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Species-Specific Relationship between Transpiration and Cadmium Translocation in Lettuce, Barley and Radish

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Abstract

Cadmium (Cd) may accumulate in plants to levels that are of concern in human diets. Our ability to predict the accumulation of Cd in plants is restricted by our poor understanding of the physiological processes that control Cd accumulation and translocation. A hydroponic experiment was carried out to test the hypothesis that the amount of Cd taken up and translocated to aboveground tissues is proportional to the volume of water transpired in lettuce (*Lactuca sativa* L.), barley (*Hordeum vulgare* L.) and radish (*Raphanus sativus* L.). Transpiration was measured as mass of water lost. Increased transpiration caused increased accumulation of Cd in plants; however, the proportion of total Cd translocated to the leaves ranged from 85% in lettuce to 66% in radish to only 21% in barley. Thus, factors controlling species-specific internal distribution of Cd are more important than transpiration in translocating Cd to aboveground tissues.

Keywords: Cadmium, Root: Shoot partitioning, Transpiration, Translocation

1. Introduction

Cadmium (Cd) is a non-essential element for almost all biota with the exception of *Thalassiosira weissflogii* (Grunow) G. Fryxell et Hasle, a marine diatom that uses Cd as a substitute for zinc (Zn) in the metalloenzyme carbonic anhydrase (Lane *et al.*, 2005). The two main sources of Cd in soils are geological parent materials and inputs from anthropogenic sources (Nriagu & Pacyna, 1988). Soils derived from Cd-rich parent materials can have concentrations up to 24 mg total Cd kg⁻¹ (Alloway & Steinnes, 1999). Anthropogenic sources include the application of manure and sewage sludge as well as certain industrial activities. In Canada, Cd-contaminated phosphorus (P) fertilizers are one of the major sources of Cd-contamination in agricultural systems and concentrations of Cd in P fertilizers could be as much as 300 mg Cd kg⁻¹ dry product (Grant & Sheppard, 2008). The mean Cd concentration in the soil extract could be as high as 0.17 µg l⁻¹, depending on the rate of P fertilizer application and the Cd concentration of the fertilizer (Lambert *et al.*, 2007). Crops grown in contaminated soil may accumulate Cd in different plant parts, such as root, leaf, grain etc., and consumers may develop a number of Cd-related chronic diseases (Åkesson *et al.*, 2006; Ogawa *et al.*, 2004; Simmons *et al.*, 2005). It is recommended to keep Cd concentrations below regulatory guidelines in vegetables, fruits, grains and other agricultural products to avoid metal toxicity (Canadian Food Inspection Agency [CFIA], 2011). Because the concentration of Cd in edible plant tissues is not always directly proportional to the concentration of Cd in the

soil (Carbonell *et al.*, 2011; Hejzman *et al.*, 2009; Smolders *et al.*, 2009; Wang *et al.*, 2006), understanding the mechanisms of Cd accumulation and translocation in plants is important to ensuring food safety.

The ability of Cd to enter plants depends on a number of biotic and abiotic factors including plant species (Grant *et al.*, 2008), microbial activity (Gao *et al.*, 2010), soil pH (Mann & Ritchie, 1993; Peijnenburg *et al.*, 2000), soil organic matter (Murray *et al.*, 2010), cation-exchange capacity (Bolan *et al.*, 2003a, 2003b), presence of chelators, e.g., organic acids (Cieśliński *et al.*, 1998), presence of competing or complexing ions (Gao *et al.*, 2011), and amounts of total and plant-available Cd in the soil (Carbonell *et al.*, 2011; Wang *et al.*, 2006). Translocation of Cd within the plant depends on three major transport processes: passive and/or active uptake of Cd into the root (Cataldo *et al.*, 1983; Zhao *et al.*, 2002), xylem transport from the roots to the shoots (Uraguchi *et al.*, 2009) and translocation to the seeds via phloem (Tanaka *et al.*, 2007). Each of these processes is directly or indirectly correlated with water transport and transpiration rate. Since Cd is highly soluble in water, it is reasonable to expect a relationship between transpiration rate and Cd accumulation in plants.

The effect of Cd on transpiration of water from leaves has been studied extensively. At low concentrations, Cd increased the permeability of the leaf cuticle and increased transpiration in sugar beet (*Beta vulgaris* L.; Greger & Johansson, 1992). At high concentrations, Cd induced stomatal closure and decreased leaf transpiration in mustard (*Brassica juncea* L.) Czern; Haag-Kerwer *et al.*, 1999), barley (*Hordeum vulgare* L.; Vassilev *et al.*, 2002), and lettuce (*Lactuca sativa* L.; Mensah *et al.*, 2008). However, the mechanism of Cd-induced stomatal closure is still poorly understood. Some studies reported increased production of abscisic acid (ABA) with increased Cd-exposure and suggested that ABA might regulate stomata closure in Cd-stressed conditions (Hsu & Kao, 2003, 2005; López-Climent *et al.*, 2011); however, in ABA-insensitive mutants of *Arabidopsis thaliana* (L.) Heynh Cd²⁺ affected guard cell regulation in an ABA-independent manner by entering the cytosol via Ca²⁺ channels (Perfus-Barbeoch *et al.*, 2002).

While the effect of Cd on leaf transpiration has been well studied, little is known about the effect of transpiration on Cd accumulation and translocation in plants. In some cases, increased transpiration resulted in increased metal content. For example, when grown in artificial wastewater treated with different combinations of Cd and Zn, young wheat (*Triticum aestivum* L.) seedlings accumulated more Cd and Zn under conditions with high vapor pressure deficit (VPD) of the atmosphere compared to low VPD (Salah & Barrington, 2006). This finding is consistent with populations of American pokeweed (*Phytolacca americana* L.) that showed a positive correlation between Cd accumulation and transpiration when grown in nutrient solution (Liu *et al.*, 2010). In contrast, no relationship was found between transpiration and Cd concentration in shoots of inbred lines of maize (*Zea mays* L.) grown in the field (Florijn & Beusichem, 1993). The lack of consensus might be due to differences in species, duration of Cd exposure as well as the way transpiration was measured in the different studies. The species included hyperaccumulator weeds as well as low accumulator crop plants and the plants were either exposed to Cd in hydroponics for a short period of time or collected from contaminated fields. Transpiration measurement methods included amount of water lost per plant per day, amount of water lost per unit leaf area per second, and amount of water lost per unit dry weight of the shoot. The relationship between Cd content and these different measurements of transpiration may vary, especially if the plants being compared have markedly different leaf surface areas.

In this study, the hypothesis that the amount of Cd taken up and translocated to aboveground tissues is proportional to the total volume of water transpired in lettuce (*Lactuca sativa* L.), barley (*Hordeum vulgare* L.) and radish (*Raphanus sativus* L.) grown in a non-toxic Cd concentration was tested. These three species were chosen because of their broad range of leaf areas, which were expected to correspond to a range in volumes of water transpired per plant, and because they represent a leaf, grain and root crop, respectively.

2. Methods and Materials

Chemicals, stock solution and reagents used were of analytical grade and all glassware was washed in soapy tap water, rinsed in tap water, soaked in 10% (v/v) hydrochloric acid overnight, rinsed in RO (reverse osmosis) water and air-dried before use.

2.1 Germination and growth conditions

Seeds of each of three plant species, lettuce (*L. sativa* L. cv. Grand Rapids), barley (*H. vulgare* L. cv. CDC McGwire, hullless 2-row feed barley) and radish (*R. sativus* L. cv. Crimson Giant Champion), were germinated on moist (RO water) filter paper in Petri dishes in the dark for 24 hours. When the radicles were approximately 1 cm long, seedlings were transferred to pots (15 cm diameter) filled with rinsed sand supplemented with nutrient solution (Table 1) adjusted to pH 6.0. The seedlings were kept in a growth chamber set to 21°C with a 16 hour light and 8 hour dark cycle. The light intensity was $187 \pm 1.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and relative humidity was set to 60%. After 7

days in sand culture, the roots were long enough to transfer the seedlings to hydroponics in 1.4 L glass jars. Different concentrations of Cd were added as CdCl₂ to the nutrient solution and pH was set to 6.0 before seedlings were transferred to the jars. In a preliminary experiment, it was determined that concentrations of Cd above 5.0 µM were toxic to lettuce and barley, and 1.0 µM Cd was toxic to radish. Therefore, the concentrations used in this experiment were 0, 0.1, 0.5, 1.0 and 2.0 µM Cd for lettuce and barley and 0, 0.05, 0.1, 0.2, and 0.5 µM Cd for radish. A total of three replicates were used for each treatment. In each jar (experimental replicate), one seedling was suspended in a folded 0.5 x 1 x 6 cm piece of foam and placed in a slot cut into a black plastic lid; this ensured that evaporative water loss was negligible. The sides of the jars were covered with black cloth to prevent algal growth. Each jar was hooked up to an aeration system and the plants were provided with fresh nutrient solution (including the corresponding Cd treatment) every second day.

2.2 Transpiration and growth record

The volume of nutrient solution lost per jar was determined by weighing the mass of each jar each time the nutrient solution was replaced, the daily transpirational water loss and the total volume of water lost were calculated from these values. Plant growth was recorded as crown diameter (lettuce) or shoot height (barley and radish) and measured every alternate day.

2.3 Tissue harvest and biomass determination

Plants were harvested 28 days after Cd treatments were applied. At harvest, roots and shoots were separated, rinsed in RO water and blotted dry. Fresh weight and total leaf area (as measured using a LI-3100 leaf area meter, LI-COR Inc., Lincoln, Nebraska, USA) for each plant were recorded. The roots were rinsed in RO water for 30 seconds then placed in 1mM CaCl₂ solution for 30 minutes followed by another 30 second wash in RO water (Taylor *et al.*, 1998). This procedure desorbs Cd from the root surface by means of a cation exchange reaction between Cd²⁺ and Ca²⁺ and would remove Cd-containing nutrient solution from the surface of the roots. All tissues were oven dried (60°C) until a constant weight was recorded.

2.4 Cadmium content

The concentration of Cd in roots and shoots was measured using a modified EPA test method SW-846 (United States Environmental Protection Agency [US EPA], 2005). The dried plant tissue was hand-chopped into fine pieces and ground using a mortar and pestle. A 0.1 g subsample was then placed in a 15 ml test tube and covered using a glass marble to prevent evaporation while allowing pressure to be released. Standard reference material (SRM) from the National Institute of Standards and Technology (NIST 1573a, tomato leaves) and reagent blanks were used to assess accuracy, quality assurance and quality control. All the test tubes were placed in a rack and 1 ml pure nitric acid (OmniTrace®, EM Science, USA) was added to each test tube to digest the organic matter. The samples were left overnight at room temperature. The following day, the test tube rack was placed in a shallow tray filled with sand and heated to 90-100°C on a hot plate until the vapors became transparent. Samples were allowed to cool to room temperature before being filtered (VWR, qualitative grade 413) into 50 ml sterile disposable centrifuge tubes. Reverse osmosis water was used to rinse the test tubes and bring the volume to 50 ml. The samples were analyzed for Cd content by inductivity-coupled plasma atomic emission spectrometry (ICP-AES) using the following conditions: Perkin-Elmer Optima 3300 Dual view ICP-AES, RF generator power -1300 Watts, plasma flow rate -15 L min⁻¹, auxiliary flow rate - 0.5 L min⁻¹, nebulizer flow rate - 0.8 L min⁻¹, pump flow rate - 1.0 L min⁻¹, analyte line - Cd 226.507 nm, plasma view - axial, with a detection limit of 0.001 ppm for Cd. The percentage recovery of Cd in the digested SRM was 84 ± 5% and no Cd was detected in the reagent blanks.

2.5 Statistical analysis

SigmaPlot (version 11.0) was used for all statistical analysis and graphics. One-way ANOVA was used to detect treatment effects and Tukey's test was used to determine significant differences between treatment means ($P < 0.05$). Correlation and linear regression analyses were used to determine the relationship between transpiration rate and cadmium content.

3. Results

3.1 Plant biomass

The effects of Cd treatment on the dry biomass of each type of tissue are shown in Table 2. For lettuce, Cd exposure decreased both the shoot and root dry biomass and the leaves showed symptoms of Cd-induced stress (chlorosis, leaf rolling, etc.) at higher Cd doses. In the case of barley, Cd exposure slightly reduced shoot mass but had no effect on root mass (mid-panel in Table 2) and all seedlings looked healthy throughout the experimental period. Radish seedlings were sensitive to Cd and did not survive when grown in concentrations above 0.5 µM Cd.

Increasing concentrations of Cd did not consistently affect shoot mass of radish and had no effect on their root mass (bottom panel of Table 2).

3.2 Plant transpiration

The total volumes of water transpired and water loss per unit leaf area are shown in Table 3. Lettuce grown in the highest dose of Cd transpired 27% less total water than did plants grown in control solution but transpiration per unit leaf area was 75% higher for plants grown with 2.0 μM Cd relative to control plants. For both barley and radish, plants grown at the highest dose of Cd transpired 36% less water volume as compared to control plants, and Cd did not affect transpiration per unit leaf area. Among the three species studied, radish transpired the largest volumes of water and barley transpired the least.

3.3 Cd content

The effects of different concentrations of Cd in solution on plant Cd content are shown in Figure 1. As expected, concentrations of Cd in the tissues of all three species increased as the concentrations of Cd in the growth medium increased. Among species, total Cd concentrations were highest in barley (Figure 1c) and lowest in radish (Figure 1e). Within the species, shoot and root concentrations were equal under most experimental treatments for lettuce (Figure 1a) whereas Cd concentrations were higher in the roots compared to the shoots in barley (Figure 1c) and radish (Figure 1e). In radish, the lateral roots had much higher concentrations of Cd than did the tap roots (Figure 1e). When the total amount of Cd accumulated in each tissue was calculated (amount = Cd concentration \times biomass) similar patterns emerged. The greatest amounts of Cd were measured in barley (Figure 1d) and the lowest amounts were measured in radish (Figure 1f). However, the three species responded differently in their ability to partition Cd among the different plant parts. Translocation of Cd from the roots to the shoots was measured by calculating shoot Cd as a percentage of total Cd (Table 4). In lettuce, 85% of the total Cd taken up by the plant was translocated to the leaves. In barley, most of the Cd taken up by the plant was retained in the roots and only 21% of the total Cd was translocated to the leaves. The pattern in radish was intermediate to the other two species; 66% of the total Cd was translocated to the leaves.

3.4 Solution Cd, transpiration and plant Cd

Regardless of the species, plants took up less than half of the total Cd supplied in the nutrient solution (Figure 2a). The total amount of Cd taken up by lettuce, barley and radish was approximately two to three times higher than the amount of Cd available through transpiration (Figure 2b), indicating involvement of membrane transport of Cd into these plants. The total amount of Cd in the plants was positively correlated with the amount of Cd available through transpiration (Figure 2b). The amounts of Cd translocated to the shoots of each species were also positively correlated with Cd available through transpiration (Figure 2c). However, the amounts of Cd in shoots of lettuce consistently exceeded the amounts predicted to be available through transpiration. Positive correlations were also found between the amount of Cd in the shoot and transpiration measured per unit leaf area in each species but the strongest correlation ($R^2=0.67$) was detected in lettuce (Figure 3). Although barley and radish transpired three times more water per unit leaf area compared to lettuce, the amounts of Cd in lettuce shoots were comparable to those in radish and up to three times higher than in barley.

4. Discussion

The main purpose of this study was to investigate the relationship between Cd content and total volume of water transpired in lettuce, barley and radish. While there was a positive correlation between Cd content and total volume of water transpired in all the three species, the intensity of the relationship was species-specific. We addressed the relationship using three approaches.

First, lettuce, barley and radish transpired different volumes of water throughout the study period and responded differently in terms of Cd accumulation. Radish transpired the largest volumes of water and accumulated the least amounts of Cd whereas barley transpired the least and accumulated the highest amounts of Cd. When shoot Cd was plotted against the amounts of water transpired per unit leaf area, the strongest correlation was observed in lettuce.

Secondly, budgeting Cd amounts showed that all three species accumulated more Cd than was available through water uptake. So, it is confirmed that transpiration alone cannot explain plant Cd accumulation and it is likely that active uptake of Cd also took place in the studied species. A number of studies reported that Cd can enter the root through other divalent cation transporters, e.g. Fe^{2+} (Nakanishi *et al.*, 2006) and Ca^{2+} (Zhao *et al.*, 2002). Ueno *et al.* (2008) studied the uptake and translocation mechanism of Cd in *Arabidopsis halleri* (L.) and suggested that Cd entered the root through an energy-dependent process that is partly shared with Zn and/or Fe transport. Lombi *et al.* (2001) investigated the uptake and translocation characteristics of Cd and Zn for the

hyperaccumulator *Thlaspi caerulescens* (L.) and raised the possibility of Cd²⁺ transporters in the root cell plasma membranes.

Thirdly, though less total Cd was measured in lettuce compared to barley and radish, lettuce shoots contained higher amounts of Cd than were measured in shoots of the other two species. This pattern could be explained if barley and radish had Cd-restriction mechanisms in the root that minimized translocation to the shoot. One of those mechanisms could be binding Cd with phytochelators, sulphur-rich compounds that are synthesized upon Cd exposure in the cytoplasm and vacuole. Salt *et al.* (1995) reported Cd-S complexes in Indian mustard root and noted that most of the Cd taken up by Indian mustard was retained in the root. Moreover, different species may accumulate Cd in different compartments within the root. For example, Cd distribution in durum wheat (*Triticum turgidum* L. var. durum) was reported to be symplastic (Van der Vliet *et al.*, 2007) whereas Cd distribution in bush beans (*Phaseolus vulgaris* L.) was reported as apoplastic (Hardiman & Jacoby, 1984). It is possible that Cd distribution in monocots is mostly symplastic whereas in dicots Cd is sequestered in the apoplast; however, this idea needs confirmation. If the theory is true then Cd in lettuce may have been translocated to the shoot through apoplastic bypass whereas in barley Cd was immobilized in the symplast of the root.

Based on the above discussion we are convinced that plant Cd accumulation depends on multiple factors including bulk flow through transpiration, solution Cd concentration and a plant-specific factor. The relative contribution of each of these factors will determine how much Cd will move into the plant and subsequently be translocated to the aboveground part. This is consistent with the findings from several other studies conducted on potato, sugar beet, winter wheat (Ingwersen & Streck, 2005) and radish (Kashem & Singh, 2002) where it was shown that, rather than one single factor, Cd accumulation was driven by multiple factors including the ones mentioned above. In our study, regardless of species, plant Cd content increased with increased Cd concentration in the nutrient solution which is supported by the findings conducted on other species (Ingwersen & Streck, 2005; Salah & Barrington, 2006). The finding that all three plant species showed a positive correlation between shoot Cd and transpiration are in line with the observations from several other studies. Salah and Barrington (2006) studied wheat grown in a range of 0-0.5 mg Cd/L and found that more Cd was taken up by the plants grown under high vapour pressure deficit (VPD) compared to the plants grown under low VPD and that increased Cd in the soil or nutrient solution increased plant Cd accumulation. Hardiman and Jacoby (1984) exposed 10 days old bush bean (*Phaseolus vulgaris* L.) in ¹⁰⁹Cd for 14 hours either at 68% or 97% relative humidity (RH) and found increased Cd content with increased transpiration. The mean Cd concentrations in the transpiration stream under both RH were similar and the authors suggested that increased Cd transport to the shoot under 68% RH occurred in response to increased mass flow of solutes in the transpiration stream. Ingwersen & Streck (2005) surveyed potato, winter wheat and sugar beet from contaminated sites and found increased Cd concentrations in the years with higher saturation deficit of the atmosphere and they suggested that about 66-82% of the relationship between Cd concentration in the crop and Cd concentration in the soil solution can be explained by the volume of water transpired. On the other hand, Florijn and Beusichem (1993) investigated different inbred lines of maize and found no correlation between Cd content of the shoots and transpiration.

Finally, until a plant-specific factor is added, Cd translocation cannot be explained completely. This plant-specific factor includes the ability of individual species to either exclude Cd in the rhizosphere through chelation with organic acid exudates from the plant in response to Cd exposure or pass Cd through the cell wall into the symplasm using cationic transporters for Ca²⁺, Zn²⁺, Cu²⁺ or Fe²⁺ present in the cell membrane (Lombi *et al.*, 2001). Once Cd enters the root, it will either enter and bind with the chelators present in the symplast and restrict Cd movement to the aboveground part or be translocated directly to the aboveground parts through apoplastic bypass.

5. Conclusions

Approximately 85% of the Cd taken up by lettuce was accumulated in the leaves, whereas 80% of the Cd in barley was retained in the roots. In radish, Cd was more evenly distributed between aboveground and below ground tissues. Cd accumulation and translocation in lettuce, barley and radish depends on multiple factors including solution Cd concentration, transpiration, and a plant-specific internal compartmentation of Cd in the root. Preferential retention of Cd in the cell wall or sequestration in the vacuole might explain the observed differences in Cd distribution. So, understanding how and where Cd is stored in the roots is worthy of further investigation as this might enhance our understanding of Cd tolerance and differential translocation in lettuce, barley and radish.

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Table 1. Composition of the nutrient solution

Macronutrients	Concentration (mM)	Micronutrients	Concentration (μ M)
Ca(NO ₃) ₂ ·4H ₂ O	1.0	H ₃ BO ₃	6.0
K ₂ HPO ₄	1.0	MnCl ₂ ·4H ₂ O	2.0
KNO ₃	0.4	ZnSO ₄ ·7H ₂ O	0.5
Mg(NO ₃) ₂ ·6H ₂ O	0.3	CuSO ₄ ·5H ₂ O	0.15
NH ₄ NO ₃	0.3	Na ₂ MoO ₄	0.1
K ₂ SO ₄	0.1		
FeCl ₃ ·6H ₂ O	0.01		
Na ₂ EDTA	0.01		

Table 2. Dry biomass (SE) of lettuce, barley and radish grown in different Cd treatments for 28 days

Treatments ($\mu\text{M Cd}$)	Shoot mass (g)		Root mass (g)			
Lettuce						
0	2.4 (0.3)a		0.6 (0.1)a			
0.1	3.6 (0.2)a		0.9 (0.1)a			
0.5	2.4 (0.2)a		0.7 (0.0)a			
1.0	2.1 (0.6)a		0.5 (0.2)ab			
2.0	1.0 (0.1)b		0.1 (0.0)b			
One-way ANOVA						
	$F_{(4, 14)}$	p	$F_{(4, 14)}$	p		
	8.567	0.003	10.213	0.001		
Barley						
0	1.8 (0.3)a		0.5 (0.1)a			
0.1	1.8 (0.1)a		0.5 (0.0)a			
0.5	1.1 (0.3)b		0.4 (0.1)a			
1.0	1.2 (0.2)ab		0.4 (0.1)a			
2.0	1.0 (0.1)b		0.4 (0.1)a			
One-way ANOVA						
	$F_{(4, 14)}$	p	$F_{(4, 14)}$	p		
	3.869	0.038	1.153	0.387		
Radish						
	Shoot mass (g)	Lateral root mass (g)	Tap root mass(g)			
0	6.1 (1.1)ab	0.8 (0.1)a	5.2 (0.6)a			
0.05	9.4 (1.2)a	1.0 (0.2)a	2.1 (0.7)a			
0.1	4.6 (0.1)ab	0.6 (0.1)a	4.5 (0.4)a			
0.2	3.4 (1.2)b	0.3 (0.1)a	2.9 (0.9)a			
0.5	4.3 (1.1)b	0.6 (0.3)a	3.1 (0.7)a			
One-way ANOVA						
	$F_{(4, 14)}$	p	$F_{(4, 14)}$	p	$F_{(4, 14)}$	p
	5.110	0.017	2.041	0.164	3.257	0.059

The results (F statistic and corresponding p value) of one-way analyses of variance for each tissue type within each species are also shown. Different lower case letters indicate significant differences in dry biomass, as determined by post-hoc tests.

Table 3. Total volume of water transpired (SE) and transpiration per unit leaf area (SE) by lettuce, barley and radish grown in different Cd treatments

Treatment ($\mu\text{M Cd}$)	Lettuce		Barley		Treatment ($\mu\text{M Cd}$)	Radish	
	Volume (ml)	Transpiration (ml/cm^2)	Volume (ml)	Transpiration (ml/cm^2)		Volume (ml)	Transpiration (ml/cm^2)
0	788 (94)a	0.64 (0.05)c	1095 (44)a	1.74 (0.18)a	0	3088 (19)a	2.18 (0.44)ab
0.1	957 (38)a	0.51 (0.05)c	1047 (12)a	1.65 (0.06)a	0.05	3138 (356)a	1.34 (0.02)b
0.5	731 (52)a	0.59 (0.03)c	683 (93)b	2.02 (0.40)a	0.1	2661 (156)ab	2.3 (0.14)ab
1.0	758 (98)a	0.88 (0.04)b	730 (67)b	2.02 (0.09)a	0.2	2187 (173)b	2.1 (0.14)ab
2.0	528 (54)b	1.12 (0.05)a	698 (32)b	2.10 (0.28)a	0.5	1971 (32)b	2.5 (0.06)a
$F_{(4,14)}$	4.529	31.875	12.601	0.728		7.517	4.066
p	0.024	0.001	0.001	0.59		0.005	0.033

The results of one-way analyses of variance (F statistic and corresponding p value) for each variable within each species are also shown. Different lower case letters indicate significant differences in transpiration per unit leaf area and total volume of water transpired, as determined by post-hoc Tukey tests.

Table 4. Shoot Cd as a percentage of total Cd (SE) in lettuce, barley and radish

Species	Proportion of total Cd in the shoot (%)
Lettuce	84.47 (1.65)a
Barley	21.26 (1.32)c
Radish	66.15 (2.97)b

One-way ANOVA	
$F_{(2,35)}$	p
296.405	0.001

Data from each Cd treatment (0.1-2.0 μM Cd for lettuce and barley, and 0.05-0.5 μM Cd from radish) were pooled (n=12 for each mean value) prior to the calculation of the proportion of total Cd that accumulated in the shoot. The results of the one-way analysis of variance (F statistic and corresponding p value) are also shown. Different lower case letters indicate significance differences in Cd translocation, as determined by post-hoc Tukey tests.

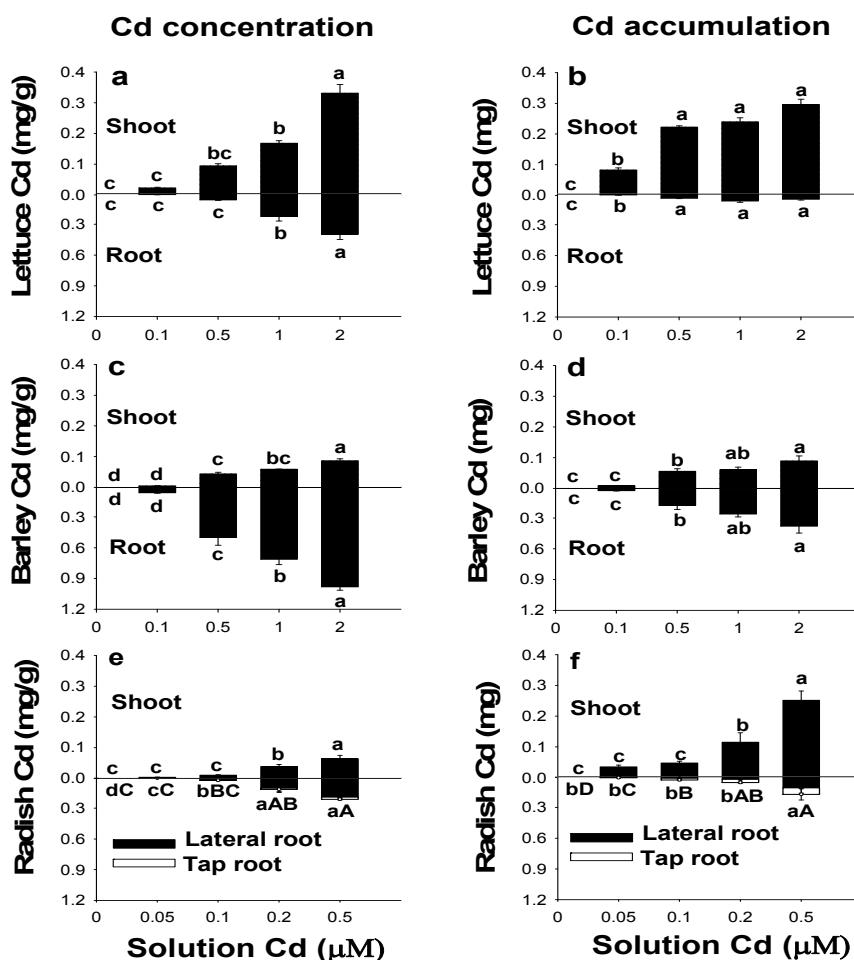


Figure 1. Accumulation of Cd in lettuce, barley and radish grown in different Cd treatments for 28 days

Concentrations of Cd (left-side panels) and total amounts of Cd (right-side panels) are shown for shoots and roots of (a,b) lettuce, (c,d) barley and (e,f) radish. Within each species, different lower case letters indicate significance differences in Cd concentration and Cd accumulation for shoots and roots, as determined by post-hoc Tukey tests. For radish, differences between lateral roots are indicated by lower case letters, and differences between tap roots are indicated by upper case letters.

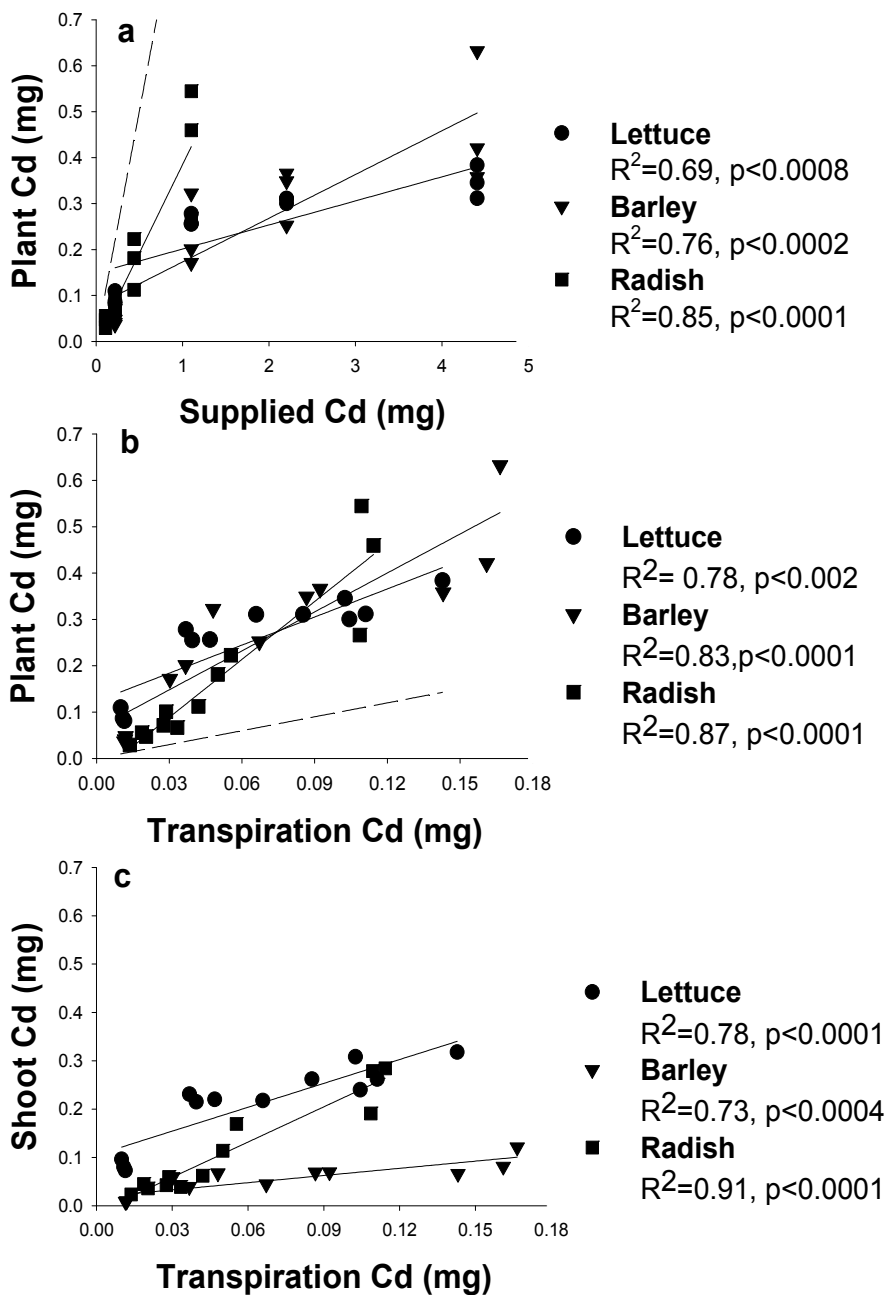


Figure 2. Relationships between Cd accumulation and Cd supply in lettuce, barley and radish

(a) The total amount of Cd in each plant is plotted against the total amount of Cd supplied in the growth medium. The dashed line illustrates the maximum amount of Cd that could have been taken up by the plants. (b) The total amount of Cd in each plant is plotted against the amount of Cd in the volume of water that was taken up by each plant. The dashed line represents the maximum Cd available through transpiration. (c) The total amount of Cd in the shoot of each plant is plotted against the amount of Cd in the volume of water that was taken up by each plant. Circles, triangles and squares illustrate lettuce, barley and radish, respectively. The solid lines represent lines of best fit for each plant species.

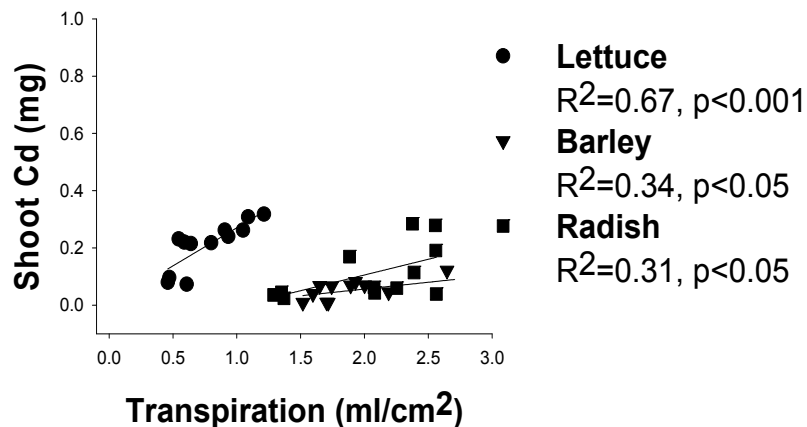


Figure 3. Relationship between Cd translocation and transpiration in lettuce, barley and radish

The total amount of Cd in the shoot of each plant is plotted against transpiration per unit leaf area. Circles, triangles and squares illustrate lettuce, barley and radish, respectively. The solid lines represent lines of best fit for each plant species.