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Root uptake and shoot accumulation of cadmium by lettuce at various Cd:Zn ratios in nutrient solution

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ABSTRACT

Cadmium (Cd) is toxic to animals and humans after it accumulates over decades in the kidney cortex. Food crops grown in Cd-contaminated soils are the primary sources of excessive Cd entry into humans. Although plant available Zn concentration in soil is an important factor which can greatly reduce Cd uptake by plant roots and its translocation into the edible parts, Cd:Zn ratio is suggested to be a more important factor in comparison with Zn concentration alone in determining Cd uptake by plants. In the present study, the physiological mechanisms of Cd absorption by roots and its translocation to leaves of lettuce (*Lactuca sativa* L.) at various Cd:Zn ratios in the rooting media were investigated. For this purpose, seedlings of hydroponically-grown lettuce were exposed to combinations of four Zn (0, 12.5, 50 and 100 μM) and four Cd (0, 0.5, 1 and 10 μM) concentrations providing different ratios of Cd:Zn. At each level of Cd, decreasing the Cd:Zn ratio by increasing Zn concentration in the nutrient solution caused significant reduction of root symplastic Cd and also reduced Cd loading into the xylem and Cd transport to and accumulation in leaves. The highest root symplastic Cd (1087 mg/kg^{-1} Dry Weight [DW]) and shoot Cd concentrations (64 mg/kg^{-1} DW) were observed at the highest Cd:Zn ratio of = 0.8 (Zn = 12.5, Cd = 10). At the Cd:Zn ratios of ≤ 0.01, shoot Cd concentration was less than the Detection Limit (< 0.02 mg/kg DW). Decreasing Cd:Zn ratio in nutrient solution was accompanied with significant increase in root apoplastic Cd and decrease in the root symplastic Cd. According to the obtained results, at the Cd:Zn ratio equal to 0.01 and less, Cd concentration in lettuce shoots decreased to < 0.02 mg/kg.

1. Introduction

Cadmium (Cd) is a toxic metal for animals and humans after excessive accumulation over decades in the kidney cortex (Rizwan et al., 2016). Food crops and tobacco grown in the Cd-contaminated soils are considered the main sources of Cd entry into humans (McLaughlin et al., 2006). Cd contamination of leafy vegetables e.g., lettuce (*Lactuca sativa* L.) is of greater importance as compared with other crops (other than rice (Chaney, 2015)), because leafy vegetables have the highest accumulation gradient (1.72) of Cd (Chaney et al., 2000). Therefore, it is necessary to decrease Cd concentration in edible parts of food crops particularly leafy vegetables below the maximum level allowed by the FAO/WHO Codex Alimentarius Commission (2005).

Availability to plants of Cd in soil is affected by several plant and soil characteristics such as pH, organic matter content, chloride concentration in soil solution, Fe and Mn hydrous oxides, Cd:Zn ratio (Chaney et al., 2001; Nan et al., 2002) and plant cultivar (Li et al., 1997). Cadmium and Zn are chemically similar and thus compete with each other for root symplastic and apoplastic uptake, vacuolar storage in root cells, xylem translocation from roots to shoots, re-translocation via phloem, and accumulation in edible parts (Das et al., 1997; Hart et al., 2002). Plant available Zn concentration is an important factor which can greatly reduce Cd uptake by plant roots and further reduce translocation of Cd from roots into the edible parts. For example, Green et al. (2003) reported that increasing Zn concentration in the nutrient solution reduced translocation of Cd into the shoots of hard red spring wheat (*Tritium aestivum* L.). Other studies with maize (Sadana et al., 1989), cereals (Adiloglu, 2002), linseed (Chakravarty and Srivastava, 1997), barley (Wu and Zhang, 2002) and tomato (Cherif et al., 2011) also showed that soil application of Zn decreased root uptake, translocation to the shoots and accumulation of Cd in the plant edible parts. Khoshgoftar et al. (2004) also reported that Zn fertilizer application decreased shoot Cd concentration (by 11–90%) while increasing shoot Zn concentration (by 75–103%) in wheat. In contrast with the above-mentioned results, there are several reports indicating that Zn addition had no clear effect on Cd uptake and its accumulation in plant tissues.

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For example, Harris and Taylor (2001) reported that soil and foliar application of Zn was ineffective in reducing Cd translocation from the roots to the grains of durum wheat; on the other hand, Hart et al. (2002) found clear evidence that higher solution Zn reduced Cd uptake and translocation by both hard red and durum wheats. Chaney et al. (2001) reported that Cd:Zn ratio is a more important factor in comparison with Zn concentration determining Cd accumulation in plant shoots. They reported there can be no invisible Cd poisoning when soil Cd:Zn remains about 1:100 and the maximum foliar Cd concentration is limited by Zn phytotoxicity. Hussain et al. (2016) also reported that concentration of Cd in the leaves of spinach was highly correlated with Cd:Zn ratio in the growth media. The highest leaf Cd concentration was observed in their study at a Cd:Zn ratio of 1:10. At a Cd:Zn ratio of 1:100, leaf Cd concentrations decreased by 46%.

Natural high Cd and high Cd:Zn ratio in soil promotes Cd uptake by crops (Burau, 1983). Therefore, reducing Cd:Zn ratio by application of Zn fertilizer in areas with high Cd would be a useful approach to reduce Cd accumulation in plants except in the case of rice (Chaney, 2015). The lower the Cd and the lower the Cd:Zn ratio, the less likely that increased bioavailable Cd will accumulate in crops (Chaney, 2010). Actual Zn deficiency has been repeatedly observed to cause higher Cd accumulation by plants (Grant et al., 1999; Oliver et al., 1994). Subsequent research on Cd applied in biosolids has shown that when high-quality (low Cd and low Cd:Zn ratio) biosolids were applied, crop Cd was only marginally increased and Zn came along with the Cd so that bioavailable Cd in leafy vegetables was not increased (Chaney et al., 1978).

To better understand the effects of Zn application (Cd:Zn ratio) on Cd accumulation in plant tissues, information on the mechanisms of root uptake and root-to-shoot translocation of Cd at various Cd:Zn ratios in the root media is necessary. There are three main transport paths for metal accumulation into the shoots and seeds: (i) uptake by roots, (ii) loading to xylem, (iii) translocation to shoots, and (iv) phloem translocation to seeds (Clemens et al., 2002). Root uptake of divalent metal cations (M^{2+}) consisted of two steps: apoplastic adsorption and symplastic uptake (Hart et al., 2002; Zhao et al., 2002). During the first stage of metal uptake from an outer medium, cations are accumulated in the plant root apoplast via retention on the negative fixed charges of the cell wall matrix (Meychik and Yermakov, 2001). Transport across the root cell membrane is the initial step of metal uptake by plant tissues (Yin et al., 2015). Cd enters root cells as Cd^{2+} through Zn-specific ZIP (Zn/Iron-regulated transporter-like Protein) transporters at soil solutions levels of Cd and Zn, and also by orthologues of AtIRT1 at higher concentration of Cd^{2+} and TcZNT1/TcZIP4 or via cation channels, such as depolarization activated calcium channels (DACC), hyperpolarization activated calcium channels (HACC), and voltage- insensitive cation channels (VICC) (Clemens et al., 1998; Cohen et al., 1998, 2004; White and Broadley, 2003; Plaza et al., 2007; DalCorso et al., 2008; Pedas et al., 2008; Verbruggen et al., 2009). When a metal enters the cytoplasm of a root cell, it may be moved into the vacuole. Up to 95% of the total Cd absorbed by the roots might be accumulated in vacuoles by HMA3 (Ueno et al., 2010). Long-distance transport of metals to shoots is mainly conducted in the non-living xylem. Xylem loading is an important step in transport of metal ions from the root to the shoot. Cd is loaded from the symplasm into the xylem by heavy metal P_{1B}-ATPase, such as orthologues of AtHMA2 and AtHMA4, and possibly also by YSL proteins (Mills et al., 2005; DalCorso et al., 2008; Wong and Cobbett, 2009; Verbruggen et al., 2009).

The present study was performed to better understanding the physiological mechanisms of Cd absorption by roots and its translocation to shoots of lettuce at various Cd:Zn ratios in the root medium. To achieve the aims, distribution of the apoplastic and symplastic Cd and Zn in the root and their translocation via xylem to the leaves of lettuce was investigated.

2. Methods and materials

2.1. Plant growth and treatments

Seeds of lettuce (*Lactuca sativa* L. cv. Grizzly) were immersed in distilled water (24 h) and stored in the dark (3 days) for germination. The germinated seeds were then transferred to a sand media and irrigated with a Johnson solution (10%-strength complete). After one week, the seedlings were transferred to 20 dm^3 plastic containers. Plant roots were placed in solutions containing (in mM) 2.5 KNO_3, 2.5 $Ca(NO_3)_2$, 1 $MgSO_4$, and 0.1 mM KH_2PO_4, and (in µM) 75 KCl, 20 FeEDTA, 10 H_3BO_3, 0.2 Na_2MoO_4, 2 $MnCl_2$ and 1 $CuSO_4$. NaOH or HCl was used to adjust the nutrient solution pH to 6. The nutrient solution was replaced every 3 days. After 10 days, the plants were exposed to different ratios of Cd:Zn (Table 1) by addition of 0, 0.5, 1 and 10 µM Cd (from $CdCl_2$) and 0, 12.5, 50, and 100 µM Zn (from $ZnSO_4$) to the nutrient solution.

2.2. Analysis of Cd concentration in lettuce roots

Three weeks after exposure to different ratios of Cd:Zn, a subset of lettuce plants (3 plants per treatment) were sampled and washed with deionized water. A sterilized knife was used to separate the lettuce roots and shoots. The root samples were further divided into two parts. One part of the root and whole shoot were washed with distilled water, dried in an oven (70 °C for 48 h) and stored for further analyses. The other part of roots was washed with an 10 mM EDTA solution, precooled in a refrigerator, as explained by Zhou et al. (2007) to determine root symplastic and apoplastic distribution of Cd and Zn. Fifty cm^3 of 10 mM EDTA (pH 6.0) was added to the fresh root samples (about 1 g) in a 100 cm^3 centrifuge tube and gently stirred (100 rpm at 4 °C) for 30 min by using a rotation bed. The EDTA rinse solution was collected to assay the apoplastic Cd and Zn. Digestion of dried plant samples was

Table 1
Effect of varied Cd:Zn levels in nutrient solution on the total, symplastic and apoplastic Cd concentration in roots of lettuce.

Concentration (µM)		Cd:Zn ratio	Root Cd concentration ($mg\ kg^{-1}$ DW)		
Zn	Cd		Total	Apoplastic	Symplastic
0	0	0	< 0.02^{i}	< 0.02^{i}	< 0.02^{h}
12.5	0.5	0.04	146^{gf} ± 7.2	87.5^{gh} ± 1.00	58^{e} ± 3.46
50	0.5	0.01	91.5^{h} ± 3.8	54^{h} ± 3.8	37.5^{efg} ± 2.41
100	0.5	0.005	121^{gh} ± 7.21	92^{fg} ± 7.22	29^{gh} ± 3.42
12.5	1	0.08	375^{d} ± 21.65	283^{d} ± 28.86	92^{d} ± 12.3
50	1	0.02	219^{e} ± 6.25	165^{e} ± 13.01	54^{ef} ± 4.12
100	1	0.01	158^{f} ± 7.21	125^{f} ± 12.50	33^{fhg} ± 1.67
12.5	10	0.8	1090^{a} ± 50	719^{a} ± 47.2	369^{a} ± 23.5
50	10	0.2	856^{b} ± 6.25	560^{b} ± 35.51	296^{b} ± 32.1
100	10	0.1	750^{c} ± 12.5	511^{c} ± 2.60	229^{c} ± 24.1

In each columns, means with similar letter are not significantly different at $P < 0.05$.