obtained from High Mowing Organic Seeds (Wolcott, VT, USA) and seeded 1gm of each variety in each experimental unit (container). The study included radish cultivars “Cherry Belle”, “White Beauty”, “Purple Plum”, “French Breakfast” and “Rudolf” as well as the known lead hyper-accumulator Red Giant Mustard (RGM) *Brassica juncea* L. As a control, one of the radish cultivars (“White Beauty”) and RGM were grown in soil-less media composed of peat moss and sand (50:50 vol:vol) and fertilized with urea as above.

Twenty days following planting, a second application of Urea was made at the same rate. Containers were irrigated to container capacity each day to maintain even soil moisture. Irrigation water that drained from containers was caught and returned to the soil. Plants were grown in a temperature-controlled greenhouse at California Polytechnic University in Pomona, CA, USA. Containers were arranged in a randomized complete block design with five replications. Temperature was maintained at 27˚C (day) and 13˚C (night). Radishes and mustard were grown until bulb formation at 37d. Entire plants were harvested including bulb and leaves. Plants from each experimental unit were independently washed five times in municipal water to remove any soil particles, dust or media. Plants were placed in individual paper bags and dried at 105˚C for 48 h. Dried plant matter was ground and shipped to UCANR lab for lead analysis. Samples were prepared to utilize nitric acid/hydrogen peroxide microwave digestion and lead tissue concentration was determined by ICP-AES as described above.

Treatment differences of tissue lead content and other variables were analyzed using Minitab 16 software and GLM and ANOVA with Tukey’s Honestly Significant Difference Test.

2.2. Lead Salt × Soil Type × Cultivar

Since lead can have both water soluble and insoluble salts, a factorial experiment was designed to examine the uptake water soluble lead (lead nitrate) and water insoluble lead sulfate in two soils by three radish cultivars.

Salinas Clay Loam (pH 7.8; CEC 34 meq/100g soil) soil was collected from an unfertilized agricultural field in Santa Paula, CA, USA (34˚19'35.8"N 119˚06'19.7"W). Cieneba Sandy Loam (pH; 6.2; CEC; 14 meq/100g soil) was collected from a non-agricultural site in Norco, CA, USA (33˚56'36.9"N 117˚31'37.8"W). Soil was added to fill 10 cm round plastic containers: 580 g Salinas clay loam and 620 g Cieneba sandy loam were weighed into containers (based on soil dry weights). Lead nitrate and lead sulfate (Thermo Fischer Scientific, Waltham, Ma, USA) were added to soils to produce soils with 600 ppm of lead. Calcium nitrate was added to lead sulfate treated soils at an equivalent nitrate loading rate to account for potential nitrate fertility effects in the lead nitrate treatments. This allows that all plants were exposed to equal amounts of nitrate (a significant fertilizer salt) while experiencing lead from different salts. Previous germination studies (not shown) indicated the extra salinity from Calcium nitrate would not interfere with germination or growth.

Radish cultivars "Cherry Belle", "Rudolf" and "French Breakfast" were seeded and irrigated as described above. In this experiment, plants grown under similar greenhouse conditions, were harvested after 60d, washed and processed as above.

The experiment was a factorial design arranged in complete randomized blocks. The main factors were: Radish variety (3 levels); lead source (2 levels) and soil type (2 levels). Each treatment combination was replicated five times for a total of 60 experimental units. The data were analyzed using GLM and ANOVA with Tukey's HSD by Minitab 16 software. Main effects and significant interactions are summarized in separate tables (Tables 1-4).

2.3. Lead Salt × Soil Type × Cultivar × Lead Concentration

A final experiment was designed to examine radish lead nitrate uptake in clay and sand soil textures at different lead loading rates to each soil. All five radish cultivars were used and sourced as previously described. Greenhouse temperatures ranged from 38˚C (day) to 26˚C (night). The same sand and clay soils were weighed and measured into containers as in methods 2.2.

A concentration range of lead nitrate was added to bring the soil to 0, 150, 300, 600 and 1200 ppm Pb. Lead nitrate was evenly distributed into each weighed soil (dry weight basis) and mixed before adding to containers. Seeds of the five radish cultivars were added as before. Plants were grown for 60d and then harvested and washed as previously described. Treatments were arranged

Table 1. Lead accumulation in radish and mustard grown in a contaminated site soil.

Cultivar/Species	Dry Weight	Tissue Pb (ppm)	Tissue lead mass (mg)
"White Beauty"	0.02a	16.8bc	0.4b
"Rudolf"	0.06a	17.7bc	0.9b
"Cherry Belle"	0.03a	31.4b	1.0ab
"Purple Plum"	0.03a	31.4b	1.0ab
"French Breakfast"	0.03a	23.80bc	0.8b
Red Giant Mustard	0.05a	56.6a	2.9a
Red Giant Mustard soil less media	0.02a	5.94c	0.1b
"White Beauty" soil less media	0.021a	8.00c	0.1b
Significance	ns	***	**

Weight is dry matter (grams) of leaves, stems and bulb. Tissue lead concentration is Pb measured in harvested dry matter. Tissue lead mass is concentration × dry weight. Means are not significantly different when followed by a different letter according to ANOVA and Tukey's HSD at Probability levels of $P < 0.01$** or $P < 0.0001$***.

Table 2. Effect of soil, lead source and cultivar on tissue lead accumulation.

Main Effect	Tissue [Pb] (ppm)	Tissue dry weight (g)	Tissue lead mass (mg)
Soil			
Cieneba Sandy Loam	225.20a	1.74b	310.3a
Salinas Clay Loam	38.00b	2.54a	107.5b
Source of Lead			
Lead nitrate	126.6a	1.8a	176.4a
Lead sulfate	136.5a	2.5a	264.9a
Cultivar			
"Cherry Belle"	156.4a	2.5a	254.7a
"Rudolf"	144.6a	1.14b	223.3a
"French Breakfast"	93.8a	2.7a	149.7a
Significance of main effects[1]			
Soil	***	*	*
Source of Lead	ns	ns	ns
Cultivar	ns	*	ns
Block	ns	ns	ns

[1]Significance at $P < 0.001$ = ***; $P < 0.05$ = * according to GLM. None of the interactions were significant. Weight is dry matter (grams) of leaves, stems and bulb. Tissue lead concentration is Pb measured in harvested dry matter. Tissue lead mass is concentration × dry weight.

Table 3. Effect of four lead uptake concentrations on five radish cultivars in two soils.

	Radish Tissue Lead Concentration ppm	Tissue dry weight (g)	Tissue lead mass (mg)
Main Effects			
Radish cultivar			
"Cherry Belle"	20.8a	3.0ab	54.4ab
"French Breakfast"	21.3a	4.0a	71.2a
"White Beauty"	20.5a	3.9a	72.0a
"Purple Plum"	16.9a	4.1a	62.1ab
"Rudolf"	17.6a	1.7b	27.6b
Lead Concentration (ppm)			
0	4.4c	3.3	14.4b
300	10.4c	3.5	35.3b
600	19.9b	4.1	81.6a
1200	43.0a	2.5	103.3a
Soil type			
Cieneba sandy loam	27.2a	2.9	76.6a
Salinas clay loam	11.6b	3.8	40.7b
Significance of main effects			
Radish cultivar	ns	**	**
Lead concentration	***	ns	***
Soil	***	ns	***
Significant Interactions			
Soil × Lead concentration	***	ns	***
Soil × variety	***	ns	ns
Lead concentration × variety	*	ns	ns
Soil × Lead concentration × variety	***	ns	ns

Table 4. Interaction of soil type and lead concentration on uptake of lead in radish cultivars.

Soil	Lead Concentration	Tissue Pb concentration (ppm)	Tissue dry weight (g)	Tissue lead mass (mg)
Cieneba Sandy Loam	0	4.4d	2.2	8.5c
	300	13.4cd	3.3	42.6c
	600	28.0b	3.8	112.0ab
	1200	63.0a	2.3	143.5a
Salinas Clay Loam	0	4.4d	4.4	20.4c
	300	7.4d	3.6	27.9c
	600	11.7d	4.4	51.3c
	1200	23.0bc	2.7	63.0bc
Significance of interaction		***	ns	***

on a single greenhouse bench in a randomized complete block design with a factorial arrangement of treatments using five replications. Dry matter and tissue lead concentration were obtained as previously described and the data analyzed with Minitab 16 software using the GLM and Tukey's HSD procedures.

3. Results

3.1. Contaminated Site Results

Soil from the Claremont, CA site was measured at the UCANR lab to contain 158ppm lead. While Red Giant Mustard accumulated more lead that radish cultivars, it did not "hyper-accumulate" than concentrations above the level measured in the Claremont soil. Radish and mustard accumulated more lead in their tissues from contaminated site soil than from soilless media. Radish cultivars were not significantly different from each other in tissue lead content or accumulated lead (tissue lead mass) All radish cultivars absorbed less lead than Giant Red Mustard.

3.2. Lead Salt × Soil Type × Cultivar

Radish cultivars grown in Cieneba Sandy Loam accumulated significantly more lead in their tissues and had a higher total mass of lead than plants grown in the Salinas Clay loam. Source of lead did not affect tissue lead concentration or tissue lead mass. Cultivars were not significantly different from each other in lead uptake, however, cultivar "Rudolf" grew significantly less biomatter. Interactions between main effects (soil, lead source and cultivar) were not significant.

3.3. Lead Salt × Soil Type × Cultivar × Lead Concentration

Increasing concentrations of lead nitrate in soil resulted in increased tissue lead and total lead mass accumulation. Radish tissue dry matter (tissue dry weight) was not affected by increasing lead nitrate concentration in either soil. Maximum lead accumulation (tissue lead mass) was seen in the 600 - 1200 ppm treatments. Lead accumulation in radish tissues followed a clear linear increase consistent with concentration increases in either soil type up to 600 ppm lead nitrate. Radish cultivars were not significantly different in their tissue lead concentrations but the tissue lead mass was greatest for cultivars "French Breakfast" and "White Beauty". Cultivar "Rudolf" accumulated the least lead (tissue dry weight x lead tissue concentration), but also grew less vigorously than the other cultivars (significantly less tissue dry weight). The effect of soil texture on radish lead uptake was significant (increased radish tissue lead concentration and tissue lead mass); radishes growing in clay soil absorbed and concentrated less lead in tissues than radishes growing in sandy loam soil. Increasing lead concentration in either soil resulted in increased tissue lead concentrations and tissue lead mass across varieties.

4. Discussion

While food insecurity in the United States declined from 2008 to 2012, it is still significant—affecting > 15% of the population [5]. Localized food production can reduce food insecurity among vulnerable populations [14]. Much of localized food production in urban agriculture settings takes place in community gardens that have been renovated from other uses or were vacant land.

In our study, radishes were grown and harvested from soil obtained from a lead-contaminated site. The site in Claremont contained soil Pb levels higher than allowed by the United States Environmental Protection Agency. The site was a potential community garden and still poses risk to any who would grow and consume food crops from there, especially vegetables such as radish that will accumulate lead in its tissues. While radish cultivars did not vary significantly in their uptake of lead from the Claremont, Ca soil, the hyper-accumulator Reg Giant Mustard accumulated significantly more Pb than radish. Specific data on uptake of toxic metals by plants, especially cultivars within a taxon is lacking in the literature. Our study verifies the potential for one of the most commonly planted vegetables by urban gardeners (radish) to absorb lead from soil. Radish is a non-mycorrhizal member of the Brassicaceae and because of its lack of affiliation with fungal soil partners, it is able to take up metals that would otherwise be sequestered by mycosymbionts [11] [15].

Although radish cultivars did not have lead uptake differences in our first experiment using Claremont, Ca soil, we did find significant varietal differences especially in total lead accumulation in experiment 3.3 with higher lead loading rates. The varieties "French Breakfast" and "White Beauty" absorbed the most lead and are both lacking in anthocyanin production--they form white tubers. Since anthocyanins can act as metal chelating agents [16], those radish cultivars lacking anthocyanins may accumulate more lead from contaminated soils.

We found that tissue lead concentration was not significantly different between cultivars in any our experiments. Radishes in experiment 3.2 absorbed and accumulated greater amounts of lead than those in experiment 3.3 despite higher soil lead concentrations in the later experiment. This may be because the experiment was conducted in summer with higher daytime and night temperatures. The effect of off-season cultivation and increased temperatures on Brassicaceae lead uptake is not known. Since lead mass accumulation is the product of tissue lead concentration x biomass, significance of the cultivar response to lead mass accumulation in experiment 3.3 could be due to the reduced biomatter accumulation in cultivar "Rudolf" which grew consistently less biomatter in all experiments than most other cultivars.

Soils are complex ion exchanging environments. All soils carry a negative charge and thus will adsorb Pb cations. While Pb is considered persistent in soil [4], this does not preclude its uptake by plants. Clay soils have higher cation exchange capacity and thus adsorb cations onto cation exchange sites. Lead is considered largely immobile in soils, but in sandy soil, particularly when low in organic matter, with neutral to acid soil reaction, Pb may enter soil solutions and thus be taken up by plant roots [17]. In our study, we found greater radish Pb uptake in sandy versus clay soil, consistent with others' findings [18]. Contaminated sandy soils appear to pose more risk to consumers of vegetables growing in those sites rather than loam, clay, or highly organic matter enriched systems that will better adsorb the Pb cations. Slightly alkaline high CEC soils strongly adsorb lead, and it is less available for immediate plant uptake.

Since soil texture affects lead uptake, not all soils pose the same risk to food supplies. Reliance only on soil testing may give inaccurate estimates of what may be absorbed by plants because so many urban soils are modified by adding organic matter, or importing other soil textural classes that are not mapped. We have shown that radish is a reliable bio-assay plant to detect lead in soil. While analyzing soil directly will give data on possible lead loading rates, it may not accurately predict the amount of lead that will be taken up by crop plants; making plant growth bioassays an important assessment tool.

Edaphic conditions such as cation exchange capacity, species of lead present, and presence of sulfur all have effects on absorption of lead from soil [11]. In our study, either species tested, whether highly soluble (lead nitrate), or less soluble (lead sulfate), were both absorbed by radish resulting in similar lead tissue concentrations. Elemental sulfur may be oxidized in soil reducing soil reaction making metals more available. This would not occur with lead sulfate since the sulfur is already fully oxidized. Metal absorption by radish is more likely affected by soil pH and organic matter percentage and type [19].

Our findings suggest radish cultivars can vary in lead uptake depending on soil lead concentration, soil type, and their overall growth which is an important factor in the calculation of lead mass accumulated by a given crop. Radish cultivars are not known to be hyper-accumulators but can absorb biologically significant levels of lead from contaminated soil. Hyper-accumulators tend to be rapid and productive growers in the Brassicaceae family and their use in phytoremediation of contaminated sites is well documented [11] Radish, as well as all farm products, should still be consumed with caution when produced in urban farms with soil lead contamination. This is especially true since the Food and Agriculture Organization and the World Health Organization (FAO/WHO) determined that "There is no known lead exposure minimum that does not cause IQ (intelligence quotient) impairment" [20].

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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