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Cadmium, Copper, and Lead Accumulation and Bioconcentration in the Vegetative and Reproductive Organs of *Raphanus sativus*: Implications for Plant Performance and Pollination

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Abstract Several studies have found high levels of cadmium (Cd), copper (Cu), and lead (Pb) in honey bee hives located near urbanized or industrial areas. Insect herbivores and pollinators may come in contact with environmental contaminants in the leaves and flowers they forage upon in these areas. Our study quantified which of these metals are accumulated in the tissues of a common weedy plant that can serve as a route of exposure for insects. We grew *Raphanus sativus* (crop radish) in semi-hydroponic sand culture in the greenhouse. Plants were irrigated with nutrient solutions containing Cd, Cu, or Pb at four concentrations (control, low, medium, high). Plant performance, floral traits, and metal accumulation were measured in various vegetative and reproductive plant organs. Floral traits and flower number were unaffected by all metal treatments. Copper accumulated at the highest concentrations in flowers compared to the other two metals. Copper and Cd had the highest translocation indices, as well as higher bioconcentration factors compared to Pb, which was mostly immobile in the plant. Copper posed the highest risk due to its high mobility within the plant. In particular, accumulation of metals in leaves and flowers suggests that herbivores and pollinators visiting and foraging on these tissues may be exposed to these potentially toxic compounds.

Keywords Cadmium · Copper · Lead · Pollinators · Floral traits · Pollution

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Introduction

Cadmium (Cd), copper (Cu), and lead (Pb) are pollutants that can occur in both water and soil. The free metal ions (Cu^{2+}, Cd^{2+}, Pb^{2+}) are the most toxic forms of these three pollutants. Chelating agents such as organic acids may complex with the ions, causing them to be more bioavailable for plant uptake from the soil (Reichman 2002). Recent studies have revealed that high concentrations of Cd, Pb, and Zn in contaminated sites near smelters are correlated with a decrease in the diversity and abundance of wild solitary bees (Moroń et al. 2012). Other pollinator species, such as the honey bee, may also be susceptible to heavy metal exposure *via* accumulating plants or airborne dusts from contaminated soils.

In previous studies, foliar herbivores fed plant tissues containing high levels of metals, metalloids, or other accumulated elements reduced developmental rates and survival (Boyd 2007; Butler and Trumble 2008), but there are few published studies that examine the effects of metals in plant tissues on insect pollinators' survival or behavior. However, the soil-borne pollutant selenium (Se) adversely affects honey bees (*Apis mellifera* L.) (Hladun et al. 2012, 2013a) as well as insect herbivores (Jensen et al. 2005; Trumble et al. 1998; Vickerman et al. 2002) in the laboratory. Honey bee larvae were very susceptible to Se in their food, and demonstrated high mortality and developmental effects at concentrations as low as 0.72 mg Se kg^{-1} (Hladun et al. 2013a). Adult honey bees also suffer sublethal effects from ingesting Se, including reduced responses to sucrose (Hladun et al. 2012). Certain species of the Brassicaceae accumulate Se in the pollen and nectar (Hladun et al. 2011). In addition, pollinators do not avoid Se-accumulating plants in the field (Hladun et al. 2013b). Nickel, a transition metal with similar properties to Cd and Cu, can accumulate in two species of Brassicaceae (Meindl et al. 2014). Other soil-borne pollutants similar to Se, such as Cd, Cu, or Pb, may accumulate in plants, providing a

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route of exposure for pollinators, which may suffer toxic effects from the ingestion of the contaminated floral resources.

Copper is an essential plant micronutrient necessary for enzyme function, metabolism, and detoxification (Taiz and Zeiger 2002), but an excess can cause toxicity. Copper is bound to amino acids and transported through the xylem (Reichman 2002). Accumulation in leaves at levels between 15 and 25 mg Cu kg^{-1} can cause toxic effects, including photosynthesis inhibition, reduction in carbohydrate and nitrogen metabolism, and chromosome damage (Påhlsson 1989). One of the primary sources of Cu is from fungicides applied to agricultural crops such as grapes (Komárek et al. 2010; Mackie et al. 2012). Repeated applications of Cu-containing fungicides in vineyards worldwide have led to the accumulation of levels as high as 3216 mg Cu kg^{-1} soil in Brazil (Komárek et al. 2010).

In contrast, Cd and Pb are not essential to plant function. Cadmium is chemically similar to zinc (Zn), which is an essential micronutrient, and therefore Cd is easily accumulated by plants. Dry leaf levels as low as 3 mg Cd kg^{-1} are associated with reduced growth (Das et al. 1997). Cadmium can inhibit photosynthesis and transpiration, thus reducing CO_2 fixation. Transpiration is reduced due to xylem blockages. In addition, Cd can replace Zn in certain enzymes, leading to less stable protein complexes (Påhlsson 1989). In agricultural soils, Cd contaminants are found in the parent rock of fertilizers (Page et al. 1987) and in sewage sludges (Chen et al. 2009). Other sources of Cd include industrial and mining wastes (McLaughlin and Singh 1999).

Lead has contaminated soils worldwide, and its occurrence in agricultural, rural, and urbanized areas has been studied extensively (Markus and McBratney 2001). Lead occurs as air pollution from motor traffic (automobile and airplane) as well as from mining and industrial sources (Bogdanov 2005), and it is listed at number two on the Comprehensive Environmental Response, Compensation, and Liability Act Priorities List of Hazardous Substances (US EPA, CERCLA 2011). Although not very toxic to plants (Påhlsson 1989), Pb can accumulate in certain species, particularly those plants that grow in close proximity to roadsides (Price et al. 1974). Lead has been found at various concentrations in the bodies of forager honey bees (Leita et al. 1996; Roman 2010), honey (Jones 1987; Leblebici and Aksoy 2008; Pohl 2009; Rashed and Soltan 2004; Roman and Popiela 2011; Tuzen et al. 2007; Zugravu et al. 2009), in flowers near honey bee hives (Rashed and Soltan 2004; Rashed et al. 2009), in apiary products such as propolis and wax (Conti and Botrè 2001), and in pollen (Morgano et al. 2010). These studies have identified a trend towards higher levels of Pb in honey bees and their products near urbanized or industrial areas, particularly in areas close to airports or roadsides. With foraging areas of tens of square km (Visscher and Seeley 1982), bees may come in contact with environmental contamination in the form of atmospheric Pb pollution, as well as in the flowers they forage upon. Lead may be carried back to the hive in the pollen, nectar, and gleaned water as well as on the surface of the forager's bodies (Pohl 2009).

Raphanus sativus L. (crop radish) has been examined as a potential phytoremediator for removal of Pb from polluted soils (Marchiol et al. 2004). This species also accumulates Cd and Cu into its foliar and root tissues (Davies 1992; Zheng et al. 2008). It is a common weed throughout California, and is cultivated throughout the world (Snow and Campbell 2005). This species is an annual, self-incompatible plant (thus ideal for pollination studies) that has been examined extensively in herbivore and pollinator studies (Hladun et al. 2013b; Stanton 1987; Strauss et al. 2004) as well as for its hybridization with wild radish, *Raphanus raphanistrum*. Wild radish often is planted as a food source for managed honey bees (Garvey 2010). Our first objective of this study was to determine if there are any effects of metal pollutants on floral traits or plant growth in crop radish that could affect pollinator or herbivore exposure. Specifically, we determined whether Cd, Cu, or Pb reduced flower availability or floral attractiveness, thus limiting potential effects on pollinators. Our second objective was to determine whether Cd, Cu, or Pb accumulated in the vegetative or reproductive plant organs, in particular the flowers, which also may serve as a route of exposure for pollinators.

Methods and Materials

Plant Materials and Growth Conditions *Raphanus sativus* (crop radish, cv. "White Globe", Livingston Seed Co., Columbus, OH USA) seeds were germinated in the greenhouse (Environmental Sciences Greenhouses, University of California, Riverside, CA, USA) in University of California Standard Soil Mix III in April 2013. After 2 wk, seedlings were removed from germination flats and roots were rinsed with tap water to remove as much soil as possible, and the seedlings then were transplanted to the irrigated sand culture after nutrients had already been added and passed through the sand so that carbonates in the sand would buffer the pH. Seedlings were transplanted to 7.5 L pots filled with silica sand (Weist Rentals and Sales, Riverside, CA, USA), one plant per pot. Four pots were irrigated from a 120 L tank filled with water and nutrient solution. The basal nutrient solution was added according to Parker et al. (1991). The solution contained 80 mg L^{-1} NH_4NO_3, 147 mg L^{-1} $CaCl_2$, 18.6 mg L^{-1} KCl, 24.6 mg L^{-1} $MgSO_4$, 1.56 mg L^{-1} NaH_2PO_4, 0.20 mg L^{-1} $MnCl_2$, 0.14 mg L^{-1} $ZnCl_2$, 0.02 mg L^{-1} $CuCl_2$, 0.19 mg L^{-1} H_3BO_3, 0.02 mg L^{-1} Na_2MoO_4, and 3.67 mg L^{-1} Fe-EDTA. The solution irrigation was activated on a daily timer, pumping solution into each pot five times a day for 5 min. Nutrient solution then drained out of the pots and back into the 120 L tanks. Water levels were maintained at 120 L in the tank by replacing evaporated water with deionized water. Solution nitrogen and phosphorus levels were checked throughout the experiments and replenished as necessary.

Tank pH was measured on a weekly basis throughout the experiment. Cadmium, Cu, and Pb had no significant effect on pH ($F<0.92$, $P>0.45$). Only sampling date ($F>22.98$, $P<0.001$) had a significant effect on pH. The pH of the tanks ranged from 8.29 to 8.50 over the course of the experiment, and residual carbonates in the silica sand buffered the nutrient solution.

Experimental Design and Treatments Two weeks after transplanting the seedlings to the sand culture, the Cd, Cu, or Pb treatments were started. Cadmium was added as cadmium chloride ($CdCl_2$, Fisher Scientific, Waltham, MA, USA), Cu was added as cupric chloride dihydrate ($CuCl_2*2H_2O$, Fisher Scientific, Waltham, MA, USA), and Pb was added as lead chloride ($PbCl_2$, Acros Organics, Geel, Belgium). Treatment water concentrations were chosen based on the Environmental Protection Agency (EPA) Maximum Contaminant Levels (MCL) for drinking water for the three metals as well as the concentrations listed in Table 1. Metals were added as a concentrated stock solution for the following final concentrations. Cadmium treatments were added at low (0.003 mg Cd L^{-1}), medium (0.03 mg Cd L^{-1}), and high (0.3 mg Cd L^{-1}) concentrations. Cu treatments were added at low (0.02 mg Cu L^{-1}), medium (0.2 mg Cu L^{-1}), and high (2 mg Cu L^{-1}) concentrations. Lead treatments were added at low (0.0005 mg Pb L^{-1}), medium (0.005 mg Pb L^{-1}), and high (0.05 mg Pb L^{-1}) concentrations. Control tanks contained nutrient solution with no additional metals added. Tank treatments were arranged in a randomized block design in order to minimize the variation in temperature and light in the greenhouse. Each tank was used as a unit of replication for all responses. Each metal treatment level was replicated with 6 tanks providing solution to up to 4 plants each. The Cu high treatment contained 7 tanks. Irrigation solution samples were collected on a weekly basis and analyzed for Cd, Cu, and Pb concentrations in order to maintain the original treatment concentrations.

Plant Performance Each week for the first 6 wk of the treatments, the total numbers of leaves produced was counted to yield average number of leaves per tank produced per week. In addition, the number of days to first flower and the total number of flowers per tank produced per week were counted for 10 wk, and then summarized within tank to calculate average flower number.

For floral traits, two flowers per pot were measured during peak flowering period using morphological measurements based on Conner and Via (1993). Floral trait measurements included display width (distance across flower from the tip of one petal to the other), petal area (estimated as length x width), corolla tube length, pistil and stamen length.

Shoot biomass (mostly stems and leaves remained at the end of the experiment) and root biomass were determined by harvesting the plants at the end of the experiment and weighing the samples. The plants were then dried in an oven at 70 °C for 4 d and weighed again.

Plant Analysis Leaves, flowers, and seeds were frozen in a −60 °C freezer (Fisher Scientific, Pittsburg, PA, USA) and then freeze-dried (Labconco Corp., Kansas City, MO, USA) at −40 °C and −25 psi for at least 3 d. Up to 20 flowers were pooled within replicate to create a sufficient tissue weight for analysis. Plant tissues were microwave digested in 110 ml teflon-lined vessels containing 5 ml concentrated HNO_3 (using protocols from Hladun et al. 2011). The vessels were heated for 20 min using a 570 W microwave oven (CEM Corp., Matthews, NC, USA). Plant tissues were diluted in a 6 M HCl matrix, heated in a 90 °C water bath for 20 min and analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (PerkinElmer Inc., Shelton CT, USA). Cadmium, Cu, and Pb concentrations in plant tissues are reported in $\mu g\ g^{-1}$. The NIST Standard Reference Material 1572 (citrus leaves) and 1573a (tomato leaves) were used as standards for plant tissues. Duplicate sample concentrations were within 10 % of each other, and recovery of Cd, Cu, and Pb in the NIST tissues was 90 % or above.

Overall metal distribution in each plant organ (leaves, flowers, seeds, shoots, roots) was calculated using the following equation:

$$\%\ \text{Overall metal distribution} = [\text{Specific plant organ}]/[\text{Total biomass}] \times 100.$$
$$[\text{Total biomass}] = [\text{Leaves}] + [\text{Flowers}] + [\text{Seeds}] + [\text{Shoots}] + [\text{Roots}]$$

The translocation index (TI) (Barman et al. 2000; Zhang et al. 2010) was used to evaluate the ability of *R. sativus* to accumulate metals in the shoots (aerial parts of the plant) using dried shoot and root biomass collected at the end of the experiment. Concentrations from low, medium, and high treatments were averaged to calculate a single TI for each metal:

$$\text{TI}\ \% = [\text{Shoots}]/[\text{Shoots}] + [\text{Roots}] \times 100$$

The bioconcentration factor (BCF) was calculated to measure the ability of each plant organ to accumulate metals from the substrate (Salt et al. 1995). Following Zhang et al. 2010, BCF was calculated for plants given a low, medium, or high treatment:

$$\text{BCF} = [\text{Specific plant organ}]/[\text{Irrigation water}]$$

Statistical Analysis Each metal was analyzed as a separate independent variable and replicated with 4 to 7 tanks each

Table 1 Summary of pollutant concentrations for cadmium, copper and lead worldwide

Contaminant	Source	Concentrations (mg kg-1)	Reference
Cadmium	EPA Maximum Contaminant Limit (MCL)	0.005	US EPA 2009
	EPA max pollutant limit sewage sludge	39	Rathod et al. 2011
	California (CA) croplands	0.5	Chen et al. 2009
	CA croplands	0.15	Chen et al. 2009
	CA croplands	2.38	Chen et al. 2009
	Industrial wastewater for vegetable irrigation - India	0.0001–0.00049	Gupta et al. 2010
	Surface soils near lead-zinc smelter	29–750	Page et al. 1987
Copper	EPA MCL	1.3	US EPA 2009
	EPA max pollutant limit sewage sludge	1500	Rathod et al. 2011
	Worldwide vineyard soils	2–3215	Komárek et al. 2010; Mackie et al. 2012
	US soils	0.3–495	Holmgren et al. 1993
Lead	EPA MCL	0.015	US EPA 2009
	Roadside concentrations in Tehran	50–400	Kapourchal et al. 2009
	CA croplands	5.99–62.2	Chen et al. 2009
	EPA max pollutant limit sewage sludge	300	Rathod et al. 2011
	Industrial wastewater for vegetable irrigation - India	0.0092–0.108	Gupta et al. 2010
	Soil from major cropping areas US	12.3	Holmgren et al. 1993; Markus and McBratney 2001
	Soil in children's play areas, EPA max pollutant limit	400	US EPA 2001
	Soil from other yard areas, EPA max pollutant limit	1200	US EPA 2001

containing up to 4 plants per tank. Each metal (Cd, Cu, and Pb) was analyzed with separate ANOVAs (PROC GLM, SAS 9.2; SAS Institute, Cary NC, USA) with type III sum of squares. Mean separations were conducted between groups (α=0.05) using Tukey's HSD *post hoc* test. Assumptions of normality were examined using the Shapiro-Wilks test.

Weekly flower and leaf emergence were analyzed using ANOVA (PROC GLM) with repeated measures. The independent variables were metal and concentration, flower or leaf number was the dependent variable, and sampling date was the repeated variable. All flower and leaf count data were normally distributed.

Shoot and root biomass were analyzed using ANOVA (PROC GLM) with treatment concentration (control, low, medium, high) as the independent variable. Fresh and dry biomass was log transformed for Cd, Cu, and Pb to achieve normality.

Metal accumulation in *R. sativus* was analyzed using ANOVA (PROC GLM). Each metal (Cd, Cu, Pb) was analyzed separately. The independent variables were treatment concentration (control, low, medium, high), tissue (leaves, flowers, seeds, shoot, root) and the interaction of concentration and tissue. The dependent variables were metal concentration in the tissue (Cd, Cu, Pb) and BCF. Tank was the unit of replication. Tissue concentrations and BCFs were log transformed.

Results

Plant Performance Copper was the only metal that caused any signs of phytotoxicity to the plant in our study, despite its role as an essential micronutrient. The levels of Cu used in treatments were ten-fold higher than levels of Cd, and 100-fold higher than levels of Pb, consistent with environmental levels of the metals (Table 1). Copper had a significant negative effect on shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight (Table 2). Copper reduced shoot fresh weight by up to 65 %, and root fresh weight by up to 62 % (Fig. 1), and had a similar effect on the dry weight (Fig. 1). Cadmium, Cu, and Pb did not have a significant effect on the number of days to first flower, the average number of flowers, or any of the floral traits (Table 2). For Cd, Cu, and Pb, sample date, but not concentration had a significant effect on flower production and the number of leaves produced. Cadmium, Cu, and Pb had no significant effect on the average number of leaves produced overall or the number of leaves produced per week. Cadmium and Pb had no significant effect on fresh or dry biomass (Table 2).

Metal Accumulation Each metal was distributed differently in *R. sativus* (Fig. 2). Cadmium accumulated significantly in the shoots, roots, flowers, seeds, and leaves (Table 3), with the

Table 2 ANOVA results for effects of each metal on plant performance in *Raphanus sativus*

Dependent variable	Metal	*F*	*df*	*P* value
Days to first flower	Cd	2.54	3,17	0.09
	Cu	1.34	3,19	0.29
	Pb	1.01	3,17	0.41
Number flowers per week	Sampling date	>3.47	9,180	**<0.02**
	Cd	2.17	3,20	0.12
	Cu	1.07	3,20	0.39
	Pb	0.14	3,20	0.94
Average number flowers	Cd	0.55	3,20	0.66
	Cu	1.17	3,20	0.34
	Pb	0.17	3,20	0.91
Floral traits	Cd	<1.79	3,14	>0.19
	Cu	<1.83	3,14	>0.19
	Pb	<1.12	3.14	>0.37
Number leaves per week	Sampling date	>103.10	5,100	**<0.001**
	Cd	0.45	3,19	0.72
	Cu	0.59	3,20	0.63
	Pb	0.39	3,20	0.76
Average number leaves	Cd	0.31	3,20	0.82
	Cu	0.59	3,20	0.63
	Pb	0.39	3,20	0.76
Shoot fresh weight	Cd	1.97	3,21	0.15
	Cu	4.46	3,21	**0.02**
	Pb	0.58	3,20	0.63
Shoot dry weight	Cd	0.97	3,21	0.42
	Cu	4.87	3,21	**0.01**
	Pb	0.68	3,20	0.58
Root fresh weight	Cd	1.29	3,21	0.30
	Cu	4.52	3,21	**0.02**
	Pb	0.75	3,20	0.54
Root dry weight	Cd	1.88	3,21	0.16
	Cu	3.06	3,21	**0.05**
	Pb	0.95	3,20	0.43

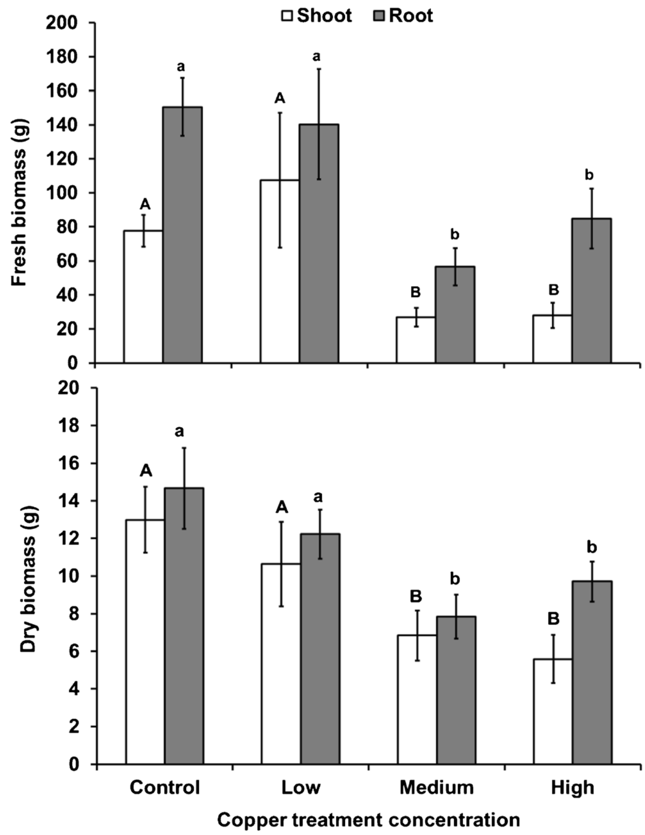

Fig. 1 Effect of Cu treatments on (**a**) shoot and (**b**) root fresh biomass, and (**a**) shoot and (**b**) root dry biomass in *Raphanus sativus* grown in semi-hydroponic sand irrigation system. *Bars* with *different letters* are significantly different based on Tukey HSD test ($P<0.05$). Replicate numbers are as follows (control, low, medium, and high treatments): Shoot and root fresh biomass (N=6, 6, 6, 7), shoot and root dry biomass (N=6, 6, 6, 7)

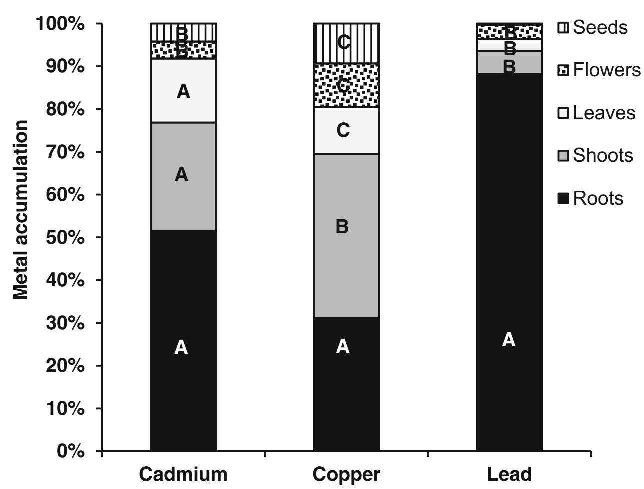

Fig. 2 Overall distribution of Cu, Cd, and Pb in *Raphanus sativus. Bars* within the same metal with different letters are significantly different based on Tukey HSD test ($P<0.05$). Replicate numbers are as follows (leaves, flowers, seeds, shoots, roots): For Cd: N=20, 19, 13, 10, 10. For Cu: N=11, 14, 7, 3, 6. For Pb: N=14, 14, 5, 5, 4

highest percent of metal accumulating in the roots (Fig. 2). Significant accumulation was achieved in all organs when plants were irrigated with as little as 0.003 mg Cd L^{-1} (low treatment), with leaves (Fig. 3) as a secondary site of accumulation after roots (Fig. 3). The TI for Cd was 35 %, consistent with translocation of Cd from root to shoot. There also was a significant effect of Cd treatment on BCFs for shoots, flowers, seeds, and leaves (Table 4). However, BCFs were significantly lower in these tissues when plants were irrigated with medium or high Cd treatments compared to low Cd treatment (Table 5).

Copper accumulated in the shoots, roots, flowers, seeds, and leaves of *R. sativus* (Table 3), and was more evenly distributed throughout the plant compared to Cd or Pb (Fig. 2).

Table 3 ANOVA results for the effects of each metal on accumulation in *Raphanus sativus*

Dependent variable - accumulation	Metal	*F*	*df*	*P* value
Shoots	Cd	9.80	3,6	**<0.05**
	Cu	12.63	3,3	**<0.05**
	Pb	87.22	3,3	**<0.01**
Roots	Cd	17.70	3,6	**<0.001**
	Cu	6.32	3,5	**<0.05**
	Pb	10.37	3,3	**<0.05**
Flowers	Cd	15.63	3,14	**<0.001**
	Cu	9.80	3,15	**<0.001**
	Pb	1.65	3,15	0.22
Seeds	Cd	7.76	3,9	**<0.01**
	Cu	6.72	3,5	**<0.05**
	Pb	0.82	3,3	0.56
Leaves	Cd	84.38	3,16	**<0.001**
	Cu	9.40	3,12	**<0.01**
	Pb	10.82	3,12	**<0.01**

All three levels of Cu treatment increased Cu uptake in leaves, flowers, seeds, and shoots (Fig. 3). Plants treated with Cu had the highest TI (46 %), suggesting more metal translocated from the roots to the shoots compared to the other two metals. There was a significant effect of Cu treatment on the BCF of shoots, roots, flowers, seeds, and leaves (Table 4). The highest BCFs occurred in the flowers, seeds, and roots, but only at the low Cu treatment level (Table 5).

Lead was poorly absorbed by the plants, with very low levels of lead in the roots even for the highest concentration tested (Fig. 3e). Lead was the least mobile metal, with a low TI, and a low BCF compared to Cd or Cu (Table 5). The low Pb levels in leaves, flowers, seeds, and shoots (Fig. 3a–d) were consistent with low absorption and low mobility.

For all three metals, the BCFs were larger for the low level metal treatment compared to the medium and high treatments. Plants were able to move more metal from the irrigation water into the flowers when the metal concentration did not approach potentially phytotoxic levels. When ranking the BCF

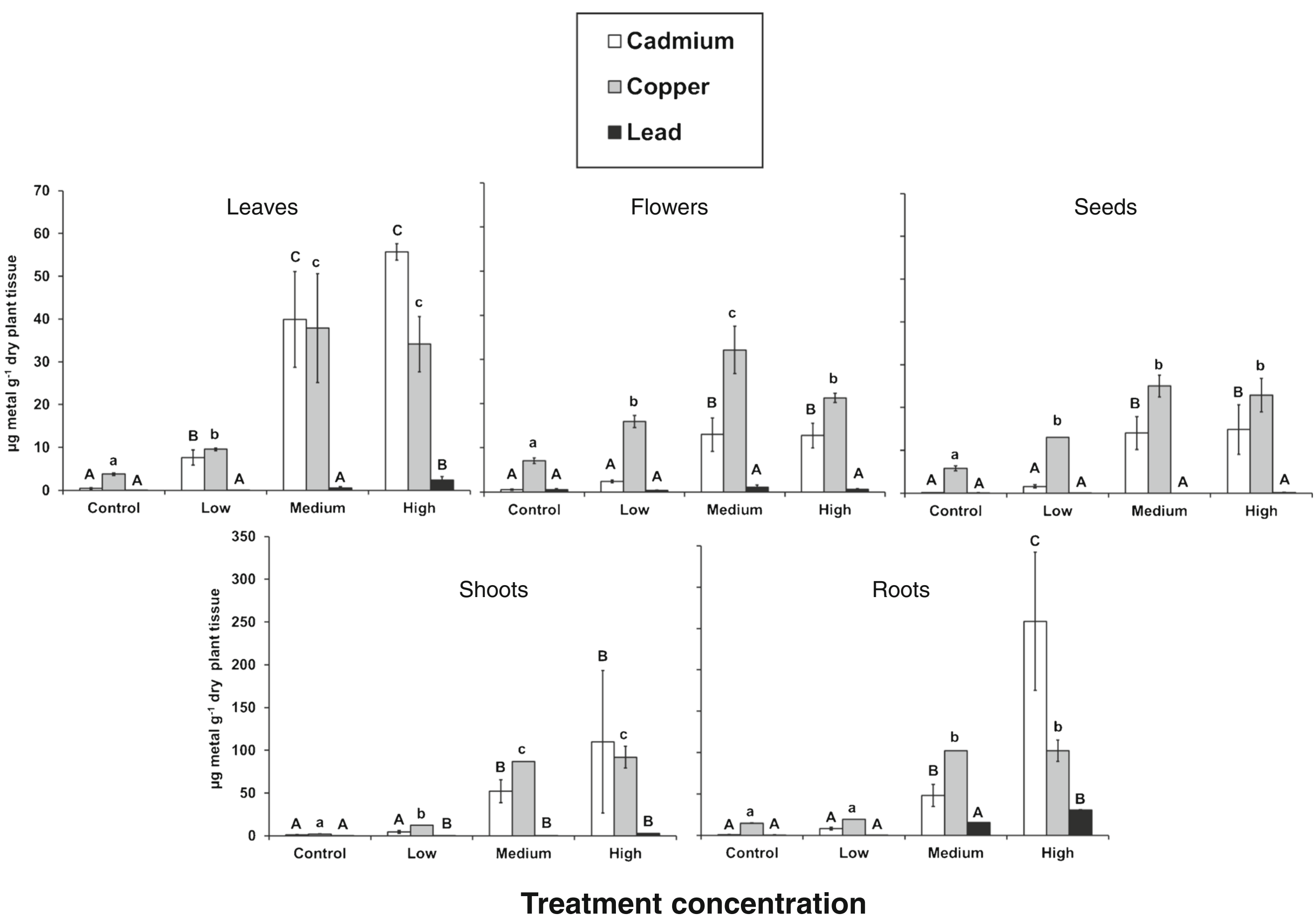

Fig. 3 Cadmium, Cu, and Pb accumulation in the leaves (*N*=2–6), flowers (*N*=4–6), seeds (*N*=1–5), shoots (*N*=1–4), and roots (*N*=1–5) of *Raphanus sativus* averaged over three time points. Shown are means ± SE. *Letters* above the means within each metal indicate statistically significant differences among groups using Tukey HSD test ($P<0.05$). All plant tissue concentrations are reported in μg of element per g of tissue dry weight (dw)

Table 4 ANOVA results for effects of each metal on the bioconcentration factor (BCF) in various plant organs of *Raphanus sativus*

Dependent variable - BCF	Metal	*F*	*df*	*P* value
Shoots	Cd	11.19	2,3	**<0.05**
	Cu	1217.79	2,2	**<0.001**
	Pb	0.87	2,2	0.53
Roots	Cd	3.99	2,4	0.11
	Cu	15.26	2,3	**<0.05**
	Pb	7.34	2,1	0.25
Flowers	Cd	20.22	2,10	**<0.001**
	Cu	97.99	2,11	**<0.001**
	Pb	15.64	2,2	**<0.001**
Seeds	Cd	7.62	2,8	**<0.02**
	Cu	630.34	2,4	**<0.001**
	Pb	0.57	2,2	0.64
Leaves	Cd	12.51	2,12	**<0.001**
	Cu	63.27	2,8	**<0.001**
	Pb	0.48	2,11	0.63

for flowers only, a pattern emerged of Cu > Cd > Pb, indicating radish has the ability to concentrate only certain metals in this reproductive organ. The overall TI ranking was Cu > Cd > Pb, suggesting that not only can plants readily accumulate Cu and Cd into the roots, they also can translocate it more easily to the aerial parts of the plant compared to Pb. Finally, when irrigated with high metal treatments, BCFs in all three metals decreased, indicating a potential phytotoxic effect at higher concentrations.

Discussion

Metal contamination of soils around the world has increased with the onset of urbanization and industrialization. Metals occur in natural and anthropogenically-disturbed ecosystems, and can be readily accumulated in a variety of crop and native plants. Our study demonstrated that weedy Brassicaceae species such as *R. sativus* may be able to maintain floral traits, and potentially attract pollinators, even when growing in contaminated areas. Pollinators may not be deterred by the presence of metals in floral rewards. Nickel concentrations in floral rewards as high as 300 mg Ni kg^{-1} did not deter floral visitors such as *Lasioglossum* bees and syrphid flies (Meindl and Ashman 2014). Selenium levels as high as 200 mg Se kg^{-1} did not deter honey bees from foraging on *R. sativus* flowers (Hladun et al. 2013b).

Previous studies of several plant species (including *R. sativus*) demonstrated high mobility of Cd from soil to the above ground foliar parts (Das et al. 1997; Gupta et al. 2010). *Silene vulgaris, Noccaea caerulescens,* and *Mattiola flavida* accumulated similar levels of Cd into the shoots when grown in soils contaminated from smelters and mining activities (Mohtadi et al. 2012b). Despite absorbing high levels of metals into the leaves, some populations of these species experienced minimal phytotoxicity, as was also demonstrated with *R. sativus* in our study. *Raphanus sativus* accumulated substantial amounts of both Cu and Cd into the shoots and flowers in a dose-dependent manner but showed no signs of phytotoxicity in the reproductive organs. In stressful environments, ruderal plants such as *Raphanus sativus* experience a tradeoff between maintaining reproduction over vegetative development (Grime

Table 5 Translocation Indices (% of metal translocated from root to shoot, TI, averaged across treatments) and Bioconcentration Factors (BCF) of metals averaged within each treatment concentration in various plant tissues grown in semi-hydroponic irrigation solution. Numbers in parentheses are the tank replicates

	TI	BCF				
		Leaves	Flowers	Seeds	Shoots	Roots
Cadmium treatment	35 %					
Low		535±123 (5) a	768±128 (5) a	80±18 (5) a	1507±559 (2) a	2758±1055 (2) a
Medium		279±56 (5) b	248±113 (4) b	70±19 (3) a	3044±67 (4) a	2038±64 (2) a
High		52±12 (5) b	47±8 (5) b	7.4±2.9 (3) b	296±174 (2) b	676±268 (3) a
Copper treatment	46 %					
Low		478±15 (4) a	797±70 (4) a	652 (1) a	628 (1) a	958±71 (2) a
Medium		189±64 (3) b	161±27 (6) b	125±13 (3) b	434 (1) b	511±188 (2) a
High		17±3 (5) c	11±1 (4) c	11±2 (3) c	46±6 (3) c	51±12 (2) b
Lead treatment	6 %					
Low		61±41 (6) a	766±143 (5) a	90±90 (2) a	26 (1) a	624 (1) a
Medium		121±61 (6) a	233±97 (4) b	0 (1) a	25±24 (2) a	3074 (1) a
High		48±18 (2) a	13±3 (5) b	3±1 (2) a	55±3 (2) a	614±393 (2) a

Means (±SE) followed by the same letter within columns of treatments are not significantly different according to Tukey HSD test (P<0.05)

1977). In our study, copper exposure reduced plant size, but flower number was preserved. If metal-accumulating plants can retain attractive floral resources even under stress, they likely will be visited by pollinators.

The levels of Cd found in *R. sativus* could have a detrimental effect not just on pollinators, but also on herbivores feeding on the shoots and roots. Cadmium reduced aphid population growth on plants grown in 200 mg Cd kg^{-1} soil (Kramarz and Stark 2003) and decreased leaf feeding damage by thrips at concentrations of 1869 mg Cd kg^{-1} shoot (Jiang et al. 2005). Although phloem feeders are more tolerant of high concentrations of Cd, leaf chewing herbivores suffer toxic effects at much lower levels. For example, metals in an artificial diet reduced survival of the diamondback moth at concentrations as low as 7.5 mg Cd kg^{-1} (Coleman et al. 2005). Gypsy moths reared on a contaminated food source had a No Observed Effect Concentration (NOEC) of 2 mg Cd kg^{-1} (Gintenreiter et al. 1993). In our study, shoots and roots accumulated over 100 mg Cd kg^{-1}, and phloem feeders as well as leaf feeding insects could be at risk. For florivores such as bees, floral concentrations as high as 13 mg Cd kg^{-1} in *R. sativus* may be toxic if the Cd reaches the pollen and nectar.

Susceptibility to Cu varies among insects. Although organisms require Cu for essential functions such as enzyme cofactors, the amount necessary is minimal (Scheifler et al. 2002), and ingestion of more metal than the required amount can be toxic (Jensen and Trumble 2003). In studies using an artificial diet, Cu reduced survival of the diamondback moth at 280 mg Cu kg^{-1} (Coleman et al. 2005). Gypsy moths reared on a contaminated food source had a NOEC of 10 mg Cu kg^{-1} (Gintenreiter et al. 1993). Cabbage aphids feeding on Cu-accumulating radish showed reduced fitness and developmental instability (Görür 2009). The minimum lethal concentration for *Spodoptera exigua* fed an artificial diet was 530 mg Cu kg^{-1} (Cheruiyot et al. 2013a). Biotransfer of Cu from a prey item (*S. exigua*) to its predator (*Podisus maculiventris*) caused sublethal effects of reduced development when prey items contained 77 mg Cu kg^{-1} (Cheruiyot et al. 2013b). In our study, Cu levels exceeded 50 mg Cu kg^{-1} in the shoots and roots, and 32 mg Cu kg^{-1} in the flowers. These levels could pose a risk to insects feeding on vegetative as well as reproductive tissues in terms of developmental or other sublethal effects.

Lead is not as phytotoxic to plants as Cd or Cu due to its limited availability and uptake from the growth substrate (Påhlsson 1989), so that lead in contaminated soils is typically not bioavailable (Davies et al. 2003). Similar to other studies using different plant species (Kapourchal et al. 2009; Mohtadi et al. 2012a, b; Salt et al. 1995; Sawidis et al. 2011), Pb was sequestered mainly in the *R. sativus* root system, where it binds to the root surface and cell walls (Påhlsson 1989). In our study with *R. sativus,* the levels in the above ground plant parts were not very high relative to the roots. Metals in artificial diets reduced survival of the diamondback moth at 15 mg Pb kg^{-1} (Coleman et al. 2005), and gypsy moth reared on a contaminated food source had No Observed Effect Concentrations (NOECs) of 4 mg Pb kg^{-1} (Gintenreiter et al. 1993). If root herbivores are as susceptible to Pb as shoot herbivores, they may suffer mortality when feeding on plants growing in Pb-contaminated soils. The Pb concentration in *R. sativus* flowers reached only about 1 mg Pb kg^{-1}, and Pb probably does not have a significant impact on pollinators.

Our study found that Cu has more potential than Cd or Pb for exposing pollinators to metal-contaminated floral resources in *R. sativus* even when the level of the contaminant is low. Although generally the literature demonstrates detrimental fitness effects on herbivores using higher levels of metals than those used in our study, low concentrations may result in chronic, sublethal effects on insect development or behavior. In addition, lower levels of contamination may allow for more bioconcentration, but with minimal phytotoxic effects on flower availability or attractiveness. Ruderal plants such as *R. sativus* growing in contaminated soils may tolerate metal stress by maintaining reproduction, and would most likely preserve their relationship with pollinators in the field. However, more research is needed to determine whether there are other cues that a metal-accumulating plant emits that might deter a floral visitor, or if the ingestion of metals through floral resources is detrimental to thepollinator.

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