Neighbour effects of purslane (*Portulaca oleracea* L.) on Cd bioaccumulation by soybean in saline soil

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ABSTRACT

Bioaccumulation of heavy metals can be affected by various crop-weed interactions in agroecosystems. An experiment was conducted to evaluate the role of belowground interaction of soybean and purslane (*Portulaca oleracea* L.) weed on cadmium (Cd) uptake and its allocation to soybean grains. The experimental treatments included two cropping systems (mono and mixed culture), two salinity levels (0% and 0.5% NaCl) and three levels of Cd in soil (control; 3 and 6 mg Cd/kg). Results showed that the promoting effect of salinity on Cd uptake by soybean and Cd allocation to grains was enhanced in the presence of purslane compared to the absence of neighbour plant. This could be due to increasing Cd-mobilization within the shared rhizosphere of plants. In the non-saline soil decreasing uptake and grain allocation of Cd in co-planted soybean was associated with enhancing of purslane Cd uptake and the depletion of Cd in soil solution. Therefore, it can be concluded that co-planted purslane can alter the uptake of cadmium to the neighboring soybean plants; its effect may be influenced by soil environmental conditions such as salinity.

Keywords: cadmium; co-planting; grain; plant interaction; salinity

The contamination of agricultural soils by heavy metals may alter the belowground interactions among plants (Su et al. 2008). Crop roots are in contact with neighboring root systems with different uptake status of heavy metals. However, under some circumstances, the co-occurrence of crops and weeds may result in influx of heavy metals to the human food chain. Accordingly the interactions among crops and weeds in contaminated rhizosphere have attracted novel attention in controlling heavy metal contamination of the food chain.

Root-induced changes in rhizosphere largely influence element dynamics and in turn the mineral nutrition of plants (Li et al. 2009). Local rhizosphere situations of metal accumulator weeds may change the uptake of heavy metals by adjacent crops in contaminated fields. In some cases accumulator weeds may not only enhance metal uptake by neighboring plants (Gove et al. 2002, El Mehdawi et al. 2011), but their coexistence might also alter heavy metal allocation in the different organs of crop plants (i.e. phytoenrichment). This may potentially be a serious problem when the changes in metal allocation can lead to an increase in the accumulation of heavy metals in edible parts of crop plants. Instead, it may be possible that accumulators protect companion plants by depletion of heavy metals in shared rhizosphere (Whiting et al. 2001b, Su et al. 2008). For instance, in the study of Whiting et al. (2001b), co-planting of *Thlaspi caerulescens* L. (a metal hyperaccumulator plant species) increased the shoot mass of *Thlaspi arvense* L. (a non-accumulator species) by 30% when grown in a soil containing toxic concentrations of zinc.

In fact the contaminant-rhizosphere interaction plays a key role in controlling trace element contamination of the food chain and can be considered as a hot spot in food health (Singh et al. 2011). Thereby, critical density of accumulator weeds in a field may be changed based on their effects on food safety. Accordingly, the knowledge of crop-weed relationships in the presence of heavy metals may provide some valuable information for more efficient ecologically-based weed management systems.

Cadmium (Cd) as a toxic heavy metal may be absorbed by some crops, translocated into edible parts and threats food safety. The mobility and bioavailability of cadmium is dependent on its concentration in soil and other soil factors such as salinity (Acosta et al. 2011). Salinity affects uptake and accumulation of heavy metals in plants, either by increasing metal solubility or altering root functions (Huang et al. 2007, Acosta et al. 2011). Thus, interaction of Cd and salinity should be taken into consideration where both stresses are expected to impact crop growth and yield.

In the current study, soybean and purslane were chosen in a co-planting culture. Due to inherent genetic and physiological characteristics, soybean was considered as main plant in mixed cultures. This crop was shown to accumulate higher concentrations of Cd in grains compared to other crops (Arao et al. 2003). Purslane (*Portulaca oleracea* L.), an accumulator weed species (Tiwari et al. 2008) widely distributed in temperate environments, often co-occurs with many thermophile crops such as soybean.

There is little information on the possible effects of plant heavy metal accumulation on crop-weed interactions. We hypothesized that the efficient removal of Cd from soil solution by Cd-accumulator purslane might change the Cd uptake of co-planted soybean. Therefore, the aim of this study was to investigate the influence of co-occurred purslane sharing rhizosphere with soybean on changing of Cd uptake and allocation by the crop under different salinity and Cd levels.

MATERIAL AND METHODS

Soil preparation. A clay loamy soil was taken from the top 20 cm layer of a crop field located at the west of Esfahan, Iran. The soil was air dried, sieved to < 10 mm and analyzed for its total Cd content and other physical and chemical properties (Table 1). The soil was treated with spraying deionized water and $Cd(NO_3)_2 \cdot 2\,H_2O$ solution to obtain Cd0, Cd3 and Cd6 treatments containing background level, 3 and 6 mg Cd/kg cadmium, respectively. Prior to planting, all soil samples were incubated at approximately 60% water-holding capacity for 6 weeks. Half of the spiked soil was treated by addition of 0.5% w/w NaCl. The electrical conductivity of soil was experimentally determined by saturated paste of soil which was almost 5 dS/m. Since there was no leaching from the pots during the experiment and the electrical conductivity of tap water used for irrigation was about 0.15 dS/m, the change of soil salinity was therefore negligible.

Plant cultivation. The pot experiment was carried out in outdoor condition in College of Agriculture, Isfahan University of Technology, Iran, during June–September 2011, using a soybean cv. Habit and a purslane ecotype as a common weed in the soybean fields of Isfahan. The crop that occurs and weed cultures included sole planting of soybean, sole planting of purslane, co-planting of both species and a non-planted treatment. The experiment design was a completely randomized design in a factorial scheme with four replications.

Pots of the sole culture were filled with 2.5 kg while pots of mixed culture were filled with 5 kg of related treated soils in order to reach equal soil amount per plant. To avoid any intraspecific competition in mono culture, soybeans were planted in half of soil applied to mixed culture. Seeds of soybean and purslane were cleaned with 1% commercial bleach for 5 min, washed with distilled water and germinated in moist sterile sand. After 10 days, three same size seedlings of soybean and

Table 1. Selected characteristics of the soil sample used in the experiment

Clay	Sand	Silt	Organic matter	pH (1:2.5)	Electrical conductivity (dS/m) (1:2.5)	N (g/kg)	P	Total Cd	Total Zn
(%)							(mg/kg)		
Hydrometer method			Ignition method			Kjeldahl method	Olsen's method	*Aqua regia* extraction method	
47	17	36	1.32	7.4	0.6	1.4	16	0.56	25

purslane were transplanted to the related pots of different soil treatments. Seedlings were sown at fixed spots according to a predefined design so that soybean and purslane plants always occupied the similar position (Figure 1). This arrangement could prevent probable differences among the treatments that could be confounded by neighbour interactions (Rinaudo et al. 2010). Pots were irrigated every day with tap water (with no cadmium) to retain the level of soil water closed to the field capacity.

Sampling and measurements. Plants were harvested after 15 weeks of transplanting. Each individual plant was removed from the soil separately. The aboveground plant tissues were rinsed with deionized water. Roots were quickly separated from the adhering soil by shaking and then were cleaned using tap water to remove parts of roots from other plants. The rhizosphere soils (i.e. strongly adhering soil) after air-drying were prepared for subsequent analysis. Separated roots were sonicated in 5 mmol $CaCl_2$ for 10 min in an ultrasonic bath (Transsonic T460/H, Elma, Germany) and rinsed with deionized water (Rivelli et al. 2012). In order to determine dry weight of plant tissues, all roots and shoots samples of both species and also grain samples of soybean were dried separately at 65°C in a ventilated oven and periodically weighed until there was no change in dry mass. Dried plants samples were ground in a stainless mill. Subsamples of 0.5 g were then digested in 4 mL HNO_3 and 1 mL $HClO_4$ at 225°C using an automated heating block (Digester DK 42/26, Velp Scientifica, Milano, Italy) (Wieshammer et al. 2007).

After separating rhizosphere by shaking soils, 20 g fresh subsamples were shaken with 50 mL of 1 mol/L NH_4NO_3 (1:2.5) at 20°C for 1 h. The mixture was immediately filtered through a Whatman 42 filter paper and acidified with concentrated HNO_3. Ammonium nitrate extraction was used as a measure of the concentration of labile Cd in the soil. Metal concentrations in the plant digests and soil extracts were determined by graphite furnace atomic absorption spectrometry (Perkin Elmer 2100, Wellesley, USA). In order to make sure about digestion and analysis, the results were validated through standard reference materials and blank samples (BCR-482, LGC Promochem, Teddington, UK; mean recovery rates of Cd: 99.1%).

Statistical analysis. Statistical analysis was performed by the SAS program software (Release 9.1, SAS Institute, Cary, USA). All treatments were tested with three-way analysis of variance (ANOVA). The difference between the means of treatments was identified using the *LSD* test ($P < 0.05$). Linear regression analysis was conducted to determine the relationships between the Cd concentrations in the soybean and the dry biomass.

RESULTS AND DISCUSSION

Plant biomass. The total biomass of soybean in all treatments was decreased as a result of increasing level of cadmium in soil (Figure 2a). The biomass of soybean was decreased by salinity in all treatments as it was expected. However, in mixed cultures rather a lesser effect of salinity on the crop biomass production appeared than in mono cultures. The effect of purslane on soybean biomass decrease in saline soil can be attributed to a possible protective role of neighbour plant when exposed to salinity. The higher biomass produced by co-planted soybean under Cd6 compared to mono culture were likely a result of greater competitive ability of soybean for resources in severe stress condition, rather than the mobilization of nutrients by purslane. In addition, Rinaudo et al. (2010) suggested that mycorrhizal host plants were able to access complementary resources when co-planted with non-mycorrhizal plant species. According to Wang and Qiu (2006) purslane is a non-mycorrhizal plant.

Total content of Cd. The content of Cd in soybean was significantly enhanced in all treatments

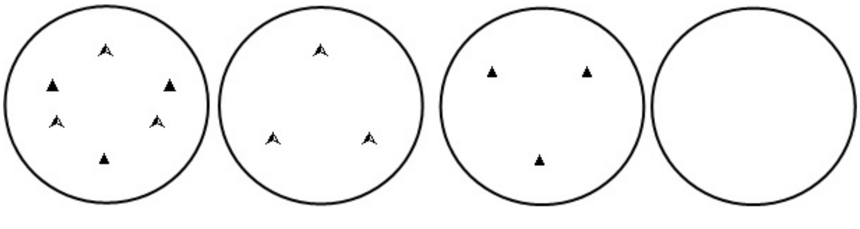
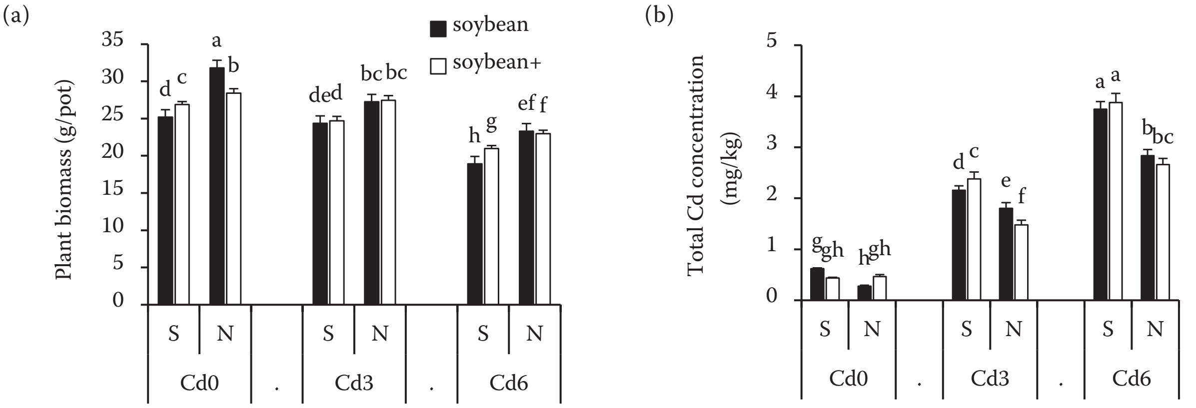


Figure 1. Experimental design of plant position cultured in pots (▲ = soybean and Δ = purslane)



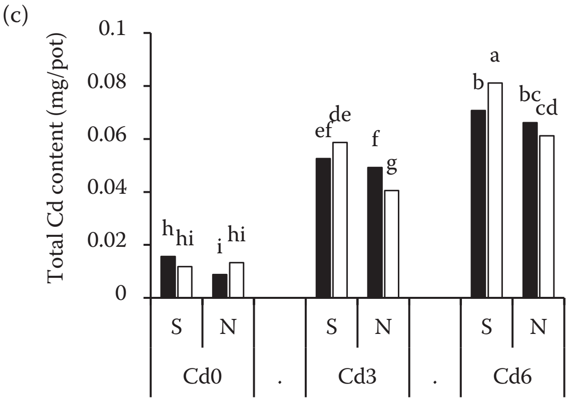


Figure 2. Biomass (a), concentration of Cd (b), and Cd accumulated (c) in soybean plants grown in pots containing mono and mixed culture (soybean and soybean+). Cd0 – control; Cd3 – 3 mg Cd/kg; Cd6 – 6 mg Cd/kg; S – saline soil; N – non-saline soil. Data are presented as means + SE, $n = 4$. Significant ANOVA values are shown by letter codes. Values showing the same letter are not significantly different (*LSD* test, $P > 0.05$)

as the level of Cd was increased in soil (Figure 2c). Salinity in the contaminated soil increased the Cd content of soybean in mixed culture while it had no significant effect on relevant mono culture. Ghallab and Usman (2007) reported that higher Cd uptake under saline conditions was attributed to the formation of Cd-Cl complexes that make Cd more available in soil. However, in some cases, a changeless Cd content was also reported in plants grown in saline soils (Xu et al. 2010, Sari and Din 2012).

The decrease of Cd content in co-planted soybean under non-saline treatments compared to soybean grown alone was a consequence of the concentration diminution rather than plant biomass change.

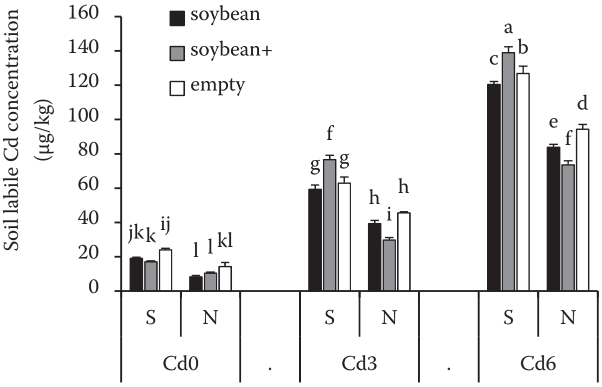


Figure 3. Soil labile Cd concentration from pots containing soybean alone (soybean), soybean grown with purslane (soybean+) and no vegetation (empty). Cd0 – control; Cd3 – 3 mg Cd/kg; Cd6 – 6 mg Cd/kg; S – saline soil; N – non-saline soil. Data are presented as means + SE, $n = 4$. Significant ANOVA values are shown by letter codes. Values showing the same letter are not significantly different (*LSD* test, $P > 0.05$)

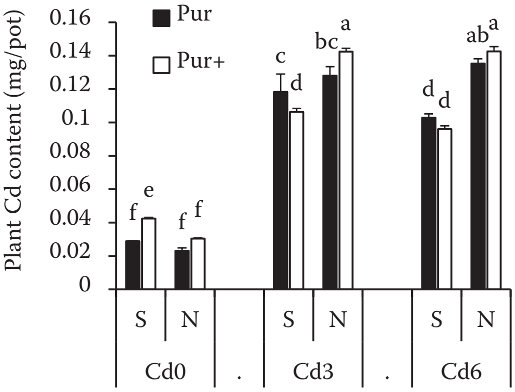


Figure 4. Accumulation of Cd in purslane plants grown in pots containing mono and mixed culture (purslane (Pur) and purslane+ (Pur+)). Cd0 – control; Cd3 – 3 mg Cd/kg; Cd6 – 6 mg Cd/kg; S – saline soil; N – non-saline soil. Data are presented as means + SE, $n = 4$. Significant ANOVA values are shown by letter codes. Values showing the same letter are not significantly different (*LSD* test, $P > 0.05$)

This reduction of Cd content can be confirmed by reduced concentration of Cd (depletion) in the soil solution (Figure 3) and increase in Cd uptake by adjacent purslane compared to mono culture of it (Figure 4). These results were also reported by other researchers (Whiting et al. 2001b, Su et al. 2008).

Besides, the salinity can modify interactions among plant species (Greenwood and MacFarlane 2009). Under saline condition, the amount of Cd accumulated by co-planted soybean was increased compared to soybean grown alone (Figure 2c). This increase at Cd6 was a function of larger plant biomass rather than an increase in the concentration of Cd in plant tissues (Figure 2a). The enhancement of Cd uptake by co-planted soybean in contaminated soils under saline condition might be attributed to the increase of the labile Cd concentration pool (Phytoenrichment) in the shared rhizosphere (Figure 3) and reduction of Cd uptake by co-occurred purslane (Figure 4). Ondrasek et al. (2012) reported that salinity, induced by NaCl, increased Cd solubility in the rhizosphere, as a consequence of dissolved organic carbon (DOC) diminution. They mentioned that salinity-induced DOC reduction might decrease Cd-DOC complexation, and consequently increase the uptake of free Cd ion (Cd^{2+}) by plant.

Cd content of grain. The accumulation of cadmium in grains of soybean was generally proportional to both Cd doses in soil and the amount of Cd accumulated in plant. In fact the trend of changes in the grain was somehow similar to that of the whole plant in various treatments. However, salinity exerted a different effect on grain Cd allocation at Cd3 when soybean grown adjacent to purslane (Figure 5). In line with our results several studies demonstrated that salinity can change Cd uptake and accumulation or may affect nutrient partitioning within the plant (Ghallab and Usman 2007, Huang et al. 2007, Sari and Din 2012). In the study of Ozkutlu et al. (2007) adding NaCl in combination with the Cd-containing solution also promoted accumulation of Cd in the grains of durum wheat. However, information is lacking on the allocation and redistribution of Cd to the grains of co-planted soybean. In our study it seems that the promoting effect of salinity on Cd allocation to grains was intensified by co-occurrence of purslane through change of physiological mechanisms that govern Cd transport within soybean, particularly those redistributed Cd to grains.

The higher grain Cd accumulation is not only attributed to the higher Cd uptake but the higher xylem translocation from root to shoot and the higher redistribution from shoot to grain by phloem are

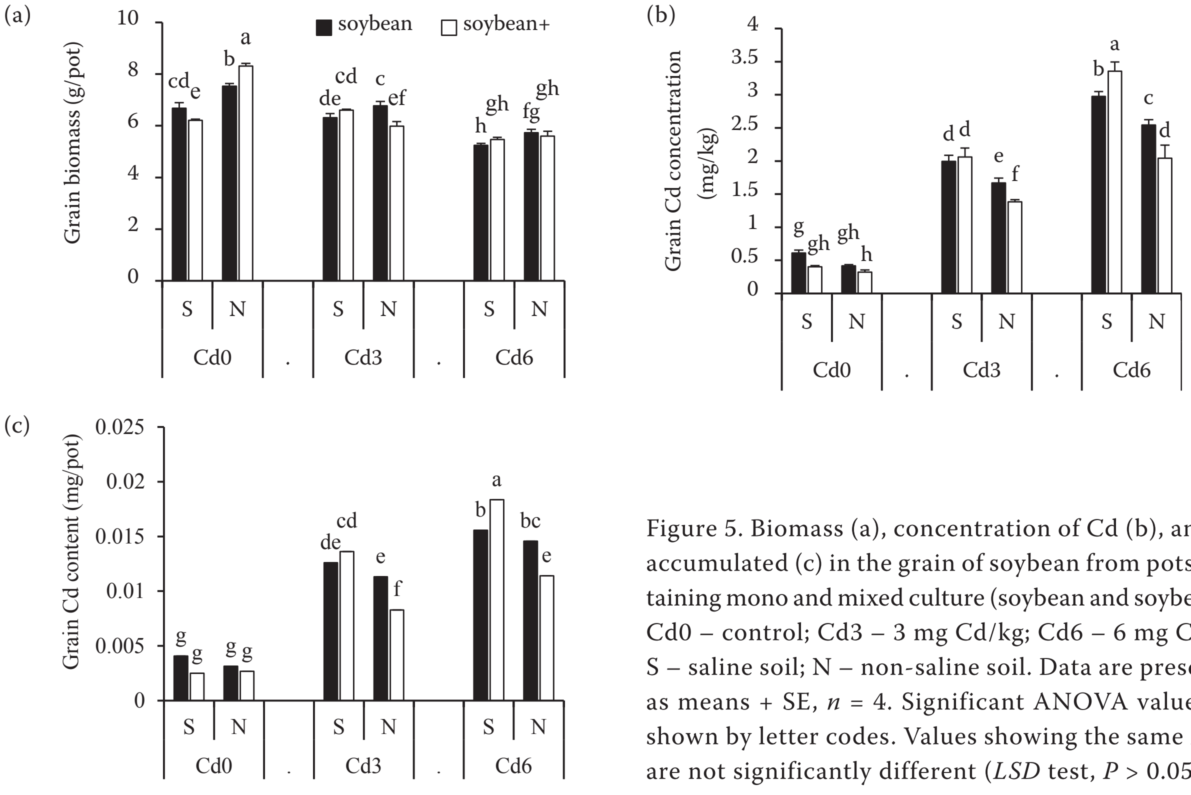


Figure 5. Biomass (a), concentration of Cd (b), and Cd accumulated (c) in the grain of soybean from pots containing mono and mixed culture (soybean and soybean+). Cd0 – control; Cd3 – 3 mg Cd/kg; Cd6 – 6 mg Cd/kg; S – saline soil; N – non-saline soil. Data are presented as means + SE, *n* = 4. Significant ANOVA values are shown by letter codes. Values showing the same letter are not significantly different (*LSD* test, $P > 0.05$)

also important (Harris and Taylor 2001, Waters and Sankaran 2011). Yan et al. (2010) indicated that in spite of higher absorption ability, the greater Cd translocation from root to shoot and subsequent higher Cd remobilization from shoot to grain, can provide higher Cd accumulation in the grains of rice.

Soil labile Cd concentration. The higher labile pool of Cd in co-planted rhizosphere under saline soil may be related to either co-plants root exudates or low Cd uptake of purslane in the presence of soybean (Figure 3). An explanation for these results is lower rate of Cd uptake by accompanied plants compared to the release rate of Cd from less available pools to soil solution. Also the lower labile pool of Cd in co-planted rhizosphere under non-saline soil was a result of higher uptake of purslane as a Cd accumulator associated with lower uptake of soybean. This seems to be due to lower rate of Cd replenishment from the less available pools in comparison with the rate of Cd uptake accompanied by plants in this condition. Generally, the re-supply of depleted Cd in the labile pool and the rate of uptake by plants play a significant role in the bioavailability of Cd (Whiting et al. 2001a, Puschenreiter et al. 2003).

In conclusion, our results indicate that in contaminated soils promoting effect of salinity on soybean Cd uptake and allocation to grains was intensified where this crop was co-planted with purslane. It can be attributed to more competitive ability of soybean than purslane and decrease of Cd content in co-planted purslane despite of increasing Cd-mobilization within the shared rhizosphere. In the non-saline soil decreasing uptake and grain allocation of Cd in co-planted soybean was associated with enhancing of purslane Cd uptake and the depletion of Cd in soil solution. An explanation for these apparently contrasting results is faster uptake rate of purslane or slower replenishment of Cd in the mixed culture. Future investigations are necessary to characterize in detail processes that may affect Cd allocation to the grains of plants co-planted under saline conditions. The results of the present study may provide new ideas for setting experiments regarding assessment of the critical density and economic threshold of accumulator weeds when exposed to heavy metals.

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