Soil and Sediment Contamination, 12(3):357-370 (2003)

Effect of Cd, Zn, and Pb Compound Pollution on Celery in a Ferric Acrisol

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A pot experiment with an orthogonal experimental design $L_9(3^4)$ was conducted to study the combined effects of Cd, Zn, and Pb on the growth and metal content of celery grown in a ferric acrisol. *The uptake of Cd, Zn, and Pb by celery was not only affected by the individual elements, but also by combinations of the elements. The effect of coexisting elements on plant uptake of the heavy metals depended on the concentration ratios of the elements. There is a given ratio where a maximum antagonism or synergism effect occurs. The combinations of elements clearly affected the dry weight of celery and the heavy metal concentration in celery. The removal rate (the ratio of plant total uptake to the total metal content in soil) was in the order of Cd > Zn > Pb, with no obvious difference between the removal rate under single pollution and that under compound pollution.*

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KEY WORDS: *Cd, Zn, Pb, compound pollution, celery.*

1532-0383/03/$.50

INTRODUCTION

THERE exists extensive information on contamination of soils and plants by single heavy metals as Cd, Zn, and Pb (Robson, 1993; Ross, 1994; Alloway, 1995; McLaughlin and Singh, 1999). Compound pollution of heavy metals is a more common phenomenon in nature, where additive, synergistic, and antagonistic effects can occur (Wallace and Berry, 1989; Smilde *et al.,* 1992; Wu *et al.,* 1995). Possible interactions of trace elements within plants and in the soil at the root surface were summarized, but it is still difficult to draw firm conclusions from the literature because of the contradictory data (Alloway, 1995).

Compound pollution of heavy metals occurs not only in mining and sewage irrigation areas, but also in suburban vegetable plots as caused by atmospheric deposition or additives. In China, studies on compound pollution have been carried out in different areas, with cereals (paddy rice, wheat, maize) and various vegetables (cabbage, rape, soybean, spinach) used as test plants on different soil types (Zheng and Chen, 1989; Zheng and Chen, 1990; Yu and Wu, 1995; Song *et al.,* 1996; You, 1997; Wu, *et al.,* 1998; Wang *et al.,* 2001). The results showed that interactions of heavy metals varied with the coexisting metals and their concentrations, soil physiochemical properties and plant species. Plant height, dry weight, and yield/biomass are often used experimental indices, with combined effects expressed in terms such as zinc equivalent, toxicity pollution index, element concentration ratio, ionic impulsion, and relative ionic strength. Comparison was also made between single pollution and compound pollution by using a concentration factor. Heavy metal pollution in Zhuzhou City was compound pollution, with Cd, Pb, and Zn as the major pollutants (result from "Heavy metal pollution in air-water-soil-plant system of Zhuzhou City, Hunan Province, China", manuscript accepted by *Water Air Soil Pollut.*). Celery is one of the main agricultural products available in the vegetable market there. Celery can easily take up heavy metals, and the accumulation of heavy metals in the edible parts can affect human health, especially in areas with a high consumption of locally grown vegetables. So far, no research on compound pollution of heavy metals was carried out in a ferric acrisol. The objective of the present study was to study the combined effects of Cd, Zn, and Pb on the growth and metal content of celery grown in a ferric acrisol. The effect of compound pollution was compared with that of single pollution by using a removal rate in addition to a concentration factor.

MATERIAL AND METHODS

Experimental Design

The topsoil (0 to 20 cm) of a ferric acrisol (FAO-UNESCO, 1978) (Typic red soil, Chinese soil taxonomy [Institute of Soil Science, 1990]) was collected from a noncontaminated site near the Central South Forestry University (CSFU), Zhuzhou

City, Hunan Province. The ferric acrisol there was derived from quaternary red clay, with a thick solum possessing marked illuviation of clay in profile, the silica-alumina ratio of clay fraction close to 2, and kaolinite as the dominate clay minerals (Institute of Soil Science, 1990). Some important soil properties are presented in Table 1. Prior to potting, the soil was air-dried and ground to pass a 1-mm nylon sieve. Because of low soil fertility and a high clay content, a given amount of granulated compound fertilizer (equal to 20 mg N, 10 mg P_2O_5, and 10 mg K_2O per kg soil) and sand were added and mixed thoroughly with the soil. After 2 weeks of incubation, 2.5 kg soil was put into each of 63 plastic pots. Seeds were sown in a nursery bed. One celery seedling was planted in each pot after having developed two real leaves. The pots were placed in an open area with plastic shed, which had a roof and was able to protect celery from ground-bound and air-borne animals. The experimental period lasted 5 months. Plants were watered with deionized water as needed.

Single Pollution of Celery by Cd, Zn, and Pb. Thirty-six pots were grouped into four treatments, with Cd, Zn, and Pb applied in their sulfate forms, as $(CdSO_4)_3 \times 8H_2O$, $ZnSO_4 \times 7H_2O$, and $PbSO_4$, respectively (Table 2). The different additions

TABLE 1
Basic Properties of the Tested Soil (Ferric Acrisol)

pH (H_2O)	Organic matter (g kg^{-1})	CEC* (cmol kg^{-1})	Background value of heavy metals (mg kg^{-1})		
			Cd	Zn	Pb
5.10	1.56	22.5	0.34	140.6	53.5

* Cation Exchange Capacity.

TABLE 2
Experimental Factors and Levels (mg kg^{-1})

Treatment	Element		
	Cd	Zn	Pb
0 (Control)	0	0	0
1 (Low dose)	1	50	50
2 (Medium dose)	5	300	400
3 (High dose)	20	800	1000

were based on the local Cd, Zn, and Pb pollution status. The experiment was replicated three times.

Compound Pollution of Celery by Cd, Zn, and Pb. An orthogonal experimental design $L_9(3^4)$ was used with three replicates (Table 3). The L_9 orthogonal array is a table of integers whose column elements (1, 2, and 3) represent the low, medium, and high levels of the column factors. Each row of the orthogonal array represents a run (treatment), that is, a specific set of factor levels to be tested. In this experiment, there were three levels of Cd, Zn, and Pb application, respectively, in their sulfate forms (same as single pollution). Altogether, there were nine treatments (element concentration combinations).

After celery harvesting, the heavy metal content in the soil and celery was measured.

TABLE 3
Orthogonal Experimental Design $L_9(3^4)$ and Heavy Metal Concentrations in Celery

Treatment	Element			Experimental indices			
No.	Cd	Zn	Pb	Dry weight (g/seedling)*	Cd concentration (mg kg^{-1})*	Zn concentration (mg kg^{-1})*	Pb concentration (mg kg^{-1})*
1	1	1	1	2.48 (0.21)	17.46 (2.62)	631.85 (38.59)	34.16 (2.42)
2	1	2	2	4.86 (0.39)	21.15 (3.10)	1239.78 (81.99)	163.29 (12.33)
3	1	3	3	4.23 (0.40)	21.94 (3.12)	2264.05 (110.20)	175.99 (15.60)
4	2	1	2	1.87 (0.19)	94.93 (13.24)	767.84 (41.39)	193.31 (19.33)
5	2	2	3	3.28 (0.26)	23.77 (3.35)	452.81 (26.64)	61.58 (5.16)
6	2	3	1	1.33 (0.15)	48.93 (6.34)	1463.70 (67.19)	198.64 (17.86)
7	3	1	3	5.55 (0.45)	89.63 (10.44)	268.46 (16.42)	163.41 (18.34)
8	3	2	1	11.77 (1.02)	62.47 (9.03)	2741.76 (145.09)	22.20 (1.22)
9	3	3	2	0.91 (0.09)	63.42 (9.51)	1337.69 (62.88)	66.72 (7.67)

* Standard deviations are presented in the parenthesis.

Analytical Methods

Soil pH was measured in a suspension of soil and water at a 1:2.5 (v/v) ratio. Soil organic matter was measured by the $K_2Cr_2O_7$-H_2SO_4 method (Nanjing Soil Research Institute, 1981; Houba *et al.,* 1988). The potential cation exchange capacity (CEC) was estimated using 1 *M* NH_4OAc (pH 7.0) (Page, *et al.,* 1982). Background values of heavy metals in the soil were determined after digestion with *aqua regia* (HNO_3: HCl = 1:3).

Plant samples were rinsed with tap water and washed thoroughly with distilled water. After air drying and weighing, the samples were cut into small pieces, mixed evenly, and oven dried at 80 to 90°C until reaching constant weight. The dried samples were finely ground with agate mortar to pass a 0.5-mm nylon sieve. About 2 g of plant samples was put in a ceramic crucible and pre-ashed on an electrothermal plate. After most of the samples were carbonized, they were transferred to a hot oven and ashed at 500°C for 12 h. The plant ash was dissolved with 3 mL of 6 *M* HCl, filtered if colloids were visible, and then diluted to a volume of 50 mL with distilled water (Nanjing Soil Research Institute, 1981; Chlopecka and Adriano, 1997).

After removing the root remains, soil samples were air-dried and ground to pass a 2-mm and then a 0.25-mm nylon sieve. About 1 g of homogenized soil samples were put in a ceramic crucible and ashed in a hot oven at 550°C for 6 h. After adding 5 mL of *aqua regia*, the samples were gently heated on an electrothermal plate until dry. The soil ash was dissolved with 5 mL of 6 *M* HCl, filtered, and then diluted to a volume of 50 mL with distilled water (Nanjing Soil Research Institute, 1981; Wu, *et al.,* 1991).

The metal concentrations of Cd, Zn, and Pb in soil and plant samples were determined by atomic absorption spectrometry (GGX-6A, geological instrument plant of Beijing, China).

Statistical Analysis and Graphical Presentation

Microsoft Excel (Microsoft Excel, 2000) was used for statistical analysis (ANOVA) and graphical presentation.

Results and Discussion

Effect of Compound Pollution on the Biomass and Heavy Metal Concentrations in Celery

Range analyses (the difference between maximum and minimum value) showed that the affecting order of Cd, Zn, and Pb application on the dry weight of celery was Zn (4.48) > Cd (3.92) > Pb (2.65). With an increase in Cd or Pb application, the dry weight of celery first decreased and then increased: Zn application had an opposite effect. As will be shown below, the effect of these applications on the Cd, Zn, and Pb concentrations in celery was Cd > Zn > Pb, Zn > Cd > Pb, and Pb > Cd > Zn, respectively (calculations based on Table 3).

Zinc is an essential micronutrient for plants, but in excess, it may cause chlorosis and retard plant growth due to the deficiency of macronutrients such as P, and thus reduce the dry weight (Marschner, 1995). A higher amount of Pb was applied to the soil than Cd and Zn, but it showed a weaker effect on the dry weight. This is probably due to the general immobility of Pb in the soil-plant system (Chaney, 1989; Davies, 1990; Huang and Cunningham, 1996; Chlopecka and Adriano, 1997), where Pb is either strongly bound to soil or retained in plant roots and thus will not affect the normal growth of celery.

Under the present experimental condition, we found different uptake patterns of metals depending on the initial level of these metals, as follows:

1. Given Cd concentrations:

   At low Cd (1 mg kg^{-1}): There was no effect of Zn and Pb applications on the Cd concentration in celery, while Zn and Pb concentrations in celery increased with increasing Zn and Pb applications.

   At medium Cd (5 mg kg^{-1}): The medium Zn application inhibited Cd uptake, while medium Pb application promoted Cd uptake by celery. Lead concentration in celery decreased with increasing Pb application, and Zn concentration in celery decreased at medium Zn application.

   At high Cd (20 mg kg^{-1}): Zinc application inhibited Cd uptake, while Pb application promoted Cd uptake by celery. Zinc concentration in celery increased at first and then decreased with increasing Zn application, and Pb concentration in celery increased with increasing Pb application (Table 3).

2. Given Zn concentrations:

   At low or medium Zn (50 or 300 mg kg^{-1}): Lead and Cd applications inhibited Zn uptake. Lead concentration in celery increased at first and then decreased with increasing Pb application, and Cd concentration in celery increased with increasing Cd application.

   At high Zn (800 mg kg^{-1}): Zinc uptake was promoted by Pb application, but inhibited by Cd application. Lead concentration in celery decreased at first and then increased with increasing Pb application, and Cd concentration in celery increased with increasing Cd application (Table 3).

3. Given Pb concentrations:

   At low Pb (50 mg kg^{-1}): Lead uptake was promoted by both medium Cd application and high Zn application. Zinc concentration in celery increased at first and then decreased with increasing Zn application, and Cd concentration in celery increased with increasing Cd application.

At medium Pb (400 mg kg^{-1}): Medium Cd application promoted Pb uptake. Cadmium concentration in celery decreased at high Cd application, and Zn concentration in celery increased with increasing Zn application.

At high Pb (1000 mg kg^{-1}): Lead uptake was inhibited by medium applications of Cd and Zn. Cadmium and Zn concentration in celery increased with increasing Cd and Zn applications (Table 3).

Our experimental results suggest that the uptake of Cd, Zn, and Pb by celery was affected not only by the elements in single applications, but also by combinations of the elements. Metals are not mutually competitive at natural concentrations. When soils are enriched with metals, ion interactions may occur between the two metals, with other trace metals or with major elements in solution, and are often competitive (Lorenz *et al.,* 1997), while Chaney (1989) thought that interactions among elements increased or decreased the potential for element toxicity. Zinc and Cd are chemically similar and may compete for binding sites in the soil system and for uptake sites in the plant (Grant *et al.,* 1998). Page *et al.* (1981) found that Zn had an antagonistic effect on Cd uptake in soils with low Cd concentrations, and either a synergistic or no effect with relatively high Cd contents. Moraghan (1993) observed in greenhouse trials that Zn application reduced Cd in flax seed where Cd was not applied, but increased seed Cd where Cd was applied. Oliver *et al.* (1994) reported that low rates of Zn fertilizer (up to 5.0 kg Zn ha^{-1}) markedly decreased the Cd concentration in wheat grain grown in areas of marginal to severe Zn deficiency, and higher rates of Zn application resulted in no further decreases in grain Cd concentration. In fact, Zn application may increase or decrease Cd uptake by plants, depending on the levels of Cd and Zn in the soil and the relative importance of competition in soil sorption or plant uptake sites (Grant *et al.,* 1998). Our experimental result supports this viewpoint. Comparatively, Pb application has less effect on the uptake of Cd and Zn by celery, which, however, did not support the synergistic effect of Pb on Cd uptake (Adriano, 1986).

The effect of coexisting elements on plant uptake of the given heavy metals depended on the concentration ratios of the elements. From Figure 1, it could be tentatively concluded that there must be a constant ratio where a maximum antagonism or synergism effect occurs. Element combinations clearly affected the dry weight of celery and heavy metal concentrations in celery. Variance analysis showed no significant influence of heavy metal application (Table 4). This is mainly because a significant interaction masked the significance of individual elements.

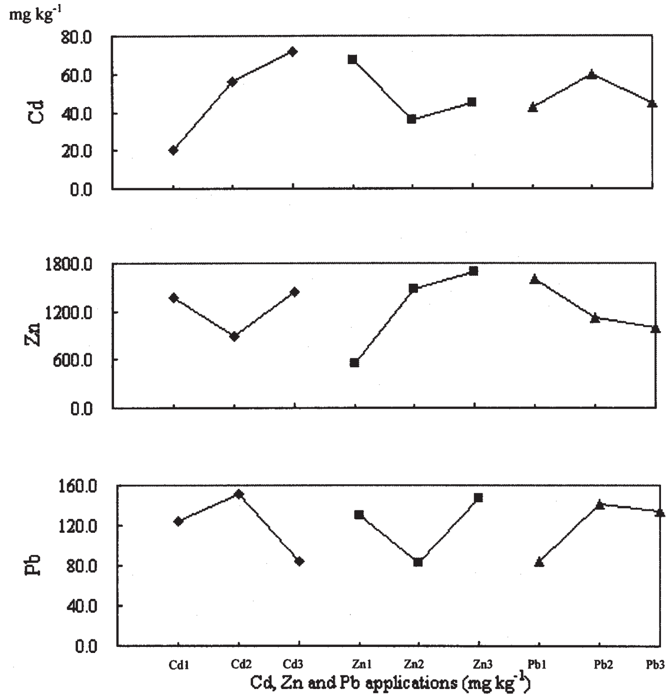

FIGURE 1

Heavy metal concentrations in celery and heavy metal applications. Note: the Y coordinate axis denotes the Cd, Zn, and Pb concentrations in celery, and the X coordinate axis denotes the Cd, Zn, and Pb applications to soil, with 1, 2, and 3 representing the low, medium, and high levels of Cd, Zn, and Pb application.

TABLE 4
Relation between Dry Weight of Celery, Heavy Metal Concentrations in Celery and the Amounts of Metals Added to the Soil

Response variable	F value and significance*		
Dry weight	F_{Cd}=1.10	F_{Zn}=1.55	F_{Pb}=0.52
Cd uptake	F_{Cd}=4.20	F_{Zn}=1.59	F_{Pb}=0.51
Zn uptake	F_{Cd}=0.25	F_{Zn}=1.01	F_{Pb}=0.30
Pb uptake	F_{Cd}=0.30	F_{Zn}=0.30	F_{Pb}=0.25

* $F_{0.05}$ = 19 $F_{0.10}$ = 9.0

The transfer of metals from soils to plants has been described by calculating concentration factors (CF) (also called accumulation factor or transfer factor) according to the following formula:

$$\mathrm{CF} = \frac{P_s\left(\mathrm{mg\ kg^{-1}\ dry\ wt}\right)}{S_t\left(\mathrm{mg\ kg^{-1}\ dry\ wt}\right)} \tag{1}$$

where P_s is the plant metal concentration originating from the soil and S_t is the total metal concentration in soil (Voutsa *et al.,* 1996; McGrath *et al.,* 1997; Emilie *et al.,* 2000).

The CF value can be a good indicator for selecting plant species, either hyperaccumulators or those avoiding excessive uptake and transport of metals, for phytoremediation purposes. However, the plant uptake is dependent on both the element concentration in plants and the biomass. Therefore, the removal rate (RR), the ratio of plant total uptake to the total metal content in soil, was calculated in the present study to compare the mobility of heavy metals in the soil-plant system.

$$\mathrm{RR} = \frac{P_s\left(\mathrm{mg\ kg^{-1}\ dry\ wt}\right) * \mathrm{Plant\ Biomass}}{S_t\left(\mathrm{mg\ kg^{-1}\ dry\ wt}\right) * \mathrm{Soil\ Volume}} \tag{2}$$

The RR results were in the order of Cd > Zn > Pb (Table 5). This supports the hypothesis that, when present in soils, Cd and Zn are easily absorbed by plants and translocated to plant tissues, while Pb is among the other elements that are strongly bound to soil or retained in plant roots, even when soils are greatly enriched (Chaney, 1989; Singh and Jeng, 1993).

With increasing Cd and Zn applications, Cd and Zn uptake by celery increased. This may be due to the soil acidity of the ferric acrisol at which soil Cd and Zn activity is positively correlated with soil Cd and Zn quantity (Wu and Aasen, l994; Wu *et al.,* 2000). However, the Cd and Zn removal rates showed a decreasing trend. This suggested that the higher soil heavy metal concentrations have blocked the plant uptake.

There was no obvious difference between the removal rate of soil heavy metals under single pollution and that under compound pollution (Table 6). However, the Cd removal rate with no Cd application was far higher than that with Cd applications. These results are inconsistent with those of several studies on comparison of single pollution and compound pollution by using CF (Wu *et al.,* 1995; Wu *et al.,* 1997a; Wu, *et al.,* 1997b).

TABLE 5
Removal Rate of Heavy Metals from Soil to Celery

Treatment	Element			Concentration Factor			Removal rate (%)		
No.	Cd	Zn	Pb	Cd	Zn	Pb	Cd	Zn	Pb
1	1	1	1	13.03	3.32	0.33	1.29	0.33	0.03
2	1	2	2	15.78	2.81	0.36	3.07	0.55	0.07
3	1	3	3	16.37	2.41	0.17	2.77	0.41	0.03
4	2	1	2	17.78	4.03	0.43	1.33	0.30	0.03
5	2	2	3	4.45	1.03	0.06	0.58	0.13	0.01
6	2	3	1	9.16	1.56	1.92	0.49	0.08	0.10
7	3	1	3	4.41	1.41	0.16	0.98	0.31	0.03
8	3	2	1	3.07	6.22	0.21	1.45	2.93	0.10
9	3	3	2	3.12	1.42	0.15	0.11	0.05	0.01

*The concentration factor (CF) is defined by eqn. (1) and the removal rate (RR) is defined by Eqn. (2).

TABLE 6
Removal Rates of Soil Heavy Metals Under Single and Compound Pollution

	Application (mg/kg)	Single pollution removal rate(%)	Compound pollution removal rate(%)*
Cd	0	8.796	
	1	2.455	1.29~3.07 (2.38)
	5	1.229	0.49~1.33 (0.80)
	20	0.522	0.11~1.45 (0.85)
Zn	0	0.147	
	50	0.209	0.30~0.33 (0.31)
	300	0.150	0.13~2.93 (1.20)
	800		0.05~0.41 (0.18)
Pb	0	0.035	
	50	0.096	0.033~0.102 (0.079)
	400	0.022	0.005~0.070 (0.036)
	1000	0.020	0.008~0.034 (0.023)

* Standard deviations are presented in the parenthesis.

Interactions of heavy metals are mainly antagonistic or synergistic. However, there also exist complicated interactions between heavy metals and macronutrients, soil properties, plant characteristics, and environmental conditions. Hence, compound pollution may not always affect the removal rate of heavy metals. Under given soil and environmental conditions, the effect of compound pollution on some plant species depended on the concentration combination of elements. Because only combinations of three elements were tested, this experimental design did not allow for a full examination of the mutual interaction between any two elements.

CONCLUSIONS

The uptake of Cd, Zn, and Pb by celery was affected not only by individual elements, but also by combinations of the elements. The effect of coexisting elements on plant uptake of the given heavy metals depended on the concentration ratio of the elements. There is a given ratio where the maximum antagonism or synergism effect occurs. The combinations of elements clearly affected the dry weight of celery and the heavy metal concentrations in celery, whereas interaction between metals masked the significant effects of individual elements.

The removal rate was in the order of Cd > Zn > Pb. Cadmium was most easily taken up by plants. In acidic soil, the uptake of Cd and Zn by celery increased but with a decreasing removal rate with an increase in soil Cd and Zn.

When using the removal rate to describe the mobility of heavy metals in the soil-plant system, there was no obvious difference between single pollution and compound pollution. Under given soil and environmental conditions, the effect of compound pollution on some plant species depended on the concentration combination of elements.

ACKNOWLEDGMENTS

I would like to thank Prof. Arne O. Stuanes, Prof. Bal Ram Singh, and Dr. Åsgeir Almås for their valuable comments. I also thank the anonymous reviewers for their helpful suggestions.

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