

Effects of Chloride and Co-Contaminated Zinc on Cadmium Accumulation within *Thlaspi caerulescens* and Durum wheat

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Cadmium is a potentially toxic heavy metal with no known benefit to humans. Soils are the main source of Cd in plants, and plant-derived foods are the main source of Cd in human diets. Cadmium is present in all soils, usually as a trace constituent. The plant availability of Cd in soil is determined by the characteristics that affect the availability of most trace metals. These characteristics include plant species, the concentration and chemical forms of metal in the soil, pH, organic matter content, clay content, its interactions with other elements, and fertilizer practices. In addition, an increase in Cl concentrations in the soil or soil solution was shown to increase Cd concentration in plants in laboratory studies, such as Swiss chard, sunflower, potato, kenaf, sorghum and wheat (Smolders et al. 1997; Hattori et al. 2006). Under field conditions, Cd concentrations in plants were found to positively relate to Cl concentration in soil as well (Norvell et al. 2000; Wu et al. 2002). Chloride is known to reduce soil sorption of Cd, probably due to the fact that chloride forms relatively

strong complexes with Cd depending on solution Cl^{-1} concentration. Chloro-Cd complexation and the resulting improved diffusion of Cd through the soil to plant roots and possibly increased uptake of Cd-chloro complexes are suspected to be the reasons for the Cl effect on Cd uptake (McLaughlin et al. 1997). Unlike Cd, Zn is an essential nutrient both for plants and animals, but if present in high concentration in plants and animals it can also be highly toxic. It is generally accepted that Zn status in soils and plants plays an important role in Cd accumulation in crop plants. Generally, Zn application decrease Cd uptake and accumulation in plants (Jiao et al. 2004; Zhang and Song 2006), which may be caused by Cd and Zn competitive adsorption in soils and absorption by roots as well as an interaction in the transport system of plant (Hart et al. 2002). However, the effects of Zn application on Cd uptake and accumulation in plants are not consistent. Nan et al. (2002) showed that increases in Zn application enhanced Cd concentration in wheat or vice versa. Similar observations were also made by other researchers (Wu and Zhang 2002). Most of studies under actual field situations, soil environments are often contaminated with multiple heavy metals, such as Cd, Zn, Pb, etc. The study of the Cd accumulation influenced by the interaction between these ions is of practical importance.

Durum wheat is an important and valuable crop in many parts of the world that can accumulate Cd to approach or exceed the toxic concentration limits in some places. Durum wheat is a non-accumulator, but *Thlaspi caerulescens* is a hyperaccumulator of Cd and Zn that can accumulate more than 100 mg Cd kg^{-1} in their leaves (Baker et al. 1994). Recently, there has been considerable interest in the use of *Thlaspi caerulescens* as a green technology for phytoremediation of surface soils contaminated with toxic

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heavy metals Cd and Zn. The phytoremediation as an environmental cleanup technology is complex. We know little about the effect of soil salinity (mainly chloride) on Cd accumulation by hyperaccumulator (*T. caerulescens*) which is practically very important in phytoremediation. The current experiment addressed the following questions: (1) Is there chloride effect on Cd accumulation in hyperaccumulator plant species such as *T. caerulescens*? (2) Is the chloride effect restricted to freshly contaminated soils by Cd? (3) Does a co-contamination with Zn modulate or minimize the chloride effect on Cd accumulation? The understanding of these questions is helpful to control Cd transport from soil to non-hyperaccumulator such as durum wheat and to promote phytoremediation of heavy metal polluted soils.

Materials and Methods

This experiment was conducted at the Institute of Plant Nutrition, Hohenheim University, Germany. NBG soil (Neckar River dredge sludge “Neckarbaggergut”), a Cd and Zn polluted soil and “Filderlehm” soil from Filderstadt and Stuttgart were used in this pot experiment. The selected soil chemical characteristics are shown in Table 1. Chloride was added into NBG soils as KCl, at 0, 0.32, 1.28 and 5.10 g KCl kg⁻¹ soil, corresponding to 0, 0.15, 0.61 and 2.43 g Cl kg⁻¹ soil, respectively. Four similar chloride rates and three Cd rates of 0, 0.2 and 1.0 mg Cd kg⁻¹ soil as freshly polluted soil were added into Filderlehm soil. In a separate experiment, the Filderlehm soil was treated with 0.2 mg Cd + 4 mg Zn and 1.0 mg Cd + 20 mg Zn kg⁻¹ soil, and each combination of Cd and Zn treatment was treated with 4 Cl⁻¹ stated above. Inorganic salts 3CdSO₄·8H₂O and ZnSO₄·7H₂O were used as Cd and Zn sources. All soils were sieved through 2mm and mixed completely with Cd, Zn and N, P, K and Mg nutrient solution that each pot received 200 mg N kg⁻¹ soil as Ca(NO₃)₂, 100 mg P kg⁻¹ soil as KH₂PO₄ and 100 mg Mg as MgSO₄·7H₂O, then sieved through 2 mm again. An amount of 1,600 and 140 g soil were added to 96 large and 96 small pots, respectively, in two steps to make the soil density at about 1.3 g cm⁻³. After 7 days incubation durum wheat (*Triticum durum* Desf. cv.Cakmak) and *Thlaspi caerulescens* J. & C. Presl were planted.

Table 1 Selected soil chemical characteristics

Soil	pH	Available Cd µg kg ⁻¹	Available Zn µg kg ⁻¹	Total Cd mg kg ⁻¹	Total Zn mg kg ⁻¹
NBG	7.6	69	217	21.2	590
Filderlehm	7.2	10	22	2.8	90

Twelve seeds per pot of durum wheat were sown into plastic pots directly (containing 1,600 g soil). After 12 days, six wheat seedlings were kept for each pot. The pots with durum wheat were put in the greenhouse under supplemental light (10 h lighting every day). Three seedlings of *T. caerulescens* (Avineres ecotype) (after 3 weeks preculture) were transplanted into each pot. The pots of *T. caerulescens* were put in the growth chamber for controlling temperature and light (27°C, 10 h lighting). Every day the weights of each pot with both plants were measured and distilled water was added to keep the soil water content at about 60% of maximum water holding capacity of the soil. After 20 days growth, the chloride solution of different rates was added to wheat soil. Ten days after transplanting, the chloride solution of different rates was added to *T. caerulescens* soil.

After 48 days growth, the plants of durum wheat and *T. caerulescens* were harvested and oven dried for analyses. Total Cd and Zn concentrations, both in soil and plant samples (digested 2 h at 500° by aqua regia, a mixture of HNO₃:HCl=11:39 in volume), were determined using flame atomic absorption spectrometry (Unicam AAS 939) (Zeien and Brümmer 1989). Available Cd and Zn in soil extracted by 1M NH₄NO₃ (shaking with medium speed for 24 h, centrifuging 10 min at 2,500 rpm) were measured by an ICP-MS (Perkin Elmer Elan 6000) (Gryschko et al. 2005). Analysis of variance was performed using the SigmaStat build 2.03.0.

Results and Discussion

The high level of chloride application had the negative effect on both durum wheat and *Thlaspi caerulescens* growth significantly (Figs. 1, 2). As the Cl application rate up to 2.43 g kg⁻¹ soil within no Cd added, the shoot dry weight of durum wheat was significantly lower than those of other three Cl treatments, which was only about one third of the no Cl application. The addition of Cd/Zn had no significant effect on the wheat growth in Filderlehm soil. Wheat growth in NBG soil suffered a significant inhibition despite of Cl rate application, which could be the toxicity of Cd and Zn. The application of highest rate Cl also caused the biomass decrease in *T. caerulescens*. The shoot dry weight of 2.43 g Cl kg⁻¹ treated soil was only about one tenth of the no Cl application within no Cd application in *T. caerulescens*. The increasing of Cd/Zn applied in soil did not result in a significant difference of shoot dry weight in *T. caerulescens*. The dry weights of *T. caerulescens* shoots grown in NBG soil were higher than those in soils freshly polluted by Cd and Zn. Compared with durum wheat, *T. caerulescens* was more susceptible to high salinity but more tolerant to the exposure of high Cd concentration.

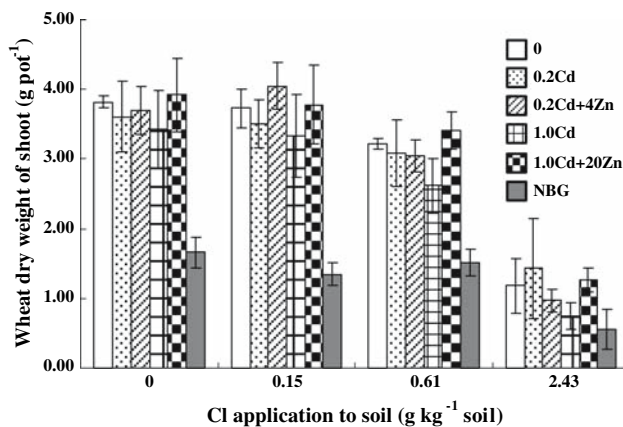


Fig. 1 Effect of different chloride, cadmium and zinc application on dry weight of durum wheat shoots grown in Filderlehm and NBG soil under greenhouse conditions. Error bars indicate SD ($n = 4$)

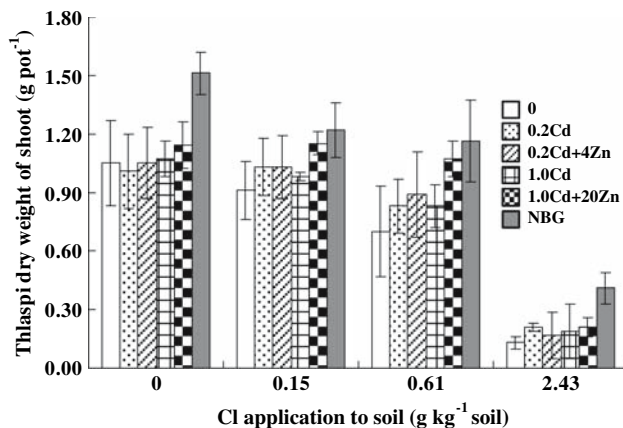


Fig. 2 Effect of different chloride, cadmium and zinc application on dry weight of *T. caerulescens* shoots grown in Filderlehm and NBG soil under greenhouse conditions. Error bars indicate SD ($n = 4$)

The addition of Cl resulted in a significant ($p < 0.001$) increase in Cd concentrations in shoots for both plants (Figs. 3, 4). The Cd concentration increased from 0.86 to 1.42 mg kg⁻¹ in wheat shoots and from 1.81 to 13.75 mg kg⁻¹ in *T. caerulescens* shoots by the Cl application with no Cd addition, which evidently displayed the promotive effect of Cl on Cd uptake both in hyperaccumulator and non-hyperaccumulator plant species. As external Cd addition rate up to 0.2 mg kg⁻¹, the Cd concentration in wheat shoot under the treatment of 2.43 g Cl per kilogram soil was about three times as high as that under no Cl application. Under 1.0 mg kg⁻¹ Cd application, the Cd accumulation in wheat also increased with the increasing of Cl application except for the highest Cl rate treatment that the Cd concentration was a little lower than that of 0.61 g Cl kg⁻¹ treatment. This may be due to the excess salinity inhibited plant growth so that the biomass was the lowest (Fig. 1), thus decreased uptake of Cd. With

the Cl application, the Cd concentration in *T. caerulescens* shoots grown in Filderlehm soil increased linearly with Cd addition. In addition, the Cd concentrations in *T. caerulescens* shoots were much higher than those in durum wheat at the same Cd and Cl level. Within no Cd application, the Cd concentration in *T. caerulescens* shoots of the treatment with 2.43 g Cl kg⁻¹ added was almost eight times as high as that of no Cl treatment, but the Cd concentration in durum wheat at the same instance just increased by 65%. Under the highest Cd application, the Cd concentration of *T. caerulescens* shoots increased by 126% with the increasing of Cl rates from 0 to 2.43 g per kilogram soil, but the Cd concentration in durum wheat shoots only increased 30%. The Cl effect on Cd uptake may be attributed to the elevated availability of Cd in soils by Cl application (Wegglar et al. 2004). *T. caerulescens* could take up much more Cd into the shoots because of its hyperaccumulation characteristics for Cd (Baker et al. 1994). According to our results, the Cl enhancing effect on Cd uptake is more evident in Cd-hyperaccumulator than non-hyperaccumulator species. Gérard et al. (2000) found that *T. caerulescens* and non-hyperaccumulator maize accessed the same available pool of Cd. It suggests that the mechanisms of the Cl enhancing effect on Cd accumulation in durum wheat be applicable in hyperaccumulator species, although the mechanism is not well understood.

The Cd concentration in the wheat shoots under the treatment of 0.2 mg kg⁻¹ Cd + 4 mg kg⁻¹ Zn was not significantly different from the treatment of 0.2 Cd mg kg⁻¹ at all Cl levels (Fig. 3). At the high Cd treatment of 1.0 mg kg⁻¹ Cd, the addition of 20 mg kg⁻¹ Zn resulted in decreased Cd concentration in shoots at no Cl addition and increased Cd accumulation in shoots under the treatment of 2.43 g Cl kg⁻¹. The inconsistent results in literature may be attributed to the competitive absorption by Zn and the promotive effect of high Cl application (Nan et al. 2002; Ozkutlu et al. 2007). The Cd concentrations of wheat shoots grown in NBG soil, which were 23.6–33.9 mg kg⁻¹, were much higher than those grown in Filderlehm soil. With the increasing of Cl rates applied, significant increase of Cd accumulation in wheat shoots were obtained, except for the highest Cl rate treatment that the Cd concentration was a little lower than that of 0.6 g Cl kg⁻¹ application, probably because of the high salinity (or Cl⁻¹) limitation to plant growth. At all the Cl levels, the addition of 4 mg kg⁻¹ Zn + 0.2 mg kg⁻¹ Cd and 20 mg kg⁻¹ Zn + 1.0 mg Cd kg⁻¹ did not result in significant changes of Cd accumulation in *T. caerulescens* shoots. Therefore, the co-contamination with Zn did not alleviate the Cl effect on the increasing accumulation of Cd in hyperaccumulator *T. caerulescens*, although *Thlaspi* could hyperaccumulate both Cd and Zn. But, the Cl application did not result in a significant difference in Cd concentration in *T. caerulescens* shoots grown in NBG soil with the increasing

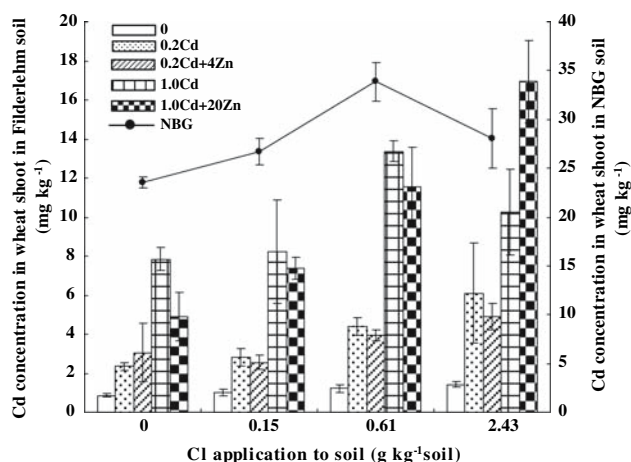


Fig. 3 Effect of different chloride, cadmium and zinc application on Cd concentrations of durum wheat shoots grown in Filderlehm and NBG soil under greenhouse conditions. Error bars indicate SD ($n = 4$)

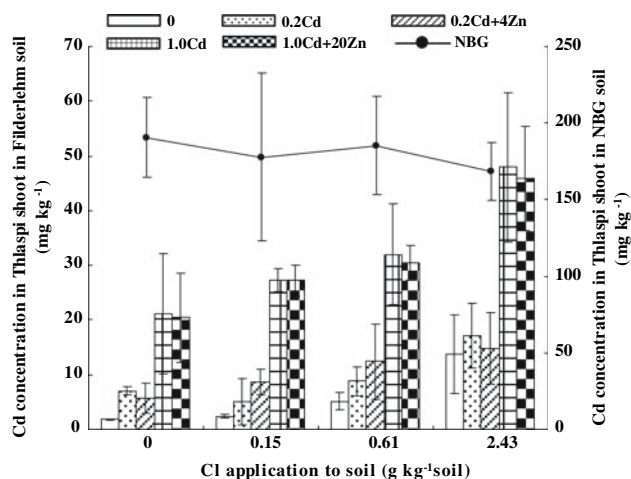


Fig. 4 Effect of different chloride, cadmium and zinc application on Cd concentrations of *T. caerulescens* shoots grown in Filderlehm and NBG soil under greenhouse conditions. Error bars indicate SD ($n = 4$)

of Cl rate supplied (Fig. 4). The NBG soil used in this experiment contained much available Cd for plant uptake. So, the reason of no effect of Cl on Cd accumulation in *T. caerulescens* grown in NBG soil probably was that *T. caerulescens* could accumulate Cd to around 200 mg kg^{-1} that was 5–8 times as compared with those in durum wheat shoots. In conclusion, the enhancing effect of Cl on elevated Cd accumulation probably was masked by the huge Cd concentrations in *T. caerulescens* shoots grown in NBG soil containing much higher level of Cd.

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