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Effects of Chlorimuron-ethyl and Cadimum on Biomass Growth and Cadimum Accumulation of Wheat in the Phaiozem Area, Northeast China

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Abstract The joint effect of chlorimuron-ethyl and cadmium (Cd) on biomass growth and cadmium accumulation in wheat (*Triticum aestivum* L.) was investigated and compared with single-factor effect of soil cadmium pollution. The results showed that dry biomass of wheat had significantly ($p < 0.01$) negative relationships with increasing concentrations of chlorimuron-ethyl and cadmium in phaiozem. The highest inhibition rates observed were 76.2%, 62.7% and 55.6% for roots, shoots and glumes, respectively, when the concentration of cadmium in soil was up to 100 mg kg^{-1}. There were synergistically inhibitory interactions between chlorimuron-ethyl and cadmium on biomass growth of wheat. The SPT values of cadmium for wheat decreased with an increase in the concentration of cadmium added to the tested soil. The accumulation of cadmium in wheat shoots, roots and glumes could be inhibited by chlorimuron-ethyl to some extent. There was an antagonistic interaction between chlorimuron-ethyl and cadmium on accumulation of cadmium in wheat.

Keywords Chlorimuron-ethyl · Cadmium · Combined pollution · Wheat *Triticum aestivum* L.

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Contamination of soil environment by heavy metals is being increased in both developed and developing countries since the Industrial Revolution (Zhou 1995; Cao et al. 2003; Zhou et al. 2004). Cadmium (Cd) is one of the most concerned heavy metals due to its strong toxic effects on human health and great mobility in soil environment and food chain (Zhou 1995; Wilson and Bell 1996; Zhou 2003). Moreover, the previous study by Guo and Zhou (2004) showed that Cd was an important pollutant occurred in phaiozem most frequently. According to Zhou et al. (2004), and Song et al. (2005), the concentration of Cd in some severe contaminated soils by mining, smelting and wastewater irrigation was higher than 100 mg kg^{-1}. In the Shenyang Smeltery, Cd accumulation in soil was up to 11.19–197.28 mg kg^{-1} (Cui et al. 2007). Simultaneously, the application of herbicides in the phaiozem area as one of the most important bases of agricultural production in China is becoming prevalent (Liang and Zhou 2003). In particular, chlorimuron-ethyl is widely used as a selective postemergence herbicide for the control of important broad-leaved weeds, such as cocklebur, pigweed, wild sunflower, and for soybeans and peanuts (Zou and Zhang 2001; Zhang et al. 2003; Wang and Zhou 2005). In that

way, the long-term field use of chlorimuron-ethyl has resulted in co-contamination of chlorimuron-ethyl and Cd in soil, surface water and groundwater, thus having an adverse effect on soil-plant systems (Ying and Williams 2000; Yen et al. 2003; Zhang et al. 2003). Much research about toxic effects of single Cd on growth and development of crops has been carried out (Leblova et al. 1986; Zheljazkov and Nielsen 1996), but it still lacks for knowledge about joint effects of chlorimuron-ethyl and Cd on crops until now. Thus, joint effects of chlorimuron-ethyl and Cd on biomass growth of crops and influences of chlorimuron-ethyl on Cd accumulation in crops were further examined using wheat (*Triticum aestivum* L) as the tested material for a typical agricultural crop, on the basis of our previous study on joint effects of chlorimuron-ethyl and Cd on germination rate and root elongation of wheat (Wang and Zhou 2005).

Materials and Methods

The tested phaiozem samples were collected from a field that has been not planted for more than a decade in the Hailun Station (47°26′N, 126°38′E) of Experimental Ecology, Chinese Academy of Sciences. The Hailun Station of Experimental Ecology located in the continental temperature monsoon zone, with a dry and cold winter and a warm and wet summer. The annual average temperature is about 1.5°C. The annual average precipitation ranges 500–600 mm.

Soil was sampled from the depth of 0–20 cm. The collected soil samples were air-dried and ground to pass through a 2 mm sieve. The initial soil pH (H_2O soil = 2.5) was 6.68, organic matter 4.79%, available N 238.5 mg kg^{-1}, available P 173.2 mg kg^{-1}, available K 189.9 mg kg^{-1}, total Cd 0.123 mg kg^{-1} and available Cd 0.0523 mg kg^{-1}. After base fertilizers including urea (0.15 g N kg^{-1} soil) and KH_2PO_4 (0.15 g P_2O_5 kg^{-1} dried weight (DW) soil and 0.1 g K_2O kg^{-1} DW soil) were added and equilibrated for 3 days, the air-dried soil was respectively, mixed with chlormuron-ethyl and Cd, filled into plastic pots (ϕ = 15 cm, and H = 10 cm), and equilibrated for 2 weeks. The soil humidity was adjusted to 60% of the water-holding capacity by adding deionized water, which was equivalent to the loss by evaporation determined by weighing the pots. In order to reduce evaporation, the soil was covered with plastic sheets. There were 1.2 kg air-dried soil samples in each pot.

All the treatments were replicated thrice to minimize experimental errors. The chemical form of Cd added to the tested soil was $CdCl_2 \cdot 2.5H_2O$. The tested concentration of Cd was 0, 5, 10, 30, 50 and 100 mg kg^{-1} dried weight (DW) soil. The tested herbicide chlorimuron-ethyl ($C_{15}H_{15}ClN_4O_6S$) as 20% of the dispersible granule was bought from the DuPont de Nemours & Co. in Shenyang, China. The tested concentration of chlorimuron-ethyl was 0, 10 (moderate quantity in agricultural use) and 30 μg kg^{-1} (excessive use in agricultural production) DW soil. All the reagents used in the experiment were of analytical grade.

The variety of the tested wheat (*Triticum aestivum* L.) was Liaoning Spring 10. In each pot, 8 wheat seeds were sown and 4 seedlings were remained and watered with tap water as required till their mature stage. The mature wheat was harvested on July 8, 2004. After they were thoroughly washed with tap water, the harvested plants were separated into roots, shoots, glumes and seeds (edible parts), roasted at 105°C for 15 min, and dried at 75°C in an oven. After having weighed them, these plant tissues were ground using a stainless carnelian grinder before the chemical analysis.

According to the standard methods suggested by Lu (2000), organic matter (OM) was determined by the $K_2Cr_2O_7$ oxidation method, available N was extracted with 1.0 N NaOH hydrolyzation diffusion method, available P was extracted with 0.5 M $NaHCO_3$ solution, available K was determined by NH_4OAC solution, and soil pH was measured at the soil to solution ratio of 1:2.5 in an aqueous suspension of soil. Dried plant samples were digested with a solution containing 87% concentrated HNO_3 and 13% concentrated $HClO_4$, and the concentration of Cd in the digested solution was measured using the atomic adsorption spectrophotometer (Hitachi 180–80, made in Japan).

The coefficient (SPT) of Cd transferring from soil to plants with/without the addition of chlorimuron-ethyl was calculated by dividing the concentration of Cd in a plant (mg kg^{-1} DW) by the concentration of Cd in soil (Rashmi et al. 2001), which was used to evaluate the influence of chlorimuron-ethyl on Cd accumulation in wheat. The data from the above experiments were statistically analyzed using the SPSS/PCCM software package. Statistical analyses included calculation of average values and standard deviation (SD). The multiple comparison procedure (LSDMOD) was used to compare the accumulation of Cd in wheat under different Cd treatments with/without the addition of chlorimuron-ethyl.

Results and Discussion

Soil pollution by single Cd had an adverse effect on reproductive growth of wheat *Triticum aestivum* L., because the dry biomass of wheat seeds decreased with increasing soil Cd, in particular, wheat became unfruitful when the concentration of Cd in soil was up to 30 mg kg^{-1}, as shown in Table 1.

Table 1 Biomass of wheat grown in soil polluted by only cadmium

Biomass (g DW)	Cd added (mg kg^{-1} DW soil)					
	0	5	10	30	50	100
Shoot	1.69 ± 0.10*a	2.00 ± 0.20a	1.66 ± 0.07ab	1.34 ± 0.11b	1.06 ± 0.07b	0.63 ± 0.08c
Root	0.42 ± 0.05a	0.47 ± 0.01a	0.44 ± 0.07a	0.27 ± 0.27b	0.19 ± 0.03bc	0.10 ± 0.02c
Glume	0.99 ± 0.07a	1.11 ± 0.07a	1.04 ± 0.06a	0.68 ± 0.04b	0.62 ± 0.06b	0.44 ± 0.08b
Seed	1.63 ± 0.11a	0.93 ± 0.26b	0.76 ± 0.07b	n	n	n

* Data were expressed as means ± standard deviation (SD); a means significant difference at $p < 0.05$ (LSDMOD), and n means unfruitful wheat in the treatment; letters besides means refer to the difference at the same concentration of chlorimuron-ethyl

The growth of wheat shoots, roots and glumes was obviously inhibited by the toxic effects of single Cd. The highest inhibition rates observed were 76.2% for roots, 62.7% for shoots and 55.6% for glumes, respectively, when the concentration of Cd in soil was up to 100 mg kg^{-1}. There were significantly negative relationships between the biomass of wheat shoots, roots and glumes and the concentration of Cd added to soil. The changing trends in wheat biomass by Cd stress can be expressed by following regression equations:

$$\mathrm{Y(shoot)} = 1.80 - 1.25\mathrm{E}-02C_{\mathrm{Cd}} \quad \left(R^2 = 0.865,\ \mathrm{n} = 18,\ p<0.01\right) \tag{1}$$

$$\mathrm{Y(root)} = 0.44 - 3.71\mathrm{E}-03C_{\mathrm{Cd}} \quad \left(R^2 = 0.825,\ \mathrm{n} = 18,\ p<0.01\right) \tag{2}$$

$$\mathrm{Y(glume)} = 2.04 - 2.01\mathrm{E}-02C_{\mathrm{Cd}} \quad \left(R^2 = 0.654,\ \mathrm{n} = 18,\ p<0.01\right) \tag{3}$$

where C_{Cd} is the concentration (mg kg^{-1}) of Cd in soil; Y (shoot), Y (root) and Y (glume) are the dry biomass (g) of wheat shoots, roots and glumes, respectively.

The biomass growth of wheat *Triticum aestivum L* was strongly inhibited by combined pollution of chlorimuron-ethyl and Cd in soil. As shown in Table 2, the dry biomass of wheat shoots, roots, glumes and seeds under the combined pollution of Cd and high chlorimuron-ethyl (30 μg kg^{-1}) was less than that under the condition of Cd combined with low chlorimuron-ethyl (10 μg kg^{-1}). The higher concentration of chlorimuron-ethyl in soil was, the greater inhibitory effects of Cd on biomass growth of wheat were. When the concentration of soil Cd was at the lower level, there was a bigger difference in biomass growth of wheat affected by various chlorimuron-ethyl concentrations; when the concentration of soil Cd was high, the difference in biomass growth of wheat affected by various chlorimuron-ethyl concentrations was small. For example, the inhibition rates of biomass growth were 1.8% for shoots, −4.8% for roots and −5.1% for glumes when the concentration of Cd in soil was 10 mg kg^{-1}, and the inhibition rates were 40.3% for shoots, 45.9% for roots and 50.0% for glumes when the concentration of chlorimuron-ethyl in soil was 10 μg kg^{-1} DW soil. However, the inhibition rates were 62.7%, 76.2% and 55.6% for shoots, roots and glumes, respectively, when the concentration of Cd in soil was 100 mg kg^{-1}, and the inhibition rates were 66.9%, 78.4% and 69.5% when the concentration of chlorimuron-ethyl in soil was 10 μg kg^{-1} DW soil. Obviously, there were synergistic effects of chlorimuron-ethyl and Cd on biomass growth of wheat.

When the concentration of chlorimuron-ethyl in soil was constant, the dry biomass of wheat shoots, roots, glumes

Table 2 Biomass changes in wheat stressed by combined pollution of chlorimuron-ethyl and cadmium

Biomass (g DW)	Chlorimuron-ethyl (μg kg^{-1})	Cd added (mg kg^{-1} DW soil)					
		0	5	10	30	50	100
Shoot	10	1.39 ± 0.21*a	1.24 ± 0.54a	0.83 ± 0.10ab	0.61 ± 0.02ab	0.62 ± 0.05ab	0.46 ± 0.13b
	30	1.04 ± 0.10a	0.51 ± 0.13a	0.46 ± 0.05a	0.50 ± 0.10a	0.33 ± 0.04b	0.32 ± 0.15b
Root	10	0.37 ± 0.08a	0.31 ± 0.09a	0.20 ± 0.04a	0.10 ± 0.02b	0.13 ± 0.01b	0.08 ± 0.03b
	30	0.25 ± 0.04a	0.12 ± 0.04a	0.09 ± 0.01a	0.09 ± 0.08a	0.06 ± 0.01a	0.06 ± 0.03a
Glume	10	0.82 ± 0.03a	1.01 ± 0.03a	0.41 ± 0.09b	0.33 ± 0.03b	0.31 ± 0.03b	0.25 ± 0.13b
	30	0.59 ± 0.09a	0.29 ± 0.10a	0.23 ± 0.02a	0.24 ± 0.04a	0.14 ± 0.01a	0.17 ± 0.11a
Seed	10	1.19 ± 0.07	0.27 ± 0.08	0.10 ± 0.01	n	n	N
	30	n	n	n	n	n	N

* Data were expressed as means ± standard deviation (SD); a means significant difference at $p < 0.05$ (LSDMOD), and n means unfruitful wheat in the treatment; letters besides means refer to the difference at the same concentration of chlorimuron-ethyl

Table 3 Correlation coefficients between added chlorimuron-ethyl/Cadmium and biomass of wheat shoots, roots and glumes

Pollutant	Wheat tissue			*p*
	Shoot	Root	Glume	
Chlorimuron-ethyl	−0.795**	−0.749**	−0.789**	<0.01
Cd	−0.687**	−0.710**	−0.661**	<0.01

and seeds decreased with increasing concentration of Cd in soil, respectively. Similarly, the dry biomass of wheat shoots, roots and glumes tended to be decreased with increasing concentration of chlorimuron-ethyl added to soil when the concentration of soil Cd was constant. Correlation coefficients between dry biomass of wheat shoots, roots and glumes and the concentration of chlorimuron-ethyl and Cd in soils were listed in Table 3. It was shown that dry biomass affected by chlorimuron-ethyl was more significantly than Cd. This was consistent with the result by Wang and Zhou (2005) that the effect of chlorimuron-ethyl on the germination of wheat seeds and the elongation of wheat shoots and roots was more obvious than Cd.

A highly significant correlation relationship between the accumulation of Cd in wheat *Triticum aestivum* and the concentration of Cd added to the test soil was observed (Fig. 1). The concentration of Cd accumulated in wheat shoots, roots and glumes increased with increasing soil Cd. In particular, when the concentration of Cd added to the tested soil was up to 30 mg kg^{-1}, wheat plants could not fructified. In other words, it can not only produce inferior wheat seeds containing Cd, but also result in the zero yield of wheat production when severe soil Cd pollution occurs. It was also shown that Cd accumulation in roots was higher than that in other tissues of wheat, accounting for 70%–90% of total Cd accumulation in wheat. On the contrary, Cd accumulation in wheat seeds was the lowest in various parts of wheat, only 0.32–6.85 mg kg^{-1}. The changing trend was consistent with some previous reports (Salt et al. 1997; Rashmi et al. 2001; Zhou et al. 2003; Guo and Zhou 2004). Perhaps, the distribution mode of Cd in wheat tissues should ascribe to self-protecting mechanisms of wheat plants resisting toxic substances.

The SPT values (Table 4) of Cd in wheat decreased with an increase in the concentration of Cd added to the tested soil. In other words, the ability of Cd transferring from soil to wheat tissues was becoming weaker with an increase in soil Cd, and wheat roots appeared to be able to restrict Cd uptake at the high soil Cd level. On the one hand, it may be ascribe to the damnification of roots when high concentration of Cd was added to the soil, and then wheat plants decreased the ability of absorbing Cd from soil, on the other hand, perhaps, wheat plants can reduce the uptake of Cd through the storage of Cd in root cortex lumens and/or

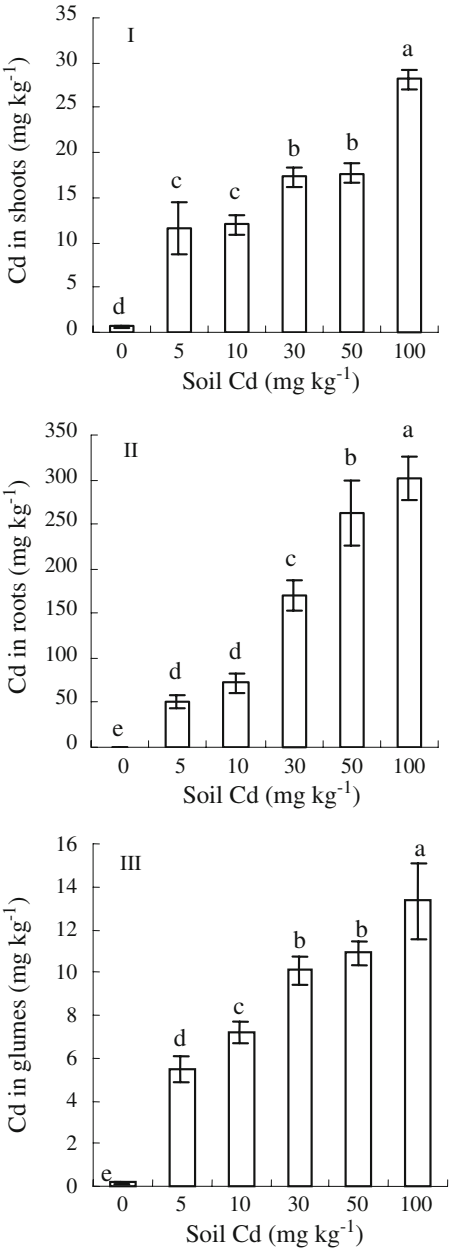

Fig. 1 The accumulation of Cd in wheat shoots (**I**), roots (**II**) and glumes (**III**) when exposed to single Cd. *Bars* with the *same letter* were not different at the 0.05% level. *Error bars* represent SD

by way of the physical barrier due to the formation of the thick mucilaginous layer over a root cap (Tereza et al. 2002). This further validated that there are self-protecting mechanisms of wheat plants resisting toxic substances.

The concentration of Cd accumulated in wheat shoots, roots and glumes when treated with chlorimuron-ethyl was lower than that without chlorimuron-ethyl (Fig. 2). The concentrations of Cd accumulated in wheat shoots, roots and glumes were 9.47, 49.70 and 5.86 mg kg^{-1}, respectively, when no chlorimuron-ethyl was added to soil, but the concentrations of Cd accumulated in wheat shoots,

Table 4 The SPT values of cadmium in wheat plants treated with/without chlorimuron-ethyl

Treatment (Cd + CE)	CK	5 + 0	5 + 10	5 + 30	10 + 0	10 + 10	10 + 30	30 + 0
SPT	4.12	2.96	1.91	1.62	1.85	1.35	1.30	1.11
Treatment (Cd + CE)	30 + 10	30 + 30	50 + 0	50 + 10	50 + 30	100 + 0	100 + 10	100 + 30
SPT	0.92	0.97	0.81	0.85	0.63	0.46	0.53	0.57

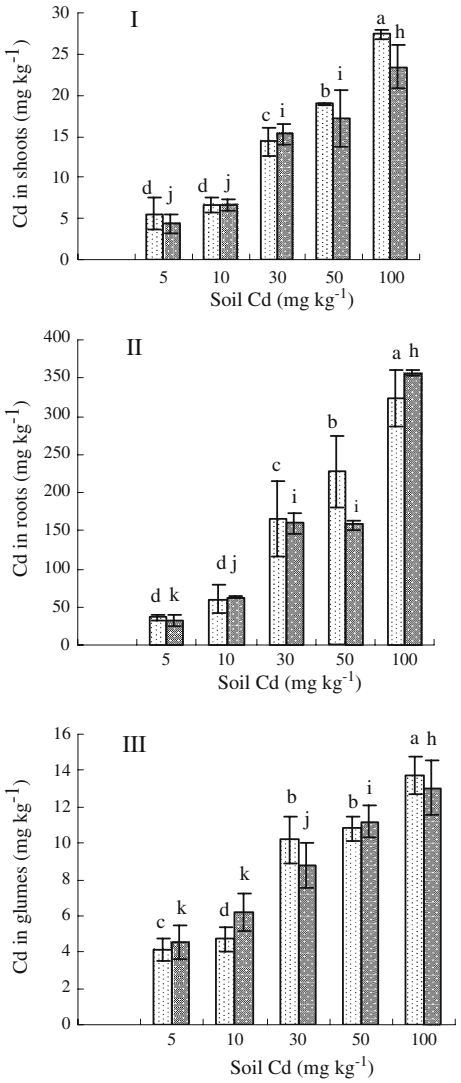

Fig. 2 The accumulation of Cd in wheat shoots (**I**), roots (**II**), and glumes (**III**) when exposed to Cd and chlorimuron-ethyl. *Bars* with the *same letter* were not different at the 0.05% level (*a–d* for CE 10 μg kg^{-1} and h-k for CE 30 μg kg^{-1}). *Error bars* represent SD. CE means chlorimuron-ethyl

roots and glumes were 5.14, 34.74 and 3.44 mg kg^{-1}, respectively, when the concentration of chlorimuron-ethyl in soil was 10 μg kg^{-1}. Moreover, the concentration of Cd in wheat shoots, roots and glumes treated with high chlorimuron-ethyl (30 μg kg^{-1}) was lower than that treated with low chlorimuron-ethyl (10 μg kg^{-1}). In other words,

Table 5 Correlation coefficients between cadmium accumulated in wheat shoots, roots and glumes and chlorimuron-ethyl added to the tested soil

Wheat tissue	Soil Cd (mg kg^{-1})				
	5	10	30	50	100
Shoot	−0.748*	−0.716*	−0.381	−0.167	−0.817**
Root	−0.721*	−0.273	−0.176	−0.842**	0.725*
Glume	−0.350	−0.197	−0.531	0.225	−0.139

* Significant level at $p < 0.05$ (1-tailed)

** Significant level at $p < 0.01$ (1-tailed)

chlorimuron-ethyl could inhibit Cd accumulation by wheat plants to a certain extent. However, the inhibition was not directly related to the concentration of chlorimuron-ethyl added to the tested soil, although some negative correlation relationships between Cd accumulated in roots and shoots and the concentration of chlorimuron-ethyl added to the tested soil was observed when soil Cd was constant ($p < 0.05$ or $p < 0.01$) (Table 5). Similarly, the SPT values of Cd were also affected by the addition of chlorimuron-ethyl. The SPT values of Cd for soil-wheat systems treated with chlorimuron-ethyl were lower than those without the addition of chlorimuron-ethyl (Table 4). This further testified that there were antagonistic effects between chlorimuron-ethyl and Cd on accumulation of Cd in wheat tissues.

Cd interacts with SH-groups that are essential for the enzyme tertiary structure, and affects enzyme conformation. Moreover some compounds or other ions can displace Cd^{2+} bound to SH-groups (Zhou et al. 2004). Chlorimuron-ethyl has quick movement towards the enzymes containing SH-groups and rapid reaction with them by displacing Cd^{2+} bound to SH-groups. Thus, the reason of inhibiting Cd accumulation by chlorimuron-ethyl may be the competition of chlorimuron-ethyl with Cd^{2+} bound to SH-groups.

Although there is a beneficial role in inhibiting Cd accumulation in wheat, chlorimuron-ethyl can exert its toxic action on the growth of wheat plants. When the concentration of chlorimuron-ethyl added to the tested soil was up to 30 μg kg^{-1}, no wheat seeds were harvested. Chlorimuron-ethyl can inhibit the formation of wheat seeds, because it is a branched-chain amino acid synthesis

(acetolactate synthase) inhibitor (Wang and Zhou 2005) and may act by inhibiting the biosynthesis of the essential amino acids valine and isoleucine, hence inhibiting plant cell division of rapidly growing tips of roots and shoots and stopping plant growth.

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