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Cadmium and Lead Uptake by Edible Crops Grown in a Silt Loam Soil

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There is increasing public concern about health effects resulting from ingestion of food containing toxic metals such as Cd and Pb. For example, a wide range of metabolic disorders and neuropsychological deficits in children have been noted (NAS 1980; EPA 1986; Nriagu 1988), and chronic exposure to Cd has been linked to kidney failure and bone disease (Ryan et al. 1982; Kido et al. 1991)). Roberts and Johnson (1978) reported that the potential harm posed by the uptake of heavy metals such as Cd and Pb by plants depended on their abundance, mobility and bioaccumulation. Chaney (1973) reported that plant uptake of heavy metals was also influenced by soil pH. Chambers and Sidle (1991) found that there was a linear relationship between soil concentrations of heavy metal and concentrations in vegetation around a zinc-lead tailing pond. The ability of the soil to retain metals depends on several factors; pH, cation exchange capacity (CEC), organic matter content, and their specific geochemical properties (e.g., occurrence of oxides or carbonates; Yassoglou et al. 1987). Overall, the metal burden of a crop depends on: (a) uptake via the root system, (b) direct foliar uptake and translocation within the plant, and (c) surface deposition of particulate matter (Jones 1991).

Numerous studies have been conducted with agronomic crops regarding heavy metals in soils and plant uptake from sewage sludge, but only a few studies have dealt with the uptake of heavy metal mixtures in vegetables (Schauer et al. 1980). This paper reports on germination/emergence, biomass and uptake of Cd and Pb in lettuce and radish grown in a loam soil spiked with known mixtures of $CdCl_2$ and $Pb(NO_3)_2$. Lettuce and radish have been used in this study because they are among the two groups of vegetable crops (leafy and root) consumed by humans. Also, earlier studies have reported that lettuce and radish bioaccumulate Cd and Pb from heavy metal polluted soils (Schauer et al. 1980; Boon and Soltanpour 1992).

MATERIALS AND METHODS

Soil samples were collected from a depth of about 0-15 cm along the banks of the Willamette River (Willamette Park, Benton County, Corvallis, Oregon). The soil was screened through a 1/4 in.(6.35 mm) mesh stainless steel screen, and stored in a plastic bag at room temperature until use. The screening removed rocks, sticks and plant debris from the soil, and also provided uniform particle size in the soil for the study. Immediately after screening, soil moisture content was measured using the gravimetric method (Gardner 1986). The soil used in this study was a Newberg silt loam soil common to Benton County, Oregon.

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The Newberg soil series consist of deep, excessively drained soils, formed in mixed sandy alluvium. These soils are present in flood plains along major rivers and streams of the Willamette Valley of Oregon (Knezevich 1975). Soil analysis and characterization was performed by the Oregon State University Soil Testing Laboratory. The soil parameters measured included pH, CEC, particle size distribution, total nitrogen, organic matter content, moisture content and soil classification (Table 1).

Table 1. Chemical Analysis of Newberg Soil

pH	6.2
CEC	29.7*
Total Nitrogen	0.17 %
Organic Matter	4.31 %
Sand(2-50mm)	32.2 %
Silt(50-2m)	50.2 %
Coarse Silt (50-20m)	16.0%
Fine+Medium Silt (20-2m)	34.2%
Clay(<2m)	16.7 %
Moisture Content	25.8 %

* milliequivalents/100 g

Table 2. Matrix for Defined Metal-Mixtures in Newberg Soil

		Pb (mg/kg)				
	0	100	200	400	800	1000
	100	100	200	400	800	1000
Cd	200	100	200	400	800	1000
(mg/kg)	400	100	200	400	800	1000
	800	100	200	400	800	1000
	1000	100	200	400	800	1000

Seeds of lettuce (<u>Lactuca sativa</u>; cultivar Buttercrunch) and radish (<u>Raphanus sativus</u>; cultivar Cherry Belle) were obtained from a local seed company. Lettuce seeds were screened using nested wire mesh screens of sizes ranging from 1/6 x 1/28 to 1/6 x 1/34 in. Four nested wire mesh screens were used, with the one containing the largest holes placed on top and followed by those with smaller holes in succession below. The radish seeds were screened similarly using a series of nested wire mesh screens of sizes ranging from 6.5 to 8 - 64ths of an in. (Brusick and Young 1982). Screened seeds were visually graded to remove empty hulls and damaged seeds and then stored in plastic Ziploc^R bags in a refrigerator at 5 °C. Seeds were not chemically pretreated, since fumigants used in seed treatment may bias test results. Prior to testing, percent germination for each plant species was determined by sowing seeds in uncontaminated silica sand. Test criteria required a minimum of 80 percent germination in silica sand for each plant species before any test was considered valid (EPA 1988).

Test temperature and humidity in the greenhouse were measured using a hygrothermograph (Belfort Instrument Co., Baltimore, MD); light intensity was measured using a light meter (LI-COR, Model LI-185B, Lincoln, NE). Soil pH was measured using the ORION^{TM} pH meter. Prior to testing, soils were analyzed using inductively coupled plasma-atomic emission spectroscopy (ICPAES; McQuaker et al. 1979) to establish baseline levels of Cd and Pb. Results indicated that the mean concentrations for Cd and Pb in the Newberg soil were 1.866 and 0.545 mg/kg, respectively.

A stock solution of Cd was prepared by weighing 65.24 g analytical grade of $CdCl_2$ (J. T. Baker, Phillipsburg, NJ) into a 1-L volumetric flask; Cd constituted 61.3% of the molecular weight (183.3 g) of $CdCl_2$. A stock solution of Pb was prepared by weighing 64.0 g analytical grade of $Pb(NO_3)_2$ (Mallinckrodt, St Louis, MO) into a separate 1-L volumetric flask; Pb constituted 62.6% of the molecular weight (331.2 g) of $Pb(NO_3)_2$. Both volumetric flasks were brought to 1 L volumes with reverse osmosis (RO) water. The concentration of Cd and Pb in each of these flasks were 40,000 parts per million (ppm).

One hundred grams of soil (based on dry weight) were initially weighed and then placed into plastic Ziploc^R bags. The amount of soil (based on wet weight) per pot was then determined by dividing the total dry weight of the soil by the moisture fraction.

Screening tests with individual metals were performed for both plant species using a logarithmic scale (0, 1, 10, 100, 1000 mg/kg soil) to establish a test range for the study. Test soils were hydrated to 45 percent moisture on a dry weight basis with 20.5 mL of solution that consisted of RO water and calculated amounts (mL) of Cd or Pb from two separate 40 g/L stock solutions.

Following preliminary screening tests with single metal exposures, experiments were conducted with Cd and Pb mixtures using concentrations ranging from 100 to 1000 mg/kg in soil (see Table 2). Test concentrations of the metals were prepared as follows; for example, the 100 : 100 mg/kg Cd and Pb treatment; 0.725 mL from each of the stock solutions were combined with 19.05 mL of reverse osmosis (RO) water in a 100 mL beaker and mixed thoroughly on a stir plate, and then poured into the Ziploc^R bag containing 100 g of soil. Other test concentrations were prepared similarly. Soils in the Ziploc^R bag were hand-mixed to ensure uniform distribution of test solution. Untreated soil served as the test controls and received only 20.5 mL of RO water treatment.

Mixed soils from each plastic Ziploc^R bag were placed in 4 in. plastic pots. Twenty pregraded seeds were then placed on the soil surface, and were covered with 25 g of 16 mesh silica sand. Six pots per treatment were placed in a 18 x 20 in. plastic plant tray on a greenhouse bench, and were irrigated daily with approximately 250 mL of nutrient solution (EPA 1987). The greenhouse was supplied with auxiliary lights for 16 hr a day, and the average light intensity was 330 uM/sec/m^2. The average day/night temperature was 28/14 °C with a relative humidity range of 45 - 50 percent.

After five days, seed germination and emergence were assessed. Uptake measurements were subsequently initiated by thinning each test pot to five plants per pot, if more than five seeds had germinated after the first 120 hours. This minimized crowding. The remaining plants were then monitored over a 30-day period for sublethal physiological effects such as chlorosis, stunting, and shoot and leaf deformities. After 30 days, plants were harvested, weighed for total biomass and washed with RO water and dried in an oven at 100°C for 24 hr. Dried plant tissues were digested by using nitric acid at 150 °C, followed by perchloric acid digestion at 250 °C (McQuaker et al. 1979). Soil samples were digested using EPA method 3050, which consisted of nitric acid digestion at 95 °C, followed by hydrogen peroxide treatment (EPA 1986). The digested plant tissues and soil samples were analyzed for total Cd and Pb contents using ICPAES (McQuaker et al. 1979).

Multiple regression analyses were performed using Statgraphics (STATGRAPHICS^{TM}, Rockville, MD) on uptake results. Tests of significance ($p=0.05$) of regression slope estimates were performed to test the null hypotheses that there are no significant differences in mean germination/emergence, plant biomass and mean uptake of Cd and

Pb by lettuce and radish at various levels of the metal mixtures. Also, tests of significance (p=0.05) of regression slope estimates were performed to test the null hypotheses that there was no interaction between Cd and Pb uptake in both lettuce and radish.

RESULTS AND DISCUSSION

Results indicated that seed germination and emergence was affected with increasing concentrations of Cd/Pb mixtures in the soil. Lettuce was slightly more sensitive to the mixture than radish (Table 3). For example, the addition of Cd alone in the soil caused a significant difference in germination rates in lettuce and radish (See Table 4).

Table 3. Germination/Emergence and Plant Biomass

Cd/Pb (mg/kg)	Mean Germ. (%)		Mean Biomass (g)	
	A	B	A	B
0/0	93.3	93.0	77.6	48.0
100/100	85.0	86.7	14.5	49.2
100/200	91.7	83.3	14.2	37.3
100/400	78.3	91.7	13.0	55.1
100/800	68.3	86.7	112.3	41.4
100/1000	75.0	91.7	10.2	25.3
200/100	90.0	90.0	5.6	35.3
200/200	68.3	76.7	6.5	21.0
200/400	75.0	70.0	3.7	16.5
200/800	70.0	70.0	2.3	17.5
200/1000	48.3	70.0	2.3	0.9
400/100	53.3	78.3	1.0	2.2
400/200	61.7	75.0	0.5	2.5
400/400	58.3	50.0	0.2	1.9
400/800	61.7	46.7	0.3	1.5
400/1000	78.3	5.0	1.7	0.3
800/100	55.0	8.3	-	-
800/200	28.3	23.3	-	-
800/400	33.3	13.3	-	-
800/800	5.0	-	-	-
800/1000	21.7	8.3	-	-

A = Lettuce; B = Radish
- = No plant growth

Table 4. ANOVA for Effect of Cd/Pb on Germination/Emergence of Plants

	Lettuce		Radish	
Parameter	F-Ratio	P-value	F-Ratio	P-value
Cd	269.6	<.0001*	362.5	<.0001*
Pb	5.3	.0234*	0.4	.5408
$(Cd)^2$	17.7	.0001*	10.5	.0019*
$(Pb)^2$	22.4	.1274	1.3	.2560
Cd*Pb	0.3	.6000	7.7	.0073*
R^2 = .82; SD = 127.2			R^2 = .75; SD = 84.35	

n = 72 (DF = 71)
* significant at P<.05

Table 5. ANOVA Table for Effect of Cd/Pb on Plant Biomass

	Lettuce		Radish	
Parameter	F-Ratio	P-value	F-Ratio	P-value
Cd	74.0	<.0001*	159.5	<.0001*
Pb	3.5	.0700	0.9	.3521
$(Cd)^2$	40.2	.0001*	68.0	.0001*
$(Pb)^2$	.20	.6887	1.2	.6962
Cd*Pb	1.9	.1685	1.8	.8729
R^2 = 0.73; SD = 10.518			R^2 = 0.83; SD = 67.87	

n = 50 (DF = 49)
* Significant at P<.05

In general, biomass results indicated that the mean plant biomass decreased in both lettuce and radish, as the concentration of Cd and Pb in the soil increased (See Table 3). Plant biomass decreased gradually, but biomass declined sharply in both lettuce and radish when the concentration of Cd reached 400 mg/kg in the soil at various concentrations of Pb (Table 3). The mean biomass for lettuce was significantly lower than the mean biomass for radish, suggesting that lettuce was more responsive to the mixture (Table 4). Statistical analysis of biomass data indicated that Cd in the soil alone caused significant reductions in mean biomass in lettuce ($p<.0001$) and radish ($p<.0001$) (See Table 5). Also, analysis indicated that there were no significant reductions in biomass in lettuce due to Pb alone in the soil ($p=.07$). Furthermore, there was no statistical evidence of an interaction between the two metals on mean biomass reduction ($p=.17$; See Table 5).

Above ground biomass of lettuce (leaves) and below ground biomass of radish (roots) was analyzed for metal uptake (Table 6). The mean uptake of Cd by lettuce and radish increased as the concentrations of Cd and Pb in the soil increased. There was moderate uptake of Cd by lettuce when the concentration of Pb reached 400 mg/kg in the soil at a Cd concentration of 200 mg/kg. However, uptake of Cd by lettuce peaked again at 400 mg/kg Cd when the concentrations of Pb in the soil increased to 800 and 1000 mg kg/kg respectively. The uptake of Cd by radish peaked initially at 200 mg/kg of Cd when the concentrations of Pb in the soil doubled, but declined as the concentration of Cd increased. However, Cd uptake by radish again increased as the concentrations of Cd and Pb in the soil increased, and peaked at 400 mg/kg of Cd when the concentration of Pb in the soil reached 1000 mg/kg. In both lettuce and radish, there was no uptake of Cd beyond 800 mg/kg Cd. Statistical analysis indicated significant differences in the mean uptake levels of Cd by lettuce ($p=.005$; see Table 7). The uptake of Cd occurred at lower concentrations in the mixture; however, uptake declined sharply when Cd concentration in the mixture reached 400 mg/kg, at different levels of Pb in the soil. Data also indicated that there were no significant differences in mean uptake of Cd by radish ($p=.43$). There was no interaction between Cd and Pb on Cd uptake by both lettuce($p=.18$) and radish ($p=.22$).

Results indicated that the mean uptake levels of Pb by lettuce increased as the concentrations of Pb increased in the mixture. However, Pb uptake was more pronounced at 800 mg/kg Pb, when Cd concentration in the mixture reached 200 mg/kg. Lead uptake declined sharply when the concentration of Cd in the mixture exceeded 200 mg/kg (see Table 6). Also, Pb uptake by lettuce increased at 400 mg/kg Cd when the concentrations of Pb in the mixture were 800 and 1000 mg/kg respectively.

The uptake of Pb by radish increased as the concentration of Pb in the mixture increased; however, Pb uptake declined when the concentration of Cd exceeded 400 mg/kg. Also, there was no uptake of Pb by both radish and lettuce, as Cd levels in the soil mixture exceeded 400 mg/kg. Statistical analysis indicated that the mean uptake of Pb by lettuce was not significant ($p=.31$; see Table 7). Data also indicated that the mean uptake of Pb by radish was not statistically significant ($p=.40$; see Table 7). Furthermore, there was no interaction between Cd and Pb on Pb uptake by both lettuce ($p=.18$) and radish ($p=.22$; see Table 7).

Cadmium and Pb have both been implicated in food chain contamination (Ryan et al. 1982; Wolnik et al. 1983; Boon et al. 1992). In the present study, the presence of Cd and Pb mixtures in the test soil was associated with uptake differences for these metals in both lettuce and radish plants. The results from this study are consistent with previously published reports that showed that lettuce accumulated significant amounts of Cd when grown on contaminated soil (John et al. 1976; Lagerwerff et al. 1972), while Pb was not. Our results support previously published findings that suggest Cd is absorbed by passive diffusion and translocated freely in the soil (Cutler et al. 1974; Jarvis et al. 1976), while Pb is more strongly adsorbed by soils and forms insoluble crystalline compounds (Santillian-Medrano et al. 1975); hence, Pb activity in the soil solution will be reduced by these physical processes (Koeppe 1981).

Table 6. Uptake of Cd and Pb by Lettuce and Radish

Cd/Pb	Plant Mean Uptake (ug/g)			
	Lettuce		Radish	
(mg/kg)	Cd	Pb	Cd	Pb
0/0	0.4	0.6	-	2.5
100/100	160.4	-	124.2	7.0
100/200	251.7	5.7	118.7	10.7
100/400	116.8	7.8	117.7	27.8
100/800	164.9	28.4	93.6	39.6
100/1000	185.2	39.0	149.3	82.9
200/100	285.2	3.0	278.6	5.5
200/200	315.6	5.3	241.8	11.2
200/400	259.1	19.9	270.0	20.7
200/800	697.9	113.7	328.8	47.4
200/1000	470.1	35.0	271.3	54.9
400/100	380.8	-	95.0	2.1
400/200	201.0	-	503.0	9.9
400/400	154.3	-	462.2	23.8
400/800	399.7	45.5	400.1	30.7
400/1000	392.0	36.3	595.9	78.1
800/100	-	-	223.4	-
800/200	-	-	-	-
800/400	-	-	-	-
800/800	-	-	-	-
800/1000	-	-	-	-

- = No plant growth

Table 7. ANOVA Table for Cd/Pb Uptake by Lettuce and Radish

	Lettuce		Radish	
Parameter	F-Ratio	P-value	F-Ratio	P-value
Cd	8.5	.0050*	0.6	.4348
Pb	1.1	.3070	0.7	.4042
$(Cd)^2$	8.7	.0053*	0.9	.3454
$(Pb)^2$	4.4	.0425	0.2	.6400
Cd*Pb	1.9	.1771	1.6	.2165
R^2 = .38; SD = 2.05			R^2 = .32; SD = 4.09	

n = 46 (DF = 45)
* significant at $P<.05$

The decline in mean Cd uptake by lettuce at 400 mg/kg may be attributed to saturation of the active binding sites on the plant root system or by early toxicological responses in the plant root. The lack of interaction between Cd and Pb in the soil, indicated that the decline in mean Cd uptake was not due to Pb antagonism. The observed uptake of Cd by radish from the mixture was consistent with previously documented reports, which showed that radish did not accumulate as much Cd as lettuce (Santillian-Medrano et al. 1975).

The lack of significant uptake of Pb by lettuce is not consistent with some reports by other researchers. For example, Roberts et al. (1974), Langerwerff and Brower (1974), and Preer (1980) reported that leafy portions of vegetable crops contained the highest levels of Pb. The lack of Pb uptake in lettuce in this study may be due to its being highly adsorbed to soil and its reduced availability for binding and uptake at the root surface. Regardless of the mechanism, Pb was not translocated to any significant extent to the above ground portions of the lettuce plant. Reports have also linked the low availability of Pb for uptake in the root zone to biochemical processes involved in Pb binding, such as inactivation and precipitation. For example, Malone et al. (1974) reported that the roots of corn plants exposed to Pb in hydroponic solution accumulated a surface Pb precipitate and Pb crystals in the cell walls. Furthermore, the low mean Pb uptake by lettuce may be attributed to the concentration of Pb by the roots, making the below ground levels of Pb greater than the above ground tissues (Koeppe 1981).

Plant uptake of metals in soil depends on biologically available metals. In the case of Pb, previous studies have reported that Pb incorporated into soils is nearly always tightly bound to organic matter and is precipitated out, thus making it biologically unavailable for uptake by plants (Zimdahl and Koeppe 1977). However, because the Newberg silt loam soil used in this study was low in organic matter and relatively low in CEC, availability of metals may have been enhanced.

When two metals such as Cd and Pb are present in soil as a mixture, it is difficult to predict their behavior. Test results from this study indicated that differences existed in the uptake of Cd and Pb between lettuce and radish. Interactions that occur between metals and nutrients, and also between metals and soluble salts in the soil, vary with crop and soil types. The lack of interaction between Cd and Pb in these exposures in Newberg silt loam soil indicated that the presence of Pb in the mixture did not affect the uptake of Cd by either plants (see Table 7). From the germination results, seeds exposed to higher levels of Cd and Pb mixtures had lower germination rates than those exposed to lower levels. Germinated plants from the group exposed to higher levels of the chemical mixtures may not have been as healthy as the group exposed to lower levels, and therefore, biomass and uptake rates could have been affected in these plants.

In conjunction with these plant related sources of variability, soil exposure medium must be taken into consideration in evaluating metals uptake by plants. The availability of Cd and Pb to plants in the soil environment is affected by soil variables such as pH, CEC and organic matter. For example, when sewage sludge amended soils are used for agricultural purposes, the soil pH should be critical, since the solubility of these metals in the soil is pH dependent. Liming of contaminated soil elevates pH, and decreases the availability of these metals to plants. Chaney et al. (1975) demonstrated that Cd concentration in soybean leaves was reduced substantially when the pH of the soil on which the plants were grown was increased by liming from pH 5.3 to pH 7.0.

The CEC and organic matter content (OM) of the soil have also been reported to affect the availability of metals in soil (Hagiri 1973). For example, Stevenson et al.(1972) reported that the amount of cadmium absorbed by oats (Avena sativa) decreased as CEC of the soil was increased by adding organic matter. Adding substances like peat moss may decrease the absorption of heavy metals by plants, because they may exhibit strong reactions to these metals.

The clay constituents of soil play an important role in the soil's ability to bind cations of heavy metals (Korte et al. 1976). The cultivation of edible crops in soils with moderate amounts of clay (that will not impact essential minerals) will decrease the chances of heavy metal accumulation in plants.

In addition, several investigators have suggested that oxides of iron and manganese exhibit highly specific adsorption affinity for trace metals, including cadmium (Jenne 1968; Forbes et al. 1976). Cultivating edible crops in iron and manganese rich soils may reduce the absorption and uptake of heavy metals such as cadmium and lead from polluted soils.

Lettuce and radish grown in a silt loam soil contaminated with Cd and Pb exhibited concentration dependent phytotoxicity. Also biomass was significantly reduced after 30 days of growth. Lettuce and radish plants that survived after 30 days bioaccumulated significant amounts of Cd from soil, whereas Pb was not. Bioaccumulation of Cd from soil by the plants was concentration dependent, however, Pb was not bioaccumulated by the plants regardless of Pb concentration in soil. Despite the low bioaccumulation of Pb in lettuce and radish, vegetables grown in soils bearing potentially toxic metals such as Cd and Pb clearly represent a scenario for human exposure through ingestion of contaminated plants.

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