

Effect of cadmium accumulation on mineral nutrient levels in vegetable crops: potential implications for human health

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Abstract Consumption of vegetables is often the predominant route whereby humans are exposed to the toxic metal Cd. Health impacts arising from Cd consumption may be influenced by changes in the mineral nutrient content of vegetables, which may occur when plants are exposed to Cd. Here, we subjected model root (carrot) and leaf (lettuce) vegetables to soil Cd concentrations of 0.3, 1.5, 3.3, and 9.6 $\mu\text{g g}^{-1}$ for 10 weeks to investigate the effect of Cd exposure on Cd accumulation, growth performance, and mineral nutrient homeostasis. The findings demonstrated that Cd accumulation in lettuce (20.1–71.5 $\mu\text{g g}^{-1}$) was higher than that in carrot (3.2–27.5 $\mu\text{g g}^{-1}$), and accumulation exceeded the maximum permissible Cd concentration in vegetables when soil contained more than 3.3 $\mu\text{g g}^{-1}$ of Cd. There was a marked hormetic effect

on carrot growth at a soil Cd concentration of 3.3 $\mu\text{g g}^{-1}$, but increasing the Cd concentration to 9.6 $\mu\text{g g}^{-1}$ caused decreased growth in both crops. Additionally, in most cases, there was a positive correlation between Cd and the mineral nutrient content of vegetables, which was due to physiological changes in the plants causing increased uptake and/or translocation. This may suggest a general mechanism whereby the plant compensated for disrupted mineral nutrient metabolism by increasing nutrient supply to its tissues. Increased nutrient levels could potentially offset some risks posed to humans by increased Cd levels in crops, and we therefore suggest that changes in mineral nutrient levels should be included more widely in the risk assessment of potentially toxic metal contamination.

Highlights • Edible parts of plants accumulated the highest Cd concentrations.

- Lettuce has a higher capacity for Cd uptake than carrot.
- Moderate Cd exposure has a hormetic effect on carrot growth.
- Cd levels positively correlated with nutrient mineral content in most cases.
- Changes in mineral nutrient levels should be included in risk assessment.

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Introduction

Trace metal contamination is widespread in ecosystems used for crop production (Wei and Yang 2010; Kabata-Pendias 2011), which may give rise to excessive levels of potentially toxic metals in agricultural products (Sarwar et al. 2010). Cadmium (Cd) is of particular concern due to its high toxicity and mobility in the soil-plant system (Kabata-Pendias 2011). Multiple anthropogenic activities such as metalliferous mining, energy and fuel use/production, sewage sludge disposal, and the application of both organic and inorganic fertilizers may contaminate soils with Cd (Environmental Agency 2007). Consequently, vegetables are grown in Cd-contaminated soil in many regions and are heavily exposed to Cd as a result (Yusuf et al. 2003; Wójcik et al. 2005; Demirezen and Aksoy 2006; Li et al. 2006). Cd is chiefly accumulated in plant roots and is generally excluded from seeds and, hence, cereal products, but certain leafy crops readily transfer Cd to the shoot (Yang et al. 2010; Laporte et al. 2015). Consequently, leafy vegetables and carrots show the highest Cd concentrations, accumulating 0.03–0.4 mg kg⁻¹ when grown in uncontaminated soils (Kabata-Pendias 2011). Vegetable crops, therefore, represent an important source of Cd in the human diet, accounting for 70–90 % of total Cd intake (Sarwar et al. 2010). Long-term exposure to Cd may give rise to a variety of health problems, such as itai-itai (ouch-ouch) disease, osteoporosis and osteomalacia, growth retardation, and diseases associated with malnutrition (Environmental Agency 2009).

A further risk factor for Cd is that this element has a higher toxicity to animals than plants (Environment Agency 2009). In addition, the relationship between the level of Cd exposure and plant growth is complex and plant biomass can be increased by elevated Cd exposure, before higher concentrations induce a negative effect (Wójcik et al. 2005; Gonçalves et al. 2009). Thus, humans may ingest potentially harmful levels of Cd when consuming visually healthy plants. Moreover, Cd can also affect the concentration and distribution of mineral nutrients within plants and this has been postulated as one mode of action in Cd toxicity (Aery and Rana 2003). However, reported results regarding the interaction of Cd with other mineral nutrients are contradictory and the mechanisms of interaction are not completely clear (Liu et al. 2003a, 2003b; Liu et al. 2011; Sikka and Nayyar 2012). For example, Cd has been reported to have both positive and negative effects on the concentrations of Cu, Zn, Mg, Mn, and Cu in plants (e.g., Monteiro et al. 2009; Li et al. 2016). The effect that altered mineral nutrient levels may have

on the health of people consuming vegetables from Cd-contaminated soils has been overlooked. Nevertheless, this may be very important to communities already consuming nutrient-deficient diets, as this may increase Cd accumulation and may also exacerbate Cd-related malnutrition (Brzóska et al. 2001; Brzóska and Moniuszko-Jakoniuk 2001).

Many investigations into the effect of Cd on mineral nutrient concentrations in plants have used high concentrations of Cd and/or atypical growing conditions (e.g., hydroponics), which can strongly influence the effect of Cd on mineral nutrient uptake and translocation (e.g., Liu et al. 2003b; Gonçalves et al. 2009). Moreover, effects of Cd on plant growth have not been taken into account. Consequently, it is not clear if changes in the concentration of mineral nutrients in response to Cd are due to concentration/dilution effects from altered growth, changes in plant physiology, or both. In the present study, therefore, we used lettuce (*Lactuca sativa*) as a leaf vegetable model and the carrot (*Daucus carota*) as a root vegetable model to test the effect of realistic levels of Cd soil contamination on Cd accumulation and mineral nutrient uptake and distribution. Specifically, the objectives were to (i) determine and compare the tissue-specific Cd bioaccumulation in the vegetables, (ii) to examine and compare the effect of Cd exposure on growth performance of the vegetables, and (iii) to investigate and compare the mineral nutrient homeostasis in the vegetables when exposed to elevated soil Cd.

Materials and methods

Greenhouse trial

A bulk sample of a sandy loam top soil (Westland Horticulture Ltd., Dungannon, UK) was split into four equal parts, each of 10 kg. Three parts were treated with Cd (in the form of a CdCl₂ solution containing 1000 mg Cd L⁻¹) to increase the soil concentration by 1, 3, and 9 μg g⁻¹ (dry weight), respectively. The fourth sample received no additional Cd to act as a control. Selected physiochemical parameters for the unamended top soil are shown in Table 1. Distilled water was added to treatments where necessary to ensure that an equal volume of liquid was added to all, and the soil was then homogenized by repeated mixing to ensure a homogenous distribution of Cd in the spiked soil. For each treatment, ca. 1 kg of soil was used to fill ten 1-L pots, which were then left for 290 days before seeding to allow the Cd to reach equilibrium with the solid phase of the soil. During this period, the pots were checked weekly and watered when the soil surface was dry. Soil samples were taken for analysis after this period.

In early April 2015, half of the pots in each treatment were seeded with crisphead lettuce (*L. sativa* L. cv. Webbs Wonderful) and the remaining pots with carrot (*D. carota* subsp. *sativus* (Hoffm.) Arcang. cv. Early Scarlet Horn).

Table 1 Selected physiochemical parameters for the unamended top soil

Physiochemical parameter	
Sand (%)	64.29 ± 1.06
Silt (%)	26.43 ± 1.75
Clay (%)	9.28 ± 2.04
Organic C (%)	5.07 ± 0.33
Total C (%)	5.80 ± 0.80
Total N (%)	0.25 ± 0.04
Total P (mg kg ⁻¹)	375.53 ± 14.14
Total K (mg kg ⁻¹)	2133.03 ± 72.11

Values are means ± SD ($n = 5$)

Thus, each Cd treatment was replicated five times for each vegetable type. The plants were watered on demand with distilled water during the experiment and were grown in a greenhouse with high-pressure sodium lighting used to supplement natural daylight to a 16-h growing period. Mean light intensity was 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Sampling and growth parameter measurement

The growth trial was terminated after 70 days, at which point whole plants were harvested and washed with distilled water to remove adhered soil. Plants were then separated into shoot and root, and both the length and fresh mass of each part were measured to the nearest millimeter and milligram, respectively. All samples were then dried at 80 °C for 72 h, and the dry mass was determined.

Determination of Cd and mineral nutrient content

To determine the Cd and mineral nutrient content of the plant tissues, ~0.25 g of dried material was digested in 10 ml of 70 % nitric acid (Fisher Scientific, Primar Plus Trace Metal grade) heated at 90 °C for 15 h. Samples were then evaporated to dryness, re-suspended in 10 ml of 5 % HNO₃, and filtered through a Whatman no. 42 filter paper. Analysis of the soil was conducted by refluxing ~0.3 g of the fine earth fraction (air dried soil <2 mm) in 12 ml of aqua regia for 48 h. Digested samples were dried and prepared for analysis using the method described for plant digests. Blanks and appropriate standard reference materials (ERM-CD281 rye grass or NWRI TH-2 sediment) were included in each batch digested for quality control.

Determination pH and organic matter contents in the soil

The pH of soil samples was determined in a suspension of 4.4 g of soil in 10 ml water left for 16 h. Organic matter

content of the soil was determined by the loss on ignition of ~6 g of oven-dried soil (24 h at 105 °C) heated at 450 °C for 12 h. All determinations were conducted in triplicate for each replicate.

Data calculation and analysis

The total Cd content in plant tissues was calculated from Eq. 1.

$$\begin{aligned} \text{Total Cd content in plant tissues} \\ = (\text{Cd content in plant tissues}) \\ \times (\text{dry matter of plant tissues}) \end{aligned} \quad (\text{Eq. 1})$$

The translocation factor (TF, %) from root to shoot for Cd was calculated from Eq. 2 and the bioconcentration factor (BCF) calculated from Eq. 3.

$$\text{TF} = 100 \times (\text{Cd content in shoot}) / (\text{Cd content in root}) \quad (\text{Eq. 2})$$

$$\text{BCF} = (\text{Cd content in plant tissues}) / (\text{Cd content in soil}) \quad (\text{Eq. 3})$$

Growth parameters of the plants were used to calculate Cd tolerance index (TI) from Eq. 4.

$$\text{TI} = (\text{growth parameter})_{\text{Cd}} / (\text{growth parameter})_{\text{control}} \quad (\text{Eq. 4})$$

where the growth parameter was the length or yield (dry mass) of plant part.

The differences in the tissue-specific growth performance, TF, BCF, TI, and Cd contents among treatments were analyzed using one-way analysis of variance (ANOVA) followed by a Tukey's honest significant difference (HSD) post hoc test for multiple comparisons.

Analysis of covariance (ANCOVA) was initially used to test the differences in mineral nutrient content among treatments using dry mass of plant tissue as the covariate to remove concentration/dilution effects caused by altered plant growth. However, the effects of covariate and interaction between the fixed factor (i.e., soil Cd treatment) and covariate were not significant ($P > 0.05$). Consequently, the covariate was removed from subsequent analysis and one-way ANOVA followed by a Tukey's HSD post hoc test was used to test the significance of differences among treatments (Table 3). Partial correlation coefficients (r) were calculated between the concentrations of Cd and mineral nutrients in the root, shoot, and the total plant, again controlling for the dry mass of the plant tissues to remove possible plant growth effects.

Normality and homogeneity of data were determined using Kolmogorov-Smirnov test and Levene's test, respectively. Difference was regarded as significant when $P < 0.05$. All statistical analyses were performed by the SPSS (vs. 18, SPSS Inc., Chicago, USA).

Results

Cd bioaccumulation, TF, and bioaccumulation factor

Mean concentrations of Cd in the soils were 0.3, 1.5, 3.3, and $9.6 \mu\text{g g}^{-1}$ for the control and 1, 3, and $9 \mu\text{g g}^{-1}$ treatments, respectively (Supporting Information STable 1). Soil pH ranged between 7.1 and 7.3 and organic matter between 18 and 24%. No statistical difference in soil pH or organic matter content was found amongst treatments ($P > 0.05$). In the high-Cd-exposure treatments, carrot roots had an evidently higher Cd content than shoots (Fig. 1a, $P < 0.05$). In lettuce, however, the Cd concentrations in the roots and shoots were very close in plants grown in the same soil Cd treatment ($P > 0.05$). Notably, a comparison of Cd accumulation in the two vegetables grown at the same soil Cd concentration showed that lettuce accumulated markedly higher concentrations of Cd compared to carrot in both root and shoot (Fig. 1a, b). Moreover, the TF in carrot steadily decreased from 129 to 29% as the soil Cd concentration increased, whilst in lettuce, the highest TF (216%) occurred at $1 \mu\text{g g}^{-1}$ Cd.

The total Cd content in carrot displayed a steady rise with increasing soil Cd (Fig. 1c, $P < 0.05$), whereas for lettuce, total Cd content reached a maximum in the $3 \mu\text{g g}^{-1}$ Cd treatment (Fig. 1d, $P < 0.05$). The proportion of Cd in root to total Cd content ranged between 17 and 41% in carrot and was clearly higher than in the lettuce (8–20%, Fig. 1c, d).

The Cd bioconcentration factor (BCF) in carrot ranged from 1.25 to 4.76 (Fig. 2a), which was lower than the values in lettuce (7.51–12.92, Fig. 2b). Carrot root showed an increasing BCF as soil Cd concentration increased, but the shoots and total plant showed steadily decreasing BCF with increasing soil Cd ($P < 0.05$). In lettuce, the root BCF was highest in the $3 \mu\text{g g}^{-1}$ Cd treatment ($P < 0.05$).

Effect of Cd exposure on growth, Cd TI, and moisture content

Carrot root length was significantly lower in plants exposed to 3 and $9 \mu\text{g g}^{-1}$ Cd compared to those exposed to $1 \mu\text{g g}^{-1}$ Cd, but the shoot length was similar among the different treatments (Fig. 3a, $P < 0.05$). In lettuce, root length was not clearly impacted by Cd exposure, whereas the shoot length decreased steadily with increasing soil Cd concentration (Fig. 3b, $P < 0.05$).

The dry yield of carrot was significantly lower in the $9 \mu\text{g g}^{-1}$ Cd treatment and was highest in plants subject to $3 \mu\text{g g}^{-1}$ Cd exposure (Fig. 3c, $P < 0.05$). For lettuce, the $9 \mu\text{g g}^{-1}$ Cd treatment also resulted in the lowest dry yield of roots and shoots (Fig. 3d, $P < 0.05$).

The highest soil Cd treatment ($9 \mu\text{g g}^{-1}$) led to the lowest tolerance index (TI) for the length of plant parts and yield

(Table 2). In addition, the TI of tissue yield in carrot was greatest at a Cd treatment of $3 \mu\text{g g}^{-1}$, whilst it decreased steadily with the increasing soil Cd concentrations in lettuce.

Effect of Cd exposure on the homeostasis of mineral nutrients

With the exception of P and Zn, soil Cd exposure significantly affected the concentration of mineral nutrients in the two crops (Table 3, $P < 0.05$). There were also significant positive correlations between the concentration of Cd and several mineral nutrients (Table 4, $P < 0.05$). Specifically, carrots exposed to $9 \mu\text{g g}^{-1}$ Cd had the highest Na, Cu, and Ni contents in all tissues; the highest K and Mg contents in the root; and the highest Ca content in the shoot and whole plant (Table 3). A significant and positive correlation was observed between the concentration of Cd and the concentration of Na in the shoot, Mg and Ni in the root, and Ca in shoot and leaf and total plant (Table 4). The lettuce showed the highest contents of Na, Ca, and Mg in shoots and total plant; the highest contents of K in the root and shoots; and the highest contents of Fe, Cu, and Ni in all tissues. Only the Na content of lettuce roots was negatively correlated with the Cd content.

Discussion

The Cd bioaccumulation in the two vegetables

Overall, our findings revealed that lettuce accumulated Cd to higher levels than carrot at a comparable soil Cd exposure, suggesting that lettuce has the higher capacity for Cd uptake. This is consistent with previous findings; i.e., lettuce is classified as “high Cd accumulator,” and carrot is classified as a “moderate accumulator” (Kuboi et al. 1986). Moreover, we found that the roots of carrot accumulated higher Cd concentrations than the shoots at medium to high soil Cd exposure, whereas the root and shoots of lettuce accumulated comparable Cd in all soil Cd treatments.

The high capacity of leafy vegetables to bioaccumulate heavy metals may be explained by their morpho-physiological traits, e.g., high leaf area and transpiration and in terms of metal uptake, detoxification (e.g., synthesis of phytochelatins), and/or efflux (Wang et al. 2006; Akhter et al. 2014). For instance, Greger and Landberg (1995) reported that there was a positive relationship between shoot Cd and the total volume of water transpired in plants. Furthermore, wheat grown under high vapor pressure deficit (VPD) was found to uptake more Cd than wheat grown under low VPD (Salah and Barrington

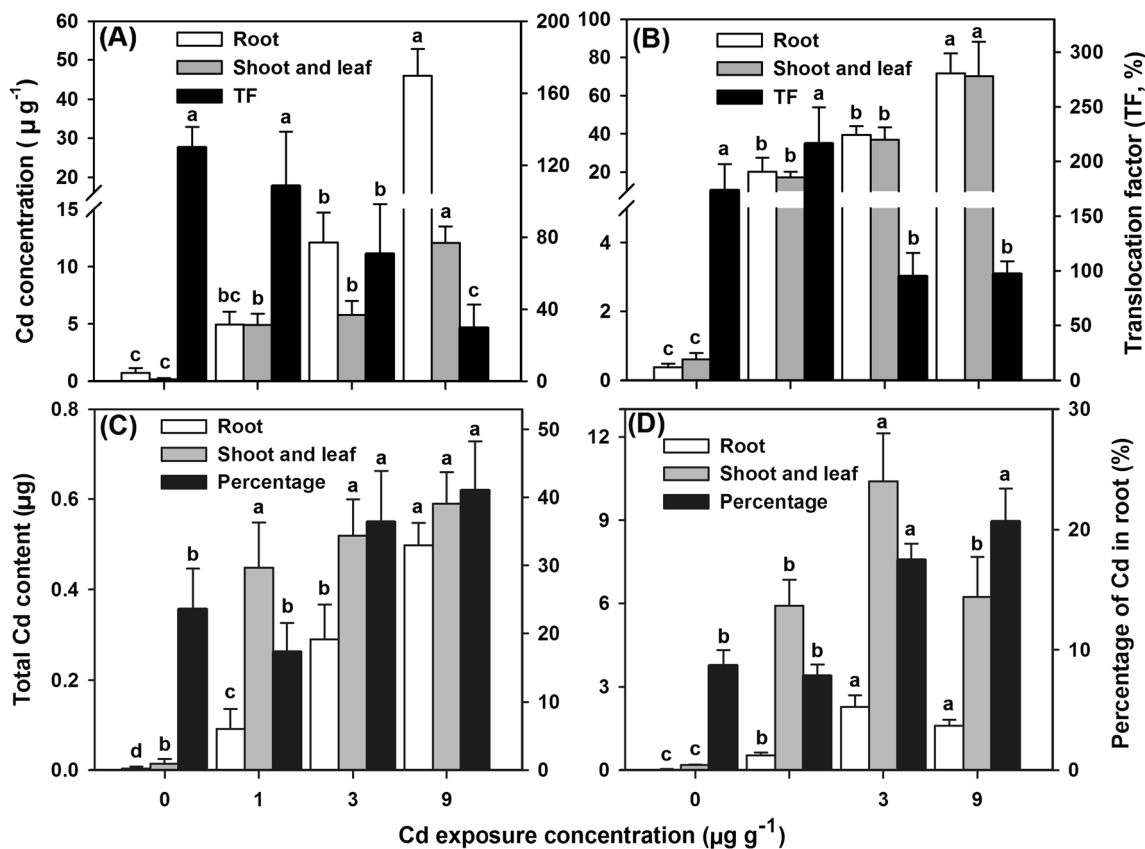


Fig. 1 The Cd concentration ($\mu\text{g g}^{-1}$ in dry matter) in the root and shoot and translocation factor (TF) of Cd from root to shoot in the carrot (a) and lettuce (b) and the total Cd content (μg) in the root and shoot and the percentage of root Cd to the gross Cd contents (%) in carrot (c) and lettuce

(d) exposed to soil Cd (0 (control), 1, 3, and 9 $\mu\text{g g}^{-1}$) for 70 days. Values are means \pm SD ($n = 5$). Significant effects of treatment on mean values for each measured parameter are denoted by *different letters* (one-way ANOVA, $P < 005$)

2006). Lettuce has a broader leaf with a larger area than carrot, so transpiration ought to be greater, which may

have largely accounted for the high Cd TF and Cd concentrations in shoots of lettuce.

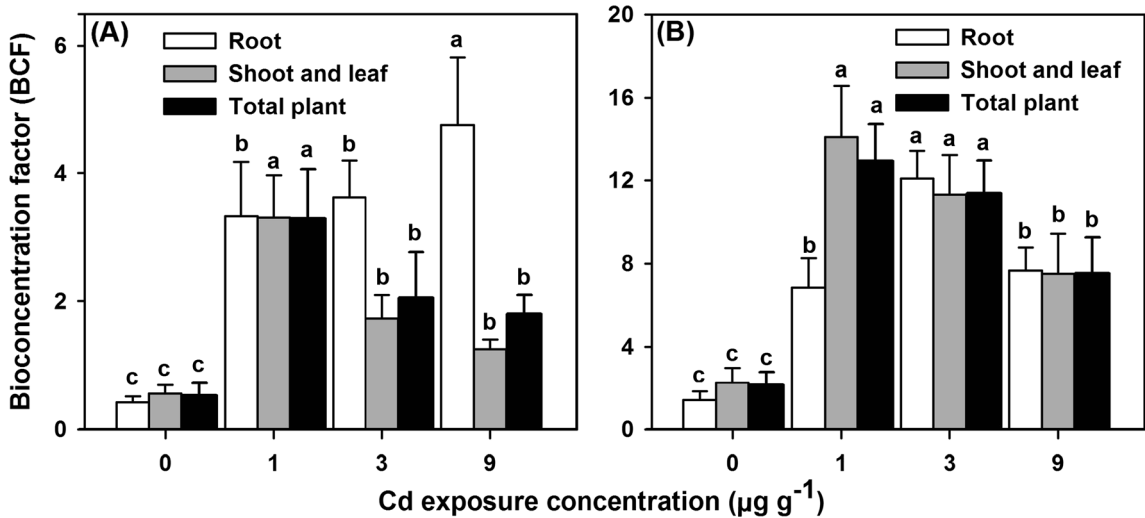


Fig. 2 The bioconcentration factor (BCF) of Cd in the root, shoot, and total plant of carrot (a) and lettuce (b) exposed to the soil Cd (0 (control), 1, 3, and 9 $\mu\text{g g}^{-1}$) for 70 days. Values are means \pm SD ($n = 5$). Significant

effects of treatment on mean values for each measured parameter are denoted by *different letters* (one-way ANOVA, $P < 005$)

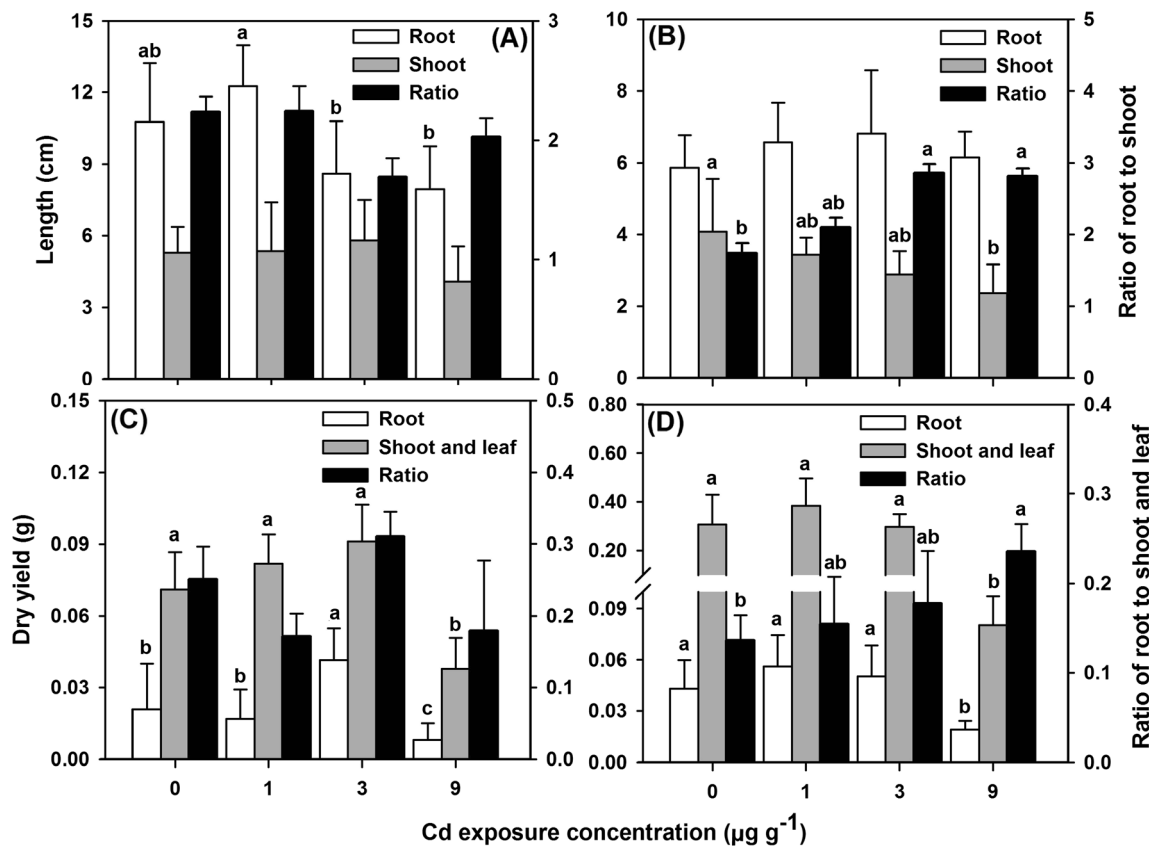


Fig. 3 Root length, shoot length, and their ratio in carrot (a) and lettuce (b) and the dry yield of root and shoot and their ratio in the carrot (c) and lettuce (d) after they were exposed to soil Cd (0 (control), 1, 3, and

9 $\mu\text{g g}^{-1}$) for 70 days. Values are means \pm SD ($n = 10\text{--}19$). Significant effects of treatment on mean values for each measured parameter are denoted by different letters (one-way ANOVA, $P < 0.05$)

Growth performance of the two vegetables exposed to soil Cd

Soil Cd exposure clearly inhibited the growth of carrot roots, whilst shoot growth of lettuce was similarly impacted. As the

root of carrot showed a clearly higher Cd content than the shoot in the high-Cd-exposure treatments, Cd accumulation in the root would seem the primary explanation for low growth (Chen et al. 2003). High Cd concentrations in roots might disturb redox equilibrium (Chen et al. 2003) and/or

Table 2 The tolerance index (TI) of carrot and lettuce exposed to soil Cd (1, 3, and 9 $\mu\text{g g}^{-1}$) for 70 days

Cd concentration in soil ($\mu\text{g g}^{-1}$)	Tolerance index (TI)				
	Root length	Shoot length	Root yield	Shoot and leaf yield	Total yield
Carrot					
1	1.14 \pm 0.25	1.01 \pm 0.30	0.81 \pm 0.19 b	1.15 \pm 0.29 a	1.23 \pm 0.28 a
3	0.80 \pm 0.13	1.10 \pm 0.21	2.00 \pm 0.09 a	1.29 \pm 0.34 a	1.50 \pm 0.30 a
9	0.77 \pm 0.19	0.79 \pm 0.18	0.39 \pm 0.14 c	0.53 \pm 0.12 b	0.67 \pm 0.11 b
Lettuce					
1	1.16 \pm 0.25	0.71 \pm 0.22 a	1.30 \pm 0.26 a	1.26 \pm 0.33 a	1.25 \pm 0.29 a
3	1.20 \pm 0.31	0.60 \pm 0.19 ab	1.17 \pm 0.24 a	0.96 \pm 0.21 a	1.07 \pm 0.36 a
9	1.08 \pm 0.20	0.48 \pm 0.16 b	0.44 \pm 0.17 b	0.26 \pm 0.11 b	0.34 \pm 0.19 b

Values of root length, shoot length, root yield, and shoot and leaf yield are means \pm SD of all individuals in five replicated pots. Mean values in the same column with different letters are significantly different from each other (one-way ANOVA, $P < 0.05$)

Table 3 The concentration (in dry matter) of nutritional elements (P, Na, K, Ca, Mg, Fe, Zn, Cu, and Ni) in the root, shoot, and total plant of carrot and lettuce exposed to soil Cd (0 (control), 1, 3, and 9 $\mu\text{g g}^{-1}$) for 70 days

Nutrient element and designed Cd exposure ($\mu\text{g g}^{-1}$)	Carrot			Lettuce		
	Root	Shoot and leaf	Total plant	Root	Shoot and leaf	Total plant
P (mg g^{-1})						
0	0.91 ± 0.25	0.78 ± 0.06	0.83 ± 0.17	0.57 ± 0.10	0.41 ± 0.04	0.43 ± 0.05
1	1.03 ± 0.22	0.93 ± 0.22	0.94 ± 0.21	0.60 ± 0.04	0.36 ± 0.07	0.40 ± 0.06
3	0.84 ± 0.14	0.71 ± 0.11	0.74 ± 0.11	0.62 ± 0.03	0.43 ± 0.05	0.46 ± 0.05
9	0.85 ± 0.15	0.70 ± 0.06	0.72 ± 0.05	0.65 ± 0.12	0.52 ± 0.16	0.54 ± 0.15
Na (mg g^{-1})						
0	8.30 ± 2.25 ab	2.46 ± 0.60 b	4.09 ± 1.91	3.27 ± 0.51	1.78 ± 0.52 b	1.97 ± 0.46 b
1	6.97 ± 1.13 ab	2.69 ± 0.41 b	3.49 ± 0.64	3.44 ± 0.53	2.19 ± 0.82 b	2.36 ± 0.72 b
3	5.57 ± 1.63 b	3.22 ± 0.88 ab	3.64 ± 1.19	2.60 ± 0.40	2.37 ± 0.8 b	2.40 ± 0.74 b
9	10.58 ± 2.03 a	4.47 ± 0.91 a	5.24 ± 1.29	2.61 ± 0.37	3.99 ± 1.05 a	3.67 ± 1.06 a
K (mg g^{-1})						
0	20.79 ± 2.91 b	35.46 ± 3.10 a	34.07 ± 2.22	12.37 ± 1.82 ab	25.59 ± 2.89 ab	23.77 ± 5.79
1	26.52 ± 3.01 ab	28.88 ± 2.53 ab	28.47 ± 2.03	10.53 ± 2.24 b	21.23 ± 2.39 b	19.53 ± 4.35
3	22.32 ± 2.97 b	24.86 ± 2.67 b	23.74 ± 1.80	12.99 ± 2.05 ab	27.68 ± 3.72 ab	25.04 ± 9.42
9	31.37 ± 3.17 a	24.98 ± 4.14 b	25.64 ± 2.02	19.96 ± 2.08 a	34.95 ± 4.05 a	32.33 ± 9.14
Ca (mg g^{-1})						
0	9.13 ± 1.66	25.69 ± 1.88	21.07 ± 3.46	7.31 ± 1.61	8.24 ± 0.87 b	8.09 ± 0.83 b
1	10.05 ± 2.28	26.92 ± 1.42	23.94 ± 3.44	6.29 ± 0.45	9.90 ± 1.60 b	9.27 ± 1.06 b
3	7.77 ± 2.48	25.52 ± 0.98	20.61 ± 3.27	7.27 ± 0.82	11.44 ± 2.04 ab	10.71 ± 2.17 ab
9	10.99 ± 3.06	33.08 ± 2.86	29.49 ± 2.69	7.88 ± 0.61	17.92 ± 2.94 a	16.84 ± 2.05 a
Mg (mg g^{-1})						
0	5.01 ± 1.26	2.28 ± 0.65	3.60 ± 1.85	3.97 ± 0.59	1.84 ± 0.11 b	2.10 ± 0.14 b
1	5.67 ± 1.71	2.25 ± 0.26	3.00 ± 0.50	4.68 ± 0.51	2.56 ± 0.27 ab	2.88 ± 0.48 ab
3	5.56 ± 1.28	1.97 ± 0.20	2.79 ± 0.34	5.25 ± 0.77	2.53 ± 0.55 ab	2.99 ± 0.15 a
9	8.81 ± 2.02	2.75 ± 0.41	3.70 ± 0.20	4.65 ± 0.43	2.91 ± 0.41 a	3.25 ± 0.27 a
Fe (mg g^{-1})						
0	2.75 ± 0.76	0.06 ± 0.03	0.75 ± 0.53	2.17 ± 0.43 b	0.05 ± 0.01 b	0.32 ± 0.10 b
1	3.26 ± 1.17	0.09 ± 0.03	0.65 ± 0.13	2.72 ± 0.33 ab	0.05 ± 0.01 b	0.45 ± 0.11 ab
3	2.08 ± 1.02	0.05 ± 0.02	0.50 ± 0.09	2.99 ± 0.19 ab	0.06 ± 0.02 b	0.50 ± 0.09 ab
9	2.90 ± 0.71	0.08 ± 0.03	0.54 ± 0.16	3.22 ± 0.21 a	0.11 ± 0.01 a	0.74 ± 0.14 a
Zn ($\mu\text{g g}^{-1}$)						
0	27.25 ± 4.95	26.88 ± 3.58	26.37 ± 3.80	28.50 ± 6.57	20.54 ± 4.05	21.48 ± 4.09
1	28.57 ± 5.64	27.25 ± 3.38	27.90 ± 4.21	23.07 ± 4.64	27.45 ± 3.57	26.62 ± 2.08
3	22.75 ± 6.92	21.94 ± 4.76	21.31 ± 3.98	19.23 ± 4.30	23.88 ± 7.22	23.09 ± 6.25
9	31.17 ± 4.07	25.79 ± 3.23	26.71 ± 5.27	25.68 ± 3.16	24.22 ± 3.62	24.38 ± 7.83
Cu ($\mu\text{g g}^{-1}$)						
0	20.90 ± 3.75 ab	17.82 ± 3.08	17.10 ± 1.95	11.05 ± 1.95 ab	4.43 ± 0.99 b	5.22 ± 1.17 b
1	18.60 ± 1.78 ab	14.00 ± 3.10	14.96 ± 2.00	7.98 ± 0.98 b	3.74 ± 1.66 b	4.34 ± 1.40 b
3	12.79 ± 2.65 b	13.12 ± 1.58	12.58 ± 1.43	10.68 ± 1.68 ab	4.06 ± 1.11 b	5.15 ± 1.56 b
9	34.17 ± 4.84 a	19.12 ± 2.20	21.14 ± 2.90	18.02 ± 2.29 a	7.39 ± 4.05 a	9.54 ± 4.79 a
Ni ($\mu\text{g g}^{-1}$)						
0	21.86 ± 3.96 b	6.47 ± 1.83 ab	11.92 ± 2.74 ab	4.78 ± 2.80 b	2.47 ± 0.68 ab	3.45 ± 0.68 ab
1	27.87 ± 6.62 b	5.86 ± 2.03 b	8.56 ± 1.61 b	6.44 ± 1.65 ab	1.47 ± 0.29 b	2.21 ± 0.42 b
3	22.08 ± 2.35 b	5.18 ± 1.24 b	8.81 ± 1.20 b	4.31 ± 1.68 b	2.82 ± 1.81 ab	3.04 ± 1.46 ab
9	49.26 ± 9.24 a	10.57 ± 1.56 a	16.58 ± 1.87 a	9.89 ± 0.80 a	4.62 ± 1.94 a	4.47 ± 1.53 a

Values are means ± SD ($n = 5$). Treatment means in the same column with different letters are significantly different from each other (ANOVA followed by a Tukey's HSD post hoc test for multiple comparisons, $P < 0.05$)

enhancement of reactive oxygen species level (Foyer and Noctor 2005), which often results in poor root growth. For lettuce, the reduction of shoot rather than root suggests a higher Cd tolerance in roots in relation to shoots as the two parts had comparable Cd contents (Monteiro et al. 2009). This confirms that the shoot is more sensitive to Cd than the root, and shoot growth could be used as a key indicator of Cd toxicity in leaf vegetables (Delp ere and Lutts 2008).

Surprisingly, the dry yields of both roots and shoots of the carrot were highest at 3 $\mu\text{g g}^{-1}$ Cd exposure, indicating a possible hormetic effect (a dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition; Calabrese 2008) on carrot growth, which was not observed in lettuce. The growth stimulation of carrot by low soil Cd concentration probably involves an adaptive compensatory process, e.g., an increase in cell division and proliferation

(Beyersmann and Hechtenberg 1997), low oxidative stress, and a positive effect on growth (Lin et al. 2007).

Effect of Cd exposure on the homeostasis of mineral nutrients

Cd has been frequently found to influence the absorption, transportation, and metabolism of mineral nutrients in plants (Liu et al. 2003a; Monteiro et al. 2009; Zorrig et al. 2010; Li et al. 2016). Cd can compete with other bivalent cations for the transport proteins necessary to move metal ions into and out of cells, and elevated Cd concentrations can thus affect the uptake and/or translocation of nutrient elements (Ramos et al. 2002; Sarwar et al. 2010). Cd may also affect the permeability of plasma membranes (Dong et al. 2006; Rezvani et al. 2012) and/or alter the conformation of proteins and, thus, the activity of enzymes (i.e., H⁺ATPases), transporters, or regulatory proteins (Astolfi et al. 2005), which may also interfere with the mineral nutrient uptake and transport.

The effect of Cd on the uptake and metabolism of mineral nutrients has been reported to be dependent on plant species, Cd concentration, metal species, plant development stage, and culture conditions (Liu et al. 2003a, 2003b; Monteiro et al. 2009; Li et al. 2016). However, reported effects are frequently contradictory and no clear understanding of the interactions between Cd and mineral nutrients is apparent. This is clearly problematic for the assessment of the risks posed by Cd contamination. The findings of present study clarify the nature Cd-mineral nutrient interactions under conditions of Cd exposure relevant to real world by showing a consistent finding in two contrasting species, which was a positive relationship between Cd and mineral nutrient content. A positive correlation between Cd and mineral nutrients has also been reported in three other species when Cd exposure concentrations were relatively low (Li et al. 2016; Liu et al. 2003a; Liu et al. 2011). Thus, under conditions of Cd contamination where crops are still likely to be grown for human consumption, it is highly

likely that plant accumulation of mineral nutrients would be enhanced.

Increased levels of elements in plant tissues can arise when growth is inhibited, but element uptake is not (Green et al. 2005). Our study demonstrated that the mass of plant tissues had an insignificant effect on mineral nutrient content, which implied that the observed changes in content were not due to reduced growth concentrating elements. Consequently, increased levels of mineral nutrients were due to physiological changes in the plants; i.e., uptake and/or translocation of mineral nutrients was enhanced. This has not been considered previously and may suggest a general mechanism whereby the plant increases nutrient supply to its tissues to compensate for disrupted mineral nutrient metabolism or an increased requirement for elements necessary for detoxification. For instance, increased P content can enhance the Cd detoxification and sequestration capacity (Sarwar et al. 2010). Ramos et al. (2002) also suggested that the higher Mn concentrations in lettuce (*L. sativa* cv.) might result in enhanced defense against Cd toxicity.

The implication for the assessment of risk and management of Cd-contaminated soil

In control group, the Cd level in carrot shoots was 0.4 μg g⁻¹, which is significantly lower than the legislative maximum permitted in Europe (EC, 2006). However, the Cd content in carrot roots (the edible part) was 3.2–27.5 μg g⁻¹ (Fig. 1a) when the plants were exposed to 1–9 μg g⁻¹ Cd in the soil, which exceeded the maximum permissible concentration (1 μg g⁻¹ dry weight (DW)). Similarly, Cd levels in the edible parts of lettuce (the shoots) grown in the Cd-treated soils also considerably exceeded the maximum permissible concentration (2 μg g⁻¹, Fig. 1b), with the Cd content ranging between 20.1 and 71.5 μg g⁻¹. Evidently, the consumption of vegetables grown in soil with >1 μg g⁻¹ Cd poses a high risk to human health. Since the UK Soil and Herbage Survey reported that estimated ambient background Cd concentrations in

Table 4 Partial correlation coefficient (*r*) between the concentrations of Cd and nutritional elements (Na, K, Na, K, Ca, Mg, Fe, Cu, and Ni) in the root, shoot, and total plant of carrot and lettuce exposed to soil Cd (0 (control), 1, 3, and 9 μg g⁻¹) for 70 days

	Na	K	P	Ca	Mg	Fe	Zn	Cu	Ni
Carrot									
Root	0.36 (0.09)	0.21 (0.24)	-0.03 (0.91)	0.39 (0.06)	<i>0.44 (0.04)</i>	0.15 (0.48)	0.12 (0.67)	0.24 (0.12)	<i>0.65 (< 0.001)</i>
Shoot	<i>0.60 (<0.001)</i>	-0.18 (0.22)	-0.11 (0.59)	<i>0.53 (0.09)</i>	0.24 (0.14)	0.10 (0.65)	-0.03 (0.90)	0.11 (0.57)	0.42 (0.07)
Total plant	0.35 (0.15)	-0.39 (0.13)	-0.30 (0.20)	<i>0.65 (<0.001)</i>	0.15 (0.52)	-0.18 (0.46)	0.15 (0.54)	0.29 (0.22)	0.44 (0.08)
Lettuce									
Root	<i>-0.41 (0.04)</i>	<i>0.63 (<0.001)</i>	0.26 (0.27)	0.23 (0.27)	0.31 (0.14)	<i>0.61 (0.011)</i>	0.01 (0.90)	<i>0.42 (0.13)</i>	<i>0.58 (0.01)</i>
Shoot	<i>0.57 (0.02)</i>	0.28 (0.16)	0.35 (0.08)	<i>0.69 (<0.001)</i>	<i>0.59 (0.008)</i>	<i>0.58 (0.005)</i>	0.21 (0.48)	<i>0.58 (0.09)</i>	<i>0.56 (0.02)</i>
Total plant	<i>0.65 (0.04)</i>	0.41 (0.07)	<i>0.48 (0.03)</i>	<i>0.84 (<0.001)</i>	<i>0.66 (0.002)</i>	<i>0.78 (<0.001)</i>	0.25 (0.33)	<i>0.56 (0.012)</i>	0.36 (0.12)

The *P* values are presented in the brackets and values of *r* in italics denote a significant correlation between the concentrations of Cd and the nutritional elements (*P* < 005)

rural soil range from 0.1 to 1.8 mg kg⁻¹ DW, with a mean of 0.39 mg kg⁻¹, and those for urban soils are between 0.1 and 2.39 mg kg⁻¹ DW, with a mean of 0.44 mg kg⁻¹ (Environment Agency 2007), monitoring and risk assessment are needed in the areas where the soil Cd is over 1 µg g⁻¹ Cd.

The higher BCF for lettuce compared to carrot suggested that lettuce poses a higher potential risk to human health than carrot when the two crops are exposed to comparable soil Cd concentrations (de Vries et al. 2007; Fairbrother et al. 2007). Thus, it is preferable to grow vegetables where the root is cropped rather than the leaf in soils contaminated with Cd up to 9 µg g⁻¹.

Along with Cd, other potentially toxic elements were also elevated in the edible parts of the vegetables. However, levels of Cu, Fe, and Zn in the two plants were below the maximum permissible concentrations (Supporting Information STable 2). Whilst there is a possibility of synergistic effects between potentially toxic elements, it is more likely that there will be an antagonistic effect on Cd toxicity through the reduction of Cd absorption/accumulation or prevention/alleviation of Cd toxicity (Sarwar et al. 2010; Rogalska et al. 2011) related to a reduced likelihood of deficiency. For instance, Fox et al. (1984) found that low levels of dietary Zn (11 µg g⁻¹) resulted in increased Cd absorption and retention in the small intestine and liver of Japanese quails (*Coturnix japonica*). Similar results were reported in rats (Brzóska et al. 2001), and Fe deficiency in mice resulted in a 2.6-fold increase in duodenal Cd concentration (Kim et al. 2007). Since increased levels of some mineral nutrients could offset some of the risk posed by Cd in vegetables, the effect of Cd exposure on mineral levels in plants should be taken into account in the assessment of the risks posed by Cd contamination.

Conclusions

Here, we characterized Cd accumulation and the concomitant changes in mineral nutrients in different types of vegetable crops (leaf vs. root vegetables). The results revealed that both plants primarily accumulated Cd in the edible parts, i.e., the shoot in lettuce and root in carrot, and this resulted in decreased growth performance in these tissues. In addition, the leafy vegetable showed a higher capacity for Cd accumulation and a higher potential risk to human health in comparison to the root vegetable. We further demonstrated a positive correlation between Cd and the content of most mineral nutrients in plant tissues. This was not due to a dilution effect; i.e., mineral nutrients were not concentrated in a smaller biomass but reflected physiological change in the uptake and/or translocation of these elements. Some of the affected elements, particularly Fe, may have an antagonistic effect on Cd uptake and toxicity in humans, helping to alleviate the potential harm

posed by increased Cd in vegetables. Based on these findings, we suggest that root vegetables pose a lower risk to human health in conditions of moderate contamination of the soil. We further suggest that changes in mineral nutrient levels should be included more widely in the risk assessment of potentially toxic metal contamination of soils, due to the potential effect that interactions between toxic metals and mineral elements may have on the safety of vegetable crops.

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