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Assessing Lead Thresholds for Phytotoxicity and Potential Dietary Toxicity in Selected Vegetable Crops

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Abstract Lead tolerance and accumulation in shoots and edible parts varied with crop species and soil type. The critical Pb concentrations at 10% yield reduction were 24.71, 28.25, and 0.567 mg kg^{-1} for pakchoi, celery, and hot pepper, respectively under hydroponic conditions, whereas were 13.1, 3.83, 0.734 mg kg^{-1} grown in the Inceptisol and 31.7, 30.0, 0.854 mg kg^{-1} in the Alluvial soil, respectively. Based on the threshold of human dietary toxicity for Pb, the critical levels of soil available Pb for pakchoi, celery, and hot pepper were 5.07, 8.06, and 0.48 mg kg^{-1} for the Inceptisol, and 1.38, 1.47, and 0.162 mg kg^{-1} for the Alluvial soil, respectively. Similarly, the total soil Pb thresholds were different from vegetable species and soil types.

Keywords Genotypic difference · Human dietary toxicity · Lead · Toxic threshold

Vegetables play an important role in the human diet, and their production in suburban areas has increased with urbanization. Heavy metal pollution in soils has increased in these areas because of increased disposal of municipal and industrial solid and liquid wastes to the soils and precipitation of pollutants from air on the soils (Jinadasa et al. 1997). Retention of heavy metals by soil depends on factors such as the nature of the inorganic and organic constituents, the nature of metals, the composition of soil solution, and pH (Sauve et al. 1997). Heavy metal accumulation in soils is of concern in agricultural production due to adverse effects on food quality (safety and marketability) and crop growth (He et al. 2005; Yang et al. 2002), and environmental health (soil flora/fauna and terrestrial animals). Metal accumulation in vegetables may pose a direct threat to human health after these are being consumed (Kursad et al. 2002; Monika and Katarzyna 2003).

Lead is a toxic element that can be harmful to plants, but plants usually show the ability to accumulate large amounts of lead without visible changes in their appearance or yield. In many plants, lead accumulation can exceed several hundred times the threshold of maximum level permissible for human (Wierzbicka 1995). The introduction of Pb into the food chain may affect human health, and thus, studies on Pb accumulation in vegetables have gained much importance (Coutate 1996). Although a maximum Pb limit for human health has been established for edible parts of crops (0.2 mg kg^{-1}) (CDPM 1994), soil Pb thresholds for producing safe vegetables are not available.

The objectives of this study were to examine the effects of Pb on plant growth and Pb accumulation in pakchoi, celery and hot pepper, the transformation of added Pb in vegetable garden soils, and the critical Pb concentrations in the soils for pakchoi, celery, and hot pepper based on human dietary toxicity.

Materials and Methods

Seeds of the three vegetable crops i.e. pakchoi (*Brassica chinensis* L.), celery (*Apiumg graveolens* L. var. *dulce*

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DC), and hot pepper (*Capsicum annuum* L.) were purchased from a vegetable seed company in Hangzhou, China. Seeds were germinated in dark at 25°C and transplanted into quartz sand bed for establishing seedlings. Healthy and uniform seedlings were selected and transplanted into a 3-L plastic containers having fresh nutrient solution. Each pot had six plants and the composition of the nutrient solution was modified from Yang et al. (1995) i.e. (in mmol L^{-1}): KNO_3 6.00, $Ca(NO_3)_2$ 3.50, KH_2PO_4 1.33, $MgSO_4{\cdot}7H_2O$ 0.50 , NaCl 0.48, and (in µmol L^{-1}): H_3BO_3 10.00, $MnSO_4{\cdot}H_2O$ 0.50, $ZnSO_4{\cdot}7H_2O$ 0.50, $CuSO_4{\cdot}5H_2O$ 0.20, $(NH_4)_6Mo_7O_{24}$ 0.01, Fe-EDTA 200. After 15 days of growth, plants were exposed to different Pb levels as $Pb(NO_3)_2$: CK (0.005), 1, 2, 4, 6, 8, 10, 20 mg Pb L^{-1}. A randomized complete block design (RCBD) was used with each treatment replicated three times. The pH was maintained at 5.5 by adjustment with 0.1 mol L^{-1} HCl or 0.1 mol L^{-1} NaOH. The nutrient solution was replaced at every 5 days. Pakchoi and celery were harvested after 6 weeks of treatment, while the hot pepper after the fruits were marketable. At harvest, roots of intact plants were soaked in solution of 0.01 M EDTA for a few minutes, and rinsed with distilled water. Plant samples were separated into shoots (leaf blades and petioles-edible portion for celery, and fruit for hot pepper) and roots. The base of stalks and roots were rinsed thoroughly with double-distilled water, blotted dry, then dried at 70°C for 72 h. Fresh weights (FW) and dry weights (DW) of shoots and roots were recorded. Samples of plant dry materials were grounded with a stainless steel mill and passed through a 60-mesh sieve for Pb analysis.

Two types of soil were used for pot experiments. The Alluvial soil (Fluvio-marine yellow loamy soil), collected from Hangzhou suburb of China, was derived from coastal saline soil matrix, while the Inceptisol (clayey), collected from Jiaxing suburb of China, was derived from fluvio-aquic soil matrix. The main agrochemical properties of the soils are shown in Table 1. Based on Pb adsorption and desorption characteristics of the soils, Pb was applied as $Pb(NO_3)_2$ at the rates of 0, 100, 200, 400, 600, 800, 1200, 2000 mg Pb kg^{-1} soil, respectively. The mixed soil samples were then incubated in a plastic container at 70% of maximum field water capacity for 12 weeks. Soil NH_4NO_3-extractable Pb was measured at intervals of 0, 1, 2, 4, 6, 8, and 12 weeks after incubation.

The incubated and air-dried soil samples were used to grow pakchoi, celery and hot pepper. The process of preparing seedlings was similar to that of the hydroponics culture experiment and three uniform 21-day-old seedlings were transplanted to each pot filled with 1 kg soil. A RCBD was used with each treatment replicated three times. Soil moisture was maintained at 60% of the maximum field water capacity. Pakchoi and celery were harvested after 30–35 days from transplanting, and hot pepper was harvested when the fruit attained marketable size. Shoots or the edible parts were separated from the roots, and fresh and dry weights were recorded. Samples of plant dry materials were ground with a stainless steel mill and passed through a 60-mesh sieve for Pb analysis.

Soil agrochemical properties were analyzed by SSICA (1980). Soil total Pb was determined by the method from Lu (1999). Soil available Pb was extracted using 1.0 mol L^{-1} NH_4NO_3 (pH 7.0, soil: extractant ratio of 1:5). Plant samples were ashed at 550°C and dissolved in 1:1 HCl. Concentration of Pb in the solution was determined using Graphite Furnace Atomic Absorption Spectroscopy (Shimadzu, AA-6800). The detection limits for Pb are from 20 ppb to 100 ppm and the procedure followed for the determination of Pb concentration was within the range of these detection limits.

Statistical analysis was performed using the SPSS statistical package (version 11.0). All values reported in this work are means of at least three independent replications. Data were tested at significant levels of $p < 0.05$ by one way ANOVA.

Results and Discussion

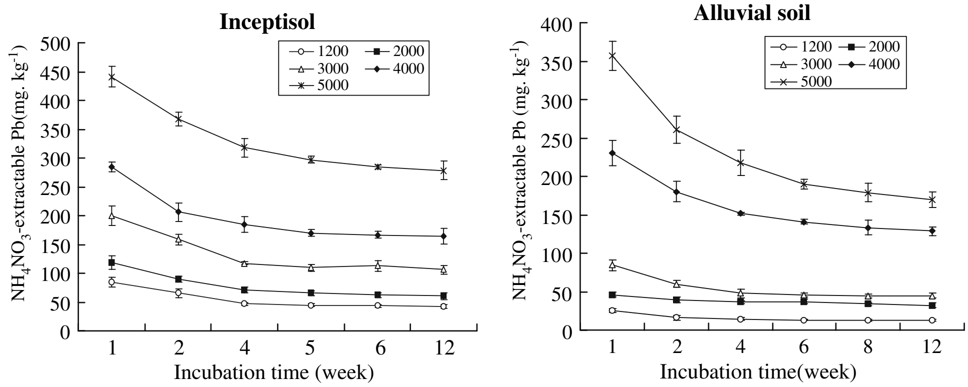
Total soil metals can be used to estimate the degree of soil exposure to heavy metal pollution, although this is not generally well correlated with metal mobility and

Table 1 The main physicochemical properties of two soils used in the study

Soil properties	IS	AS	Soil properties	IS	AS
Organic matter (%)	4.09	1.58	Total Pb (mg kg^{-1})	58.8	67.6
Total N (%)	0.235	0.102	Available Pb (mg kg^{-1})	0.312	0.027
Total P (%)	0.0704	0.0811	pH (H_2O)	5.91	8.08
Total K (%)	1.68	0.98	CEC (cmol kg^{-1})	19.28	11.59
Available N (mg kg^{-1})	128.4	79.6	Soil particle size (2–0.02 mm) (%)	17.68	36.89
Available P (mg kg^{-1})	17.2	10.2	Soil particle size (0.02–0.002 mm) (%)	45.46	46.20
Available K (mg kg^{-1})	126.4	76.3	Soil particle size (<0.002 mm) (%)	36.86	16.91

IS: Inceptisol (Clayey); AS: Alluvial soil (silty)

Fig. 1 Changes of NH_4NO_3-extractable Pb with incubation time in the Inceptisol (clayey) and Alluvial soil (silty). Data are means of three replicates ± SE. The bar depicted $LSD_{(0.05)}$.The numbers in the legend refer to the Pb addition rates of 1200, 2000, 3000, 4000, and 5000 mg Pb kg^{-1} soil, respectively



bioavailability (Davies 1992). The amount of soil Pb removed by a chelating agent like DTPA or EDTA is considered to be the plant-available portion. Some results showed that significant and positive correlations were noted between shoot Pb and soil NH_4NO_3-extractable Pb levels (Song 2002, personal communication). In this study, NH_4NO_3 was selected as the reagent to extract Pb, and the NH_4NO_3 extractable Pb decreased with incubation time, especially in the first four weeks. After a 12-week incubation period, more than 95% of added Pb was not extractable by NH_4NO_3 (Fig. 1). This may have resulted from transformation of the added soluble Pb fraction to slowly available fractions of Pb in the soil.

High Pb levels in growth media caused toxicity to all the three vegetable crops, resulting inhibited plant growth with chlorosis in new leaves, and brown, stunted, coralloid roots. Shoot fresh weight (FW) were also progressively decreased with increasing Pb levels in the nutrient solution (Fig. 2). Significant differences in the Pb tolerance were also noted among three vegetable crops. Shoot fresh weight of pakchoi and celery decreased to about 78.3%, 94.8% of the control, respectively, when treated with the Pb level of 4 mg L^{-1}. Fruit fresh weight of hot pepper decreased to about 85.4%

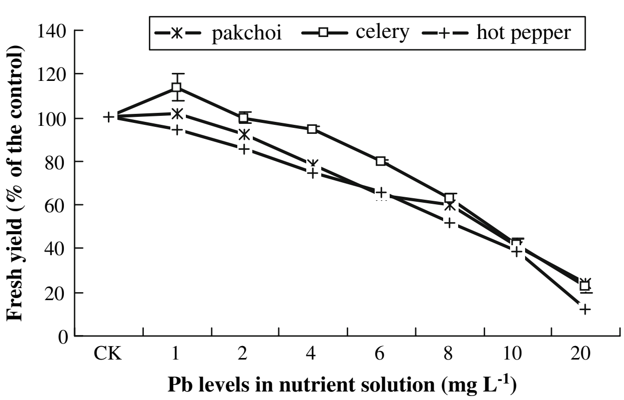


Fig. 2 Growth response of different vegetable species to Pb levels in nutrient solution. Plants were treated for 6 weeks. All data are means of three replicates ± SE

when grown under Pb supply of 2 mg L^{-1}. These results indicate that hot pepper is more sensitive to the Pb toxicity than pakchoi or celery. The sensitivity of the three vegetable crop species to Pb toxicity was decreased in the following order: hot pepper > pakchoi > celery.

Significant and negative correlations were found between biomass yields of edible parts (or stem for celery and fruit for hot pepper) and soil available or total Pb (Table 2). The biomass of shoot edible parts of the three vegetables was negatively and closely correlated with total soil Pb or soil available Pb, with the correlation coefficients being −0.90 to 0.99**. The critical soil available Pb concentrations at 10% reduction of fresh matter yield were 20.98, 21.94, 1.55 in the Inceptisol and 18.54, 30.78, 1.18 mg kg^{-1} in the Alluvial soil for pakchoi, celery (stem), and hot pepper (fruit), respectively. These results indicate that hot pepper is less tolerant to Pb toxicity than pakchoi and celery when plants were grown in the soil, and these results are consistent to those noted in the nutrient solution.

Lead concentrations in shoots and roots varied both with different Pb levels and the type of vegetables (Table 3). Lead concentrations were increased in both shoots and roots, but root concentration increased more sharply than shoot with the increasing Pb supply levels. For the three vegetable species tested, lead was mainly accumulated in the roots, and a small proportion of absorbed Pb was transported to the shoots. Large differences in shoot and root Pb concentrations were observed among the three crop species. Pakchoi contained higher Pb in both roots and shoots than celery and hot pepper at external Pb levels of <20 mg L^{-1}. For example, Pb concentration in the roots of pakchoi was 1.3 times higher than that of celery. Lead concentration in the shoots was lower than that in the roots, especially concentration in the fruits, being less than 1% of that in the roots. These Pb distribution tendencies were in agreement with the earlier reports by other researchers (Zheljazkov and Nielsen 1996; Christina 1995).

Lead accumulation coefficients (AF) in the shoots or fruits of the vegetable species were relatively smaller when

Table 2 Correlations between shoot growth (FW) and soil total or available Pb among three species

Vegetable specie		Soil total Pb		Soil available Pb	
		Regression equation	R^2	Regression equation	R^2
Pakchoi (shoot)	IS	$Y = -0.012x + 70.995$	0.9942**	$Y = -0.2236x + 64.884$	0.9444**
	AS	$Y = -0.009x + 54.14$	0.9552**	$Y = -0.282x + 48.589$	0.9867**
Celery (stem)	IS	$Y = -0.0045x + 24.63$	0.9583**	$Y = -0.0844x + 22.271$	0.9377**
	AS	$Y = -0.0023x + 12.78$	0.9080**	$Y = -0.066x + 11.386$	0.8939**
Celery (leaf)	IS	$Y = -0.003x + 14.526$	0.9830**	$Y = -0.0533x + 13.07$	0.9333**
	AS	$Y = -0.0012x + 6.089$	0.8926**	$Y = -0.0294x + 5.517$	0.9299**
Celery (shoot)	IS	$Y = -0.0077x + 39.13$	0.9653**	$Y = -0.1429x + 35.195$	0.9131**
	AS	$Y = -0.0037x + 19.23$	0.9249**	$Y = -0.1076x + 17.049$	0.9362**
Hot pepper (fruit)	IS	$Y = -0.0589x + 171.32$	0.9591**	$Y = -1.5075x + 148.74$	0.9464**
	AS	$Y = -0.0246x + 69.02$	0.9576**	$Y = -1.4565x + 59.826$	0.9355**

IS: Inceptisol; AS: Alluvial soil; ** significant at $p < 0.01$

Table 3 Lead concentration in the shoots and roots of different vegetables grown in nutrient solution

Pb levels (mg/L)	Pakchoi (mg/kg)		Celery (mg/kg)			Hot pepper (mg/kg)		
	Root	Shoot	Root	Stem	Leaf	Root	Leaf	Fruit
0.005	0.28 g	0.16 g	0.34 g	0.13 h	0.137 h	0.268 h	0.15 h	0.066 gh
1	56.67 f	17.42 f	23.6 f	5.88 g	4.77 g	16.6 g	1.92 g	0.082 g
2	197.6 de	55.28 e	123.1 de	10.75 ef	9.97 f	64.1 f	3.03 ef	1.42 ef
4	325.6 d	75.93 de	287.8 d	14.57 e	54.4 e	121.8 e	4.93 de	2.21 de
6	532.2 c	99.21 cd	511.7 c	31.51 d	83.77 d	228.5 d	6.71 cd	3.42 d
8	711.6 c	129.9 c	710.6 bc	67.8 c	137.02 c	501.4 c	8.32 c	4.84 c
10	1012.1 b	302.5 b	870.2 b	159.4 b	178.83 b	850.5 b	12.35 b	6.83 b
20	1689.7 a	451.1 a	1278.3 a	258.9 a	315.23 a	1474.9 a	21.82 a	11.08 a

"a, b, c...," denote the differences that are significant with LSD ($p < 0.05$)

grown at soil Pb levels of 0–1200 mg kg^{-1}, and dramatically increased at soil Pb levels above 2000 mg kg^{-1}. When the soil Pb levels were below1200 mg kg^{-1}, AF value of Pb in the edible parts of the three vegetable species varied in the order: pakchoi > celery > hot pepper (Table 4).

Phytotoxicity of heavy metals is related to the amount of toxicants taken up and accumulated in plant tissues (Xiong 1997). Significant and positive correlation between the reduction in shoot fresh weights and tissue Pb concentration were observed for each of the three vegetable species grown in nutrient solution. The critical tissue Pb concentrations at 10% reduction of FW yield were 24.71, 28.25, and 0.567 mg kg^{-1} for pakchoi shoot, celery stem, and hot pepper fruit, respectively. Under soil culture conditions, significant and positive correlations were also noted between plant tissue Pb and soil available Pb levels, with $r = 0.946$–0.987** for pakchoi, 0.86–0.90** for celery, and 0.915–0.936** for hot pepper, respectively. These results are in agreement with findings by other scientists (Davies 1992) and clearly shows that celery is more tolerant to Pb than the other two species, especially hot pepper under both solution and soil culture conditions.

For the soil–plant system, the potential dietary toxic threshold of heavy metal is the highest permissible content in the soil (total or bioavailable concentration) that does not cause any phytotoxicity (i.e., inhibit plant growth and decrease yield), or heavy metal in edible parts of the crops that does not exceed food safety standard (CDPM 1994). The critical food Pb threshold for human health has been established to be 0.2 mg kg^{-1} (CDPM 1994). According to the regression equations between shoot FW yields and Pb concentration in plant tissues or soil, soil Pb thresholds for phytotoxicity (10% yield reduction) and potential dietary toxicity in the edible parts of the vegetables could be calculated. Soil total and available Pb thresholds for potential dietary toxicity in the edible parts of the vegetable crops were about 1/3–1/2 of those for phytotoxicity (at 10% yield reduction) (Table 5). Among the three vegetable crops, hot pepper had much lower soil total and available Pb thresholds, compared with other two vegetable species. However, the threshold of celery was highest among the three

Table 4 Lead accumulation coefficients (AF) of different vegetable crops grown under various Pb levels on soils

Soil available Pb (mg kg^{-1})		Pb accumulation coefficient (AF) from soil							
		Pakchoi		Celery			Hot pepper		
		Root	Shoot	Root	Stem	Leaf	Root	Leaf	Fruit
CK	IS	0.0069	0.0014	0.0068	0.0020	0.0022	0.051	0.0012	0.001
	AS	0.0052	0.0009	0.0055	0.0013	0.0018	0.034	0.0013	0.0009
100	IS	0.0038	0.0006	0.0064	0.0011	0.0014	0.102	0.0021	0.001
	AS	0.0037	0.0006	0.0047	0.0009	0.0011	0.086	0.0014	0.0006
200	IS	0.0078	0.0007	0.0072	0.0008	0.0010	0.088	0.0039	0.001
	AS	0.0061	0.0006	0.0059	0.0008	0.0012	0.086	0.0025	0.0007
400	IS	0.0072	0.0011	0.0177	0.0008	0.0010	0.092	0.012	0.0018
	AS	0.0051	0.0006	0.0185	0.0007	0.0012	0.149	0.0083	0.0025
600	IS	0.0358	0.0036	0.0255	0.0012	0.0014	0.106	0.015	0.0026
	AS	0.0367	0.0010	0.0313	0.0016	0.0024	0.181	0.0110	0.0037
800	IS	0.0589	0.0065	0.0310	0.0016	0.0022	0.118	0.0277	0.0062
	AS	0.0890	0.0044	0.0463	0.0029	0.0048	0.247	0.0215	0.0076
1200	IS	0.0610	0.0127	0.0372	0.0019	0.0031	0.177	0.0446	0.010
	AS	0.0849	0.0163	0.0498	0.0050	0.0080	0.265	0.0372	0.013
2000	IS	0.0910	0.0199	0.0400	0.0040	0.0065	0.222	0.0866	0.015
	AS	0.1331	0.0243	0.0611	0.0078	0.0127	0.392	0.0755	0.0286

IS: Inceptisol; AS: Alluvial soil; AF: Pb in plant tissues/total Pb in soil; Soil available Pb extracted by 1.0 mol L^{-1} NH_4NO_3 (pH 7.0)

Table 5 Soil Pb thresholds for yield reduction and potential dietary toxicity in edible parts of the vegetables

Crop species	Total Pb (mg kg^{-1})				Available Pb (mg kg^{-1})			
	PDT		SFMYR		PDT		SFMYR	
	IS	AS	IS	AS	IS	AS	IS	AS
Pakchoi	537.2b	593.2a	900.3ab	1134.9b	5.068c	1.376a	20.9b	18.5b
Celery (stem)	595.8a	593.6a	936.2a	1496.5a	8.056a	1.473a	21.9b	30.8a
Celery (shoot)	574.7a	586.7a	893.5b	1105.7b	6.616b	1.199a	25.5a	20.6b
Hot pepper (fruit)	314.5c	353.6a	423.0c	443.6c	0.477d	0.158b	1.55c	1.18c

PDT: Potential Dietary Toxicity ($\leq$0.2 mg kg^{-1}); SFMYR: Shoot Fresh Matter Yield Reduction (by 10%); IS: Inceptisol; AS: Alluvial soil

vegetable crops. So far, the critical food Pb threshold for human health was established on the basis of total metal concentration. Therefore the results of soil thresholds analyzed on the basis on current hygienic standards of Pb levels for human health are valuable for improving diagnostic standards of soil quality.

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