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Effect of Zinc–Cadmium Interactions on the Uptake of Zinc and Cadmium by Winter Wheat (*Triticum aestivum*) Grown in Pot Culture

Y. G. Zhu,[1] Z. Q. Zhao,[1] H. Y. Li,[2,3] S. E. Smith,[3] F. A. Smith[3]

[1] Department of Soil Environmental Sciences, Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, Beijing 100085, People's Republic of China
[2] Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang, People's Republic of China
[3] Department of Soil and Water, The University of Adelaide, Glen Osmond 5064, Australia

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Cadmium (Cd) is a non-essential element with no beneficial effects on plants, and is generally considered as a toxic contaminant in food for humans and animal feed (McLaughlin and Singh 1999). The accumulation of Cd in edible parts of crop plants is increasingly becoming a public health issue in many nations (Jin et al. 2002). Soil contamination by Cd is due to both natural and anthropogenic sources. Anthropogenic sources include the application of chemical fertilizers and pesticides, irrigation with Cd-contaminated wastewater, atmospheric deposition and the application of sewage sludge in arable land. At Rothamsted in England, increases in Cd concentrations in wheat grain from 50 to 80 μg kg-1 between 1880 and 1980 were attributed to the use of chemical fertilizers (Jones and Johnston 1989). Cd can be easily transferred via food-chains and accumulated in human bodies. Typical health problems resulting from excessive body burden of Cd include: renal dysfunction, liver damage, lung edema, anemia and hypertension (Basta, et al. 1998).

Unlike Cd, zinc (Zn) is an essential element both for plants and humans, but if present in high concentration in plants and humans it can also be highly toxic. While Zn deficiency is a widespread problem in calcareous soils in many parts of the world, Zn contamination can be a serious problem in areas near mining and smelting industries or long-term application of sewage sludge. It is generally accepted that Zn status in soils and plants plays an important role in Cd accumulation in crop plants. Oliver et al. (1994) demonstrated that application of Zn to wheat grown on marginally or severely Zn-deficient soils in South Australia

Correspondence to: Y. G. Zhu

decreased Cd concentration in grain by up to 50%. Other studies showed that Zn application could reduce Cd accumulation in flax seeds (Grant and Bailey 1997) and durum wheat grain (Choudhary et al. 1994). However, other studies showed that there was no interaction or even synergism between Zn and Cd (Williams and David 1976; White and Chaney 1980).

Most of the studies on the uptake of Cd by plants have been made in isolation, without considering Cd co-existence with other contaminants, such as Zn. However, in many actual field situations, soil environments are often contaminated with multiple heavy metals, such as Cd, Zn, Pb, etc. In such cases, these metal ions will interact with each other, affecting the fate of each metal in soil-plant systems. The aim of the present study was therefore to investigate the interactions between Zn and Cd under wide range of soil Zn concentrations from adequacy to contamination levels.

MATERIALS AND METHODS

This study was a pot experiment with a loamy soil (originally 0-25cm depth) from the Luancheng Experimental Station, Chinese Academy of Sciences (CAS), Shijiazhuang. The soil was air dried and sieved (<2mm stainless steel mesh) prior to analysis of its chemical properties (Table 1). The soil was amended to give four levels of Cd: 0, 15, 30 and 50 mg kg^{-1}, and five levels of Zn: 0, 2, 10, 100 and 1000 mg kg^{-1}. Thus, there were 20 treatments altogether. Zinc was supplied as zinc sulphate and cadmium was supplied as cadmium chloride. There was uniform application of N-P-K fertilizer at amounts equal to 200 mg N kg^{-1} soil (added as urea), 133 mg P_2O_5 kg^{-1} (added as $CaHPO_4$) and 133 mg K_2O kg^{-1} (added as K_2SO_4). Each treatment had three replicates.

The treated soils were mixed thoroughly, put into plastic pots (1 kg /pot) and saturated with deionized water. Soil was allowed to equilibrate in the greenhouse for one week before sowing the seeds. Six germinated seeds of winter wheat *(Triticum aestivum,* L.), Kenong. 9204, (from Institute of Genetics and Developmental Biology, CAS, Shijiazhuang, China), were planted in each pot. The pots were randomly arranged in a greenhouse and rearranged several times during the growth period. When the seedlings had grown to about 3cm, they were thinned to three per pot. The seedlings were irrigated with deionized water every three days. The growth temperature was 18°C on average during 14/10h light/dark cycles. The plants were harvested after nine weeks.

The plants were separated into shoots and roots and rinsed thoroughly with deionized water, and the fresh weights were determined. The samples were then

oven dried at 70 °C for 48h, and the dry weight of shoots and roots were recorded. Dried plant samples were finely ground in a stainless steel mill.

Sub-samples (0.25g) of finely ground plant materials were digested at 160 °C in 5ml high-purity mixed acid (HNO_3 : $HClO_4$ = 6 :1). The digest was diluted to 50ml using high-purity water, and the concentrations of Cd in the solution were determined by ICP-MS (Inductively Coupled Plasma Mass Spectrometry, Agilent 7500a, USA).

All data were subjected to analysis of variance (ANOVA) using commercially available GENSTAT (6th ed., NAG Ltd, England)

RESULTS AND DISCUSSION

The chemical properties of the soil used in this experiment were summarized in table 1.

Table 1. Selected chemical properties of the soil used in the pot experiment

pH	OM %	Available N mg kg^{-1}	Available P mg kg^{-1}	Available K mg kg^{-1}	CEC cmol/kg (+)	Tot. Zn mg kg^{-1}	Tot. Cd mg kg^{-1}
7.7	1.4	80.0	24.1	198. 7	10.0	176.6	0.1

Table 2. Shoot biomass (g pot^{-1}) of winter wheat plants grown in pot culture with different additions of Cd and Zn.

Cd addition mg kg^{-1} soil	Zn addition (mg kg^{-1} soil)				
	0	2	10	100	1000
0	2.89±0.11	2.93±0.06	3.09±0.04	2.58±0.04	0.23±0.01
15	2.34±0.05	2.40±0.07	2.35±0.05	2.42±0.04	0.24±0.01
30	1.31±0.03	1.43±0.05	1.63±0.03	1.62±0.06	0.24±0.01
50	1.32±0.06	1.23±0.03	1.25±0.07	1.02±0.04	0.23±0.02
	Analysis of variance				
Cd	$P < 0.001$				
Zn	$P < 0.001$				
Cd × Zn	$P < 0.001$				

At any level of Zn addition up to and including 100 mg kg^{-1}, shoot biomass decreased significantly with Cd addition (Table 2); shoot biomass with 50 Cd mg kg^{-1} was at most 50% of that with no added Cd. Application of 0-100 mg Zn kg^{-1} did not significantly affect shoot biomass, but 1000 mg Zn kg^{-1} caused sharp decreases. Root biomass showed very similar pattern of changes as shoot biomass, with the

exception of the treatment with 50 mg Cd plus 1000 mg Zn kg^{-1}, in which root biomass was reduced compared with the treatment with 0 Cd plus 1000 mg Zn kg^{-1} (Table 3).

Table 3. Root biomass (g pot^{-1}) of winter wheat plants grown in pot culture with different additions of Cd and Zn.

Cd addition mg kg^{-1} soil	Zn addition (mg kg^{-1} soil)				
	0	2	10	100	1000
0	1.13±0.05	1.06±0.05	1.06±0.03	0.99±0.05	0.13±0.01
15	0.92±0.05	0.82±0.07	0.84±0.02	0.86±0.01	0.15±0.01
30	0.51±0.03	0.49±0.01	0.54±0.02	0.62±0.02	0.17±0.02
50	0.51±0.04	0.51±0.03	0.51±0.04	0.47±0.02	0.09±0.01
	Analysis of variance				
Cd	$P < 0.001$				
Zn	$P < 0.001$				
Cd × Zn	$P < 0.001$				

With no Cd addition, Cd concentrations in shoots were undetectable. This is due to the low background Cd concentration in the soil (around 0.1 mg kg^{-1}). Cd concentrations in shoots increased greatly with Cd addition (Table 4). Application of Zn at 2 and 10 mg kg^{-1} did not have significant effects on Cd concentrations in shoots. With 15 and 30 mg Cd kg^{-1}, 100 and 1000 mg Zn kg^{-1} reduced shoot Cd concentrations and with 50 mg Cd kg^{-1} there was a reduction by 1000 mg Zn kg^{-1}.

Table 4. Cadmium concentrations (mg kg^{-1}) in shoots of winter wheat plants grown in pot culture at different levels of Zn and Cd additions.

Cd addition mg kg^{-1} soil	Zn addition (mg kg^{-1} soil)				
	0	2	10	100	1000
0	n.d.*	n.d.	n.d.	n.d.	n.d.
15	39.8±2.07	40.1±1.56	39.4±1.60	28.1±0.65	22.5±0.37
30	70.1±2.67	69.8±4.54	64.8±4.00	54.3±3.64	37.3±2.72
50	76.4±1.68	77.6±1.17	72.7±1.85	74.6±6.33	41.5±3.44
	Analysis of variance				
Cd	$P < 0.001$				
Zn	$P < 0.001$				
Cd × Zn	$P = 0.007$				

* Not detectable.

Cd concentrations in roots were much higher than in shoots (Tables 4 & 5), and also increased with Cd addition. With 15 and 30 mg Cd kg^{-1}, Zn applications at 2 and 10

mg kg^{-1} did not significantly affect root Cd concentrations; while Zn applications of 100 and 1000 mg kg^{-1} reduced Cd concentrations. With 50 mg Cd kg^{-1}, applications of 10 to 1000 mg Zn kg^{-1} reduced root Cd concentrations.

Zinc concentrations in shoots increased significantly with increasing Zn applications (Table 6). With low levels of Zn, Cd addition had little effect on shoot Zn concentrations, but with100 and 1000 mg Zn kg^{-1}, Zn concentrations in shoots tended to decrease with increasing Cd addition. This was most pronounced with 1000 mg Zn kg^{-1}. Zn concentrations in roots showed a different pattern of changes from concentrations in shoots (Table 7). With 0 and 100 mg Zn kg^{-1}, there was no consistent change in root Zn concentration with changing Cd additions, but with1000 mg Zn kg^{-1}, Cd addition significantly reduced Zn concentrations in roots.

Table 5. Cadmium concentrations (mg kg^{-1}) in roots of winter wheat grown in pot culture at different levels of Zn and Cd additions.

Cd addition mg kg^{-1} soil	Zn addition (mg kg^{-1} soil)				
	0	2	10	100	1000
0	1.2±0.2	1.7±0.3	0.6±0.1	4.7±0.8	2.8±1.7
15	195±1.9	221±15.9	185±1.1	150±5.3	121±4.4
30	419±1.4	389±8.1	392±6.1	281±5.0	251±13.8
50	579±20.4	666±60.7	479±35.6	495±35.8	282±12.3
	Analysis of variance				
Cd	$P < 0.001$				
Zn	$P < 0.001$				
Cd × Zn	$P < 0.001$				

Significant Zn-Cd interactions were evident in this experiment in terms of both plant biomass and Zn/Cd concentrations in plant tissues. In this study, Zn application did not improve plant growth in the absence of Cd application, showing that the soil used is not Zn-deficient. This result is consistent with some previous studies, which showed that Zn did not have inhibitory effects on plant uptake of Cd (Nan et al. 2002), and may relate to the use of soils that are not deficient in Zn. It has been suggested that one option to reduce Cd accumulation in wheat grains was to effectively improve plant Zn nutrition via soil or foliar applications (Clarke et al. 1997; Grant et al. 1998; Cakmak et al. 2000). However, results from this study demonstrated that the inhibitory effects of the higher levels of Zn application, designed to minimize plant uptake of Cd, might have some limitations depending on the actual Zn nutritional status of the plant. Although increase in Zn application to100 and 1000 mg kg^{-1} significantly reduced Cd concentrations in plant tissues, the reduction in Cd concentration did not actually improve plant growth (no biomass gain). This could be due to the fact that Zn application at 100 mg kg^{-1}

started to have phytotoxicity to wheat plants (Table 2). However, the interactions between Zn and Cd observed in this study may be complicated further by the companion anion applied to the soil with Zn, SO_4^{2-}, which was shown to possibly increase plant uptake of Cd (Zhao et al. 2003).

Table 6. Zinc concentrations (mg kg^{-1}) in shoots of winter wheat grown in pot culture at different levels of Zn and Cd additions.

Cd addition mg kg^{-1} soil	Zn addition (mg kg^{-1} soil)				
	0	2	10	100	1000
0	104±5.1	115±2.7	147±3.8	397±16.0	1950±61
15	103±5.4	121±5.4	159±6.2	455±7.7	1730±31
30	102±5.4	109±2.1	142±12.1	350±20.4	1235±85
50	97±4.6	107±9.2	139±4.4	342±15.7	708±55
Analysis of variance					
Cd	P < 0.001				
Zn	P < 0.001				
Cd × Zn	P < 0.001				

Table 7. Zinc concentrations (mg kg^{-1}) in roots of winter wheat grown in pot culture at different levels of Zn and Cd additions.

Cd addition mg kg^{-1} soil	Zn addition (mg kg^{-1} soil)				
	0	2	10	100	1000
0	50.8±3.1	75.6±6.1	98.4±12.3	563±46.6	5409±375
15	66.3±3.9	64.2±5.8	186.7±3.5	694±13.0	4338±338
30	98.4±8.8	96.4±14.2	135.9±12	5189±4.9	3275±120
50	68.6±2.5	82.5±16.9	145.9±9.2	673±5.1	2220±410
Analysis of variance					
Cd	P < 0.001				
Zn	P < 0.001				
Cd × Zn	P < 0.001				

The amounts of Cd added to the soil in this experiment were relatively high than those in most agricultural soils, but, high concentration of Cd in arable soils do occur in areas in vicinity of mining and smelting sites in China. For example soils in an area near a smelter in Nanning, Guangxi Province in China, have average Cd concentrations up to 22 mg kg-1, yet local people still grow vegetables for their own consumption (*Cui Yujing, personal communication*). Additions of Cd reduced plant growth significantly except at the highest Zn application in the present study. At the highest Zn application, plant biomass was only 10% of that for the control plants and Zn toxicity, not Cd toxicity, may be the dominant factor affecting plant

growth. Competitive effects of Cd on Zn accumulation by wheat plants were also evident, particularly at the highest Zn application (Table 6), but this competition did not release any stress from soil Zn contamination. Soil and plant phosphorus status may also affect plant uptake of Cd (Grant and Bailey 1997; Yang et al. 1999; Maier et al. 2002). In this study, the soil is high in P (data not shown). High phosphorus may also alter the interactions between Zn and Cd, which has not been studied extensively so far.

In summary, the results show that attempts to ameliorate Cd toxicity by applying Zn fertilizer may be complicated by individual soil types and nutrient status (e.g. P). The inhibitory effects of soil Zn on Cd concentrations in plant tissues occurred only at toxic Zn levels, but this inhibition did not improve plant growth suggesting that Zn concentration became the dominant factor controlling plant growth.

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