



Contribution of root uptake to cadmium accumulation in two peanut cultivars: evidence from a split-column soil experiment

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Abstract

Cadmium (Cd) accumulation and internal Cd translocation in the peanut (*Arachis hypogaea* L.) are highly related to root uptake, which may largely depend on the cultivar variation and the depth of the Cd-contaminated soil. A split-column soil experiment was conducted using two common Chinese peanut cultivars (*Huayu-20* and *Huayu-23*) known to relocate Cd to different tissues. The growth medium was separated into four layers and Cd solution was solely applied to one layer to determine the key depth affecting the Cd accumulation in a plant via root uptakes. The results showed that the biomass of *Huayu-23* was significantly higher biomass (3.28–94.0%) than that of *Huayu-20*, especially in the aerial parts (stems and leaves) and kernels, implying the dilution of Cd. Following the addition of Cd to the soil, the Cd concentrations in peanut tissues increased on average by 28.9–172 and 28.3–111% in *Huayu-20* and *Huayu-23*, respectively. The largest presence of Cd in a peanut plant was observed in the aerial parts, followed by the kernels. *Huayu-20* accumulated more Cd in plant tissues than did *Huayu-23* due to the former's high Cd translocation. These findings imply that peanut cultivars vary widely in biomass, Cd accumulation, and the percentage distribution of Cd among various plant tissues, especially kernels. Different Cd treatments in the full depth of the root zone induced significant alterations in Cd accumulation of peanut tissues, especially kernels, for both cultivars. The percentage distribution of Cd accumulation by kernels was significantly higher in the deeper layer than in the top layer of the root zone for both peanut cultivars. This study suggests that soil modifications performed during agronomic activities should take into account the full depth of root exploration as well as the peanut cultivars to manage plant Cd uptake.

Keywords Root uptake · Cadmium accumulation · Genotypic variation · Split-column soil · Peanut

Introduction

Peanut (*Arachis hypogaea* L.) is consumed by many people throughout the world due to the high contents of protein and

oil in its kernels (Giuffrè et al. 2016; Krishna et al. 2015). Unfortunately, cadmium (Cd) accumulation in peanut kernels can pose potential health risks if Cd-contaminated peanuts are directly consumed (Wang et al. 2016b). Cd is a non-essential toxic element and is widely reported to threaten food security and human health (Mahar et al. 2016; Wang et al. 2016a). Cd in peanut kernels may show high levels even if the source peanuts were cultivated in only slightly Cd-contaminated regions (McLaughlin et al. 2000). Thus, Cd accumulation in peanut kernels may present a challenge for safe peanut consumption and warrant attention (Zhang et al. 2013). Identifying the mechanisms involved in Cd accumulation is necessary to reduce Cd contents in peanut kernels.

Crop roots are the first contact organ with Cd-contaminated soil, and they play crucial roles in Cd accumulation and translocation (Daud et al. 2013). Research has demonstrated that peanut roots can very effectively extract Cd from cultivated soil and transfer it to kernels (Angelova et al. 2004). Variations in the extraction and transfer of Cd are widespread among

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peanut cultivars (Shi et al. 2014). Our previous research discovered that root uptake of Cd and internal Cd translocation are potentially the predominant mechanisms accounting for cultivar variations in Cd accumulation in peanut kernels (Wang et al. 2016b). Interestingly, previous research indicated that Cd uptake by peanuts occurs mainly via the true root system in the deeper soil layer (Bell et al. 1997; Mclaughlin et al. 2000). Based on these findings, Mclaughlin et al. (2000) suggested that soil modification should take the full depth of root exploration into account when using agronomic techniques to manage Cd uptake. They found that only 1–3% of Cd in the kernels was directly absorbed by pods, regardless of cultivar. Furthermore, they found generally higher Cd concentrations in the peanut kernels of plants from field trials than in those of plants from pot trials in which the soil had been collected from the cultivated layer (0–20 cm) of the field site. They attributed this finding to the presence of significant levels of Cd to approximately 60 cm in the soil profile and a general decline in soil pH with depth (Bell et al. 1997). However, since the publication of this research in 2000, no further research on this issue has been performed.

To fill this research gap, this research tested the hypothesis that Cd accumulation in peanut plants depends heavily on peanut cultivar and root uptake, which might vary with the depth of root exploration. A good method for simulating the full depth of plant root exploration is to conduct split-column soil experiments, as demonstrated in studies on the distribution and transport of heavy metals, nutrient elements, and soil microbial properties (Porfiri et al. 2015; Ronchi et al. 2015; Haller et al. 2016). To test the hypothesis, this research conducted a split-column soil experiment and aimed to (1) investigate the effects of cultivar and Cd-polluted soil depth on Cd accumulation and Cd distribution among different tissues in two peanut cultivars that differ in Cd translocation; and (2) determine the relationships between plant Cd accumulation and root Cd uptake based on the results of the split-soil column experiment.

Materials and methods

Plant materials and pretreatments

Two Chinese peanut (*Arachis hypogaea* L.) cultivars, i.e., *Huayu-20* and *Huayu-23*, were selected due to their insensitivity to Cd stress and low Cd uptake. These two cultivars are widely planted for local consumption or for profitable export in Shandong province, a core peanut production base in China. They were also selected due to their differences in internal Cd translocation, which potentially results in different responses to Cd stress. The efficiency of internal Cd translocation is high and low for *Huayu-20* and *Huayu-23*, respectively. Prior to planting, peanuts seeds were surface-sterilized

with 2% (v/v) of NaClO for 10 min and thoroughly rinsed with deionized water. The sterile seeds were then germinated as described in our previous research (Wang et al. 2016b).

Basic soil characteristics and soil preparation

The experimental soil was collected from a long-term experimental farm of Qingdao Agricultural University in Shandong Province, China (36° 59' N, 120° 43' E). All of the collected soils were air-dried and ground. Cd concentrations and basic soil characteristics were analyzed according to the methods in our previous research (Wang et al. 2014; 2016b). Subsamples of soil were sieved through a 0.147-mm mesh sieve for analysis of the Cd concentrations in raw soil and basic soil characteristic. Briefly, the experimental soil was classified as a sandy loam (FAO classification) with a high level of organic matter (7.5 g kg⁻¹), a pH of 7.2, and an average soil water holding capacity (WHC) of 41.6%. The total Cd concentration and the extractable Cd concentration of the selected soil were 0.12 and 0.002 mg kg⁻¹, respectively.

Then, addition subsamples of soil were sieved through a 2-mm mesh sieve. To obtain the total Cd concentration of 1 mg kg⁻¹ in soil, 250 l of Cd²⁺ solution was added to 250 kg of soil at a concentration of 1 mg l⁻¹. Cd in soil was then homogenized by incubating the mixture of soil-Cd solution at 25 °C for approximately 60 days. The total Cd concentration in soil was adjusted to 1 mg kg⁻¹. The soil was then ground and sieved through a 2-mm mesh sieve again for the split-column soil experiments.

Split-column soil experiment

To determine the relationship between Cd in peanut plants and in various soil layers, split-column soil experiments were implemented using polyvinyl chloride (PVC) tubes. As shown in Fig. 1, the inner diameter (ID) and thickness of the PVC tube were 300 and 8 mm, respectively. The overall height of each PVC tube and the full depth of a soil column were 65 and 60 cm, respectively. The Cd-polluted soil (with a total Cd concentration of 1 mg kg⁻¹) and unpolluted soil were weighed and added into the corresponding layer of the PVC tubes according to the bulk densities of soil, which were 1.15 and 1.58 g cm⁻³ for the surface (cultivated) and deeper soil, respectively. The inner surface of each tube was pre-lined with plastic film to allow the full collection of the experimental soil. In addition, the bottom end of each tube was covered by a piece of nylon gauze of 1-mm meshes. The nylon gauze prevented contact between the soils of adjacent tubes while allowing the peanut roots to pass through. The vacant spaces between columns were filled and leveled using the farm soil to avoid a negative impact of column warming from direct solar radiation.

Fig. 1 Distribution and structure of the split-column soil

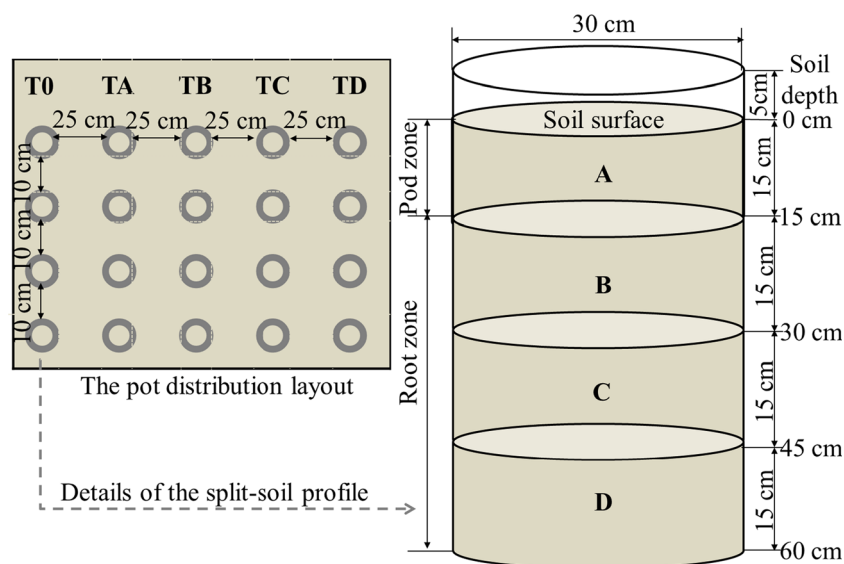


Figure 1 also illustrates the details of each PVC tube with the experimental soils. A 5-cm vertical section of each PVC tube was aboveground and without soil addition. The soil layers in each PVC tube were correspondingly divided into four top-down layers: a cultivated layer (A, 0–15 cm, pod zone) and three deeper soil layers (B–C–D, 15–60 cm, root zone). The depth of each soil layer and the total depth of all soil layers were 15 and 60 cm, respectively. The total Cd concentrations in different soil layers of each treatment are shown in Table 1. The experiment followed a completely random design with five treatments: T0, TA, TB, TC, and TD. The treatment T0 was designed as the control treatment, with no Cd-polluted soil in any layer. Each treatment was performed with four parallel replicates. There were 5 treatments and 20 tubes in total.

Prior to planting, the soil in the layer A of each tube was supplemented with analysis-grade urea, potassium dihydrogen phosphate, and potassium sulfate at rates of 0.13 g of N, 0.05 g of P, and 0.14 g of K per kilogram of soil, respectively. Tap water was then applied to the tubes to obtain a soil moisture content of 70% of the maximum field water holding capacity. Five pre-germinated peanut seeds were sowed isometrically the cultivated layer (A) of each tube. Three well-developed seedlings finally were retained in each

tube, and the remaining seedlings were shredded in the tubes. Each tube was irrigated with 500-ml tap water every 5–7 days during the dry season.

Plant biomass and Cd analysis

The peanut plants were harvested 129 days later. Peanut plants were cleaned using a neutral detergent or deionized water and were separated into seven parts: roots, leaves, stems, pegs (gynophores), shells, testas, and kernels. The root subsamples were cleaned and rinsed with deionized water. These plant materials were then dried at 105 °C in a forced draught until reaching constant weight. The dried plant materials were ground, sieved through a 100-mesh sieve, and weighed. The subsamples were digested with an HNO₃-HClO₄ acid mixture (5:1). The total Cd concentrations in the digested solutions were determined by atomic absorption spectrophotometry (AAS, TAS-990AFG, PERSEE, China). Quality assurance and quality control procedures were performed using duplicates, method blanks, and standard reference materials (GBW07605). The average recovery of Cd was 99.4 ± 6.11%. The standard error of the duplicated samples was less than 5%.

Table 1 Total Cd concentrations of each soil layer in the PVC tubes

Treatment	Total Cd concentration in the soil of each layer in the PVC tubes (mg kg ⁻¹)			
	A	B	C	D
T0	0	0	0	0
TA	1	0	0	0
TB	0	1	0	0
TC	0	0	1	0
TD	0	0	0	1

Statistical analysis

Descriptive data and statistical analyses were performed using SPSS v.16.0 and Origin v.8.0, providing the means ± S.D. (standard deviation) of the four replicates. All plant Cd concentrations were expressed on a dry weight basis. Statistically significant differences between two treatments were identified by one-way analysis of variance (ANOVA) using Duncan’s test at a significance level of 0.05. Differences were considered significant at the significance values greater than 0.05.

Results

Peanut plant biomass

As shown in Table 2, the biomass (dry weight) of *Huayu-23* was significantly greater (3.28–94.0%) than that of *Huayu-20*, both with respect to the entire peanut plant and to the tissues (roots, leaves, stems, pegs, shells, testas, and kernels). No significant difference in biomass was observed between the different Cd treatments for the entire peanut plant or for the individual tissues. However, significant differences were observed between the TC and TD treatments in the biomass of the roots and pegs for *Huayu-23*. There also were significant differences between the TB and TC treatments in the biomass of the stems and pegs for *Huayu-23*.

Peanut plant cadmium accumulation and distribution

Cd concentrations in peanut tissues are provided in Table 3. A significant cultivar difference in Cd concentration was observed only for the peanut shells, with higher Cd concentrations for the cultivar with high internal Cd translocation, *Huayu-20*. The plant Cd uptake coefficients of *Huayu-20* were significantly higher, by approximately 10%, than those of *Huayu-23*. The Cd concentrations in peanut tissues averagely increased by 28.9–172 and 28.3–111% when Cd was added to the soil for *Huayu-20* and *Huayu-23*, respectively, with the concentrations decreasing in sequence of leaves > roots > stems > testas > pegs > kernels > shells. Compared with the control treatment, the Cd treatments induced significant alterations in the Cd concentrations of peanut tissues and plant Cd uptake coefficients in both cultivars, regardless of whether Cd was applied to the cultivated (A, pod zone) or deeper soil layers (B, C, and D, root zone). Compared with applying Cd to the cultivated layer, applying Cd to the deeper layers altered Cd concentrations in roots by 25.8–35.0 and 20.0–79.5% for *Huayu-20* and *Huayu-23*, respectively, and altered the concentrations in kernels by 39.9–46.6 and 22.6–39.6% for *Huayu-20* and *Huayu-23*, respectively. When applying Cd to the deeper layers, significant differences in plant Cd uptake coefficients were observed between the TD (layer 45–60 cm) and TB/TC (layer 15–30 or 30–45 cm) treatments.

To analyze Cd distribution in peanut plants in more detail, the percentage distributions of Cd accumulation among different tissues of the plants were calculated, as illustrated in Fig. 2. Approximately half of Cd taken up by a peanut plant was

Table 2 Biomass of the two peanut cultivars in response to the different treatments (dry weight, g pot⁻¹)

Treatment	Roots	Leaves	Stems	Pegs	Shells	Testas	Kernels	Total
<i>Huayu-20</i>								
T0	6.0 ± 0.2abc	28.4 ± 0.5a	27.9 ± 0.5a	4.6 ± 0.1a	19.2 ± 0.7a	1.8 ± 0.1a	76.2 ± 2.3a	164.0 ± 1.7a
TA	5.9 ± 0.2abc	28.1 ± 0.5a	27.0 ± 0.7a	5.4 ± 0.2a	19.2 ± 1.4a	1.8 ± 0.1a	75.2 ± 4.3a	163.2 ± 6.0a
TB	5.8 ± 0abc	28.5 ± 0.3a	26.8 ± 1.1a	5.3 ± 0.4a	18.2 ± 0.4a	1.8 ± 0.1a	74.9 ± 4.0a	160.3 ± 2.8a
TC	5.6 ± 0.1a	27.2 ± 0.6a	27.4 ± 0.1a	4.4 ± 0.1a	17.6 ± 0.8a	1.7 ± 0.1a	73.4 ± 2.5a	157.3 ± 3.1a
TD	5.6 ± 0.1a	26.4 ± 0.4a	26.4 ± 0.2a	4.3 ± 0.1a	18.6 ± 0.8a	1.7 ± 0.1a	74.2 ± 3.1a	157.2 ± 4.0a
<i>Huayu-23</i>								
T0	6.1 ± 0.3bc	39.3 ± 0.6b	36.8 ± 2.0b	9.5 ± 0.5b	26.1 ± 0.7b	2.5 ± 0.1b	95.1 ± 2.3b	214.9 ± 4.8b
TA	5.9 ± 0.2abc	41.0 ± 0.2b	39.3 ± 1.6bc	9.0 ± 0.5bc	24.5 ± 0.5b	2.2 ± 0.1b	92.4 ± 1.8b	214.2 ± 0.4b
TB	6.0 ± 0.1abc	40.0 ± 1.8b	26.8 ± 1.1b	8.9 ± 0.5b	24.9 ± 0.5b	2.2 ± 0b	92.7 ± 1.7b	210.9 ± 4.1b
TC	5.7 ± 0.1abc	39.6 ± 1.2b	40.6 ± 1.5c	10.4 ± 0.5c	25.7 ± 0.2b	2.2 ± 0.1b	93.3 ± 3.7b	217.2 ± 2.4b
TD	6.2 ± 0.1c	39.0 ± 1.9b	39.6 ± 1.6bc	8.9 ± 0.2bc	26.1 ± 0.6b	2.3 ± 0.2b	92.3 ± 2.3b	214.3 ± 6.6b
Average								
<i>Huayu-20</i>	5.79 ± 0.06	27.7 ± 0.28	27.1 ± 0.3	4.8 ± 0.1	18.6 ± 0.4	1.7 ± 0	74.8 ± 1.3	160.4 ± 1.6
<i>Huayu-23</i>	5.98 ± 0.07*	39.8 ± 0.52*	38.5 ± 0.7*	9.4 ± 0.2*	25.5 ± 0.3*	2.3 ± 0.1*	93.2 ± 1.0*	214.3 ± 1.7*

Data in the same column followed by the same letter show no significant difference at the 0.05 significance level; an asterisk indicates the two peanut cultivars differ significantly at the 0.05 significance level. The same notation is employed in the following tables

Table 3 Cadmium concentrations in peanut tissues of the two different cultivars under different treatments (mg kg⁻¹, DW)

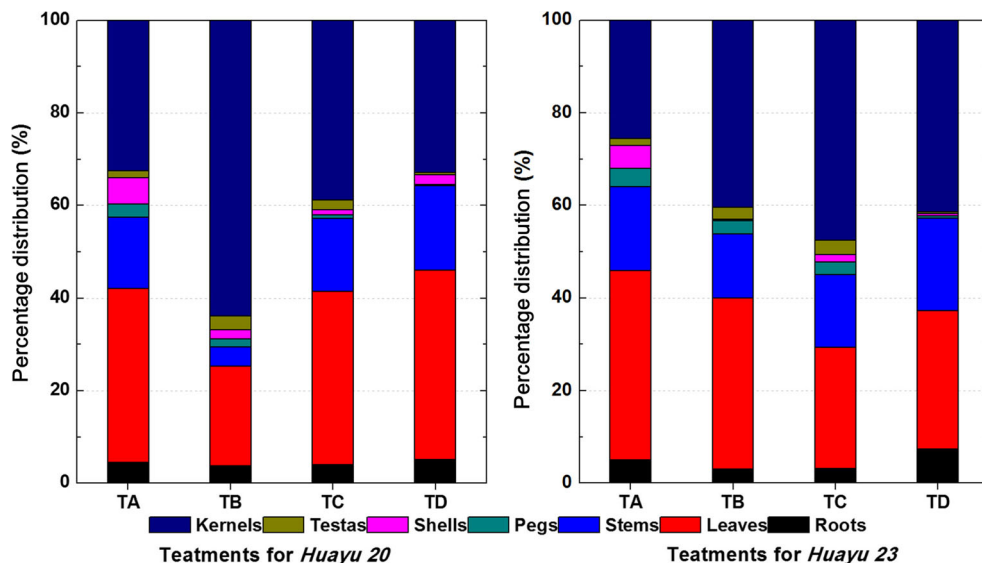
Treatment	Roots	Leaves	Stems	Pegs	Shells	Testas	Kernels	Plant Cd uptake coefficient (%) ^a
<i>Huayu-20</i>								
T0	0.75 ± 0.01a	1.03 ± 0.07a	0.62 ± 0.06a	0.61 ± 0.05a	0.26 ± 0.03b	0.40 ± 0	0.19 ± 0.03a	–
TA	1.63 ± 0.06f	2.60 ± 0.05g	1.31 ± 0.07e	1.12 ± 0.04c	0.61 ± 0e	1.38 ± 0	0.70 ± 0.07f	0.35 ± 0.02c
TB	1.06 ± 0.02cd	1.35 ± 0.08b	0.72 ± 0.06ab	0.66 ± 0.09ab	0.32 ± 0.02c	1.13 ± 0	0.56 ± 0.03e	0.08 ± 0.05a
TC	1.11 ± 0.02d	1.67 ± 0.03d	0.88 ± 0.07bcd	0.71 ± 0.10ab	0.31 ± 0.01c	0.96 ± 0	0.42 ± 0.01d	0.06 ± 0.01a
TD	1.21 ± 0.02e	1.80 ± 0.01e	0.97 ± 0.10d	0.68 ± 0.07ab	0.32 ± 0.01c	0.56 ± 0	0.39 ± 0.02c	0.23 ± 0.11b
<i>Huayu-23</i>								
T0	0.74 ± 0.04a	1.30 ± 0.01b	0.72 ± 0.09ab	0.60 ± 0.04a	0.17 ± 0.02a	0.41 ± 0	0.19 ± 0.02a	–
TA	1.78 ± 0.11g	2.45 ± 0.11f	1.23 ± 0.08e	1.18 ± 0.04c	0.43 ± 0.06d	1.33 ± 0	0.53 ± 0.01de	0.23 ± 0.07b
TB	1.00 ± 0.02bc	1.73 ± 0.06de	0.90 ± 0.03 cd	0.79 ± 0.07b	0.19 ± 0.02a	1.03 ± 0	0.41 ± 0.01d	0.04 ± 0.01a
TC	0.95 ± 0.04b	1.49 ± 0.03c	0.77 ± 0.12abc	0.63 ± 0.15a	0.20 ± 0.01a	0.89 ± 0	0.35 ± 0.03bc	0.02 ± 0.01a
TD	1.07 ± 0.04cd	1.54 ± 0.14c	0.81 ± 0.09abc	0.66 ± 0.03ab	0.18 ± 0.02a	0.50 ± 0	0.32 ± 0.01b	0.03 ± 0.02a
Average								
<i>Huayu-20</i>	1.15 ± 0.30	1.69 ± 0.55	0.90 ± 0.25	0.76 ± 0.20	0.37 ± 0.13	0.89 ± 0.37	0.45 ± 0.18	0.18 ± 0.04
<i>Huayu-23</i>	1.11 ± 0.37	1.70 ± 0.42	0.89 ± 0.20	0.77 ± 0.23	0.23 ± 0.11*	0.83 ± 0.35	0.36 ± 0.11	0.08 ± 0.03*

^a Percentage ratio of Cd present in the whole plant to Cd in soil if the accumulation of indigenous Cd is disregarded

accumulated by the aerial parts (the sum of the percentage distributions of Cd accumulation in the leaves and stems). Approximately 32.5–63.9 and 25.5–47.4% of Cd in the peanut plants were in kernels for *Huayu-20* and *Huayu-23*, respectively. The root systems accumulated approximately 3.75–5.14 and 2.98–7.33% for *Huayu-20* and *Huayu-23*, respectively. No significant cultivar difference in the accumulation of each tissue was observed between *Huayu-20* and *Huayu-23*. The percentage distribution of Cd accumulation in kernels was significantly higher in the TC treatment than in the remaining treatments for *Huayu-20*; whereas, it was

significantly lower in the TA treatment than in the other treatments for *Huayu-23*. No significant difference among the different Cd treatments was observed in the percentage distribution of Cd accumulation in roots for *Huayu-20*; whereas, significant differences were observed for *Huayu-23*. These findings imply that the root system plays crucial roles in Cd accumulation and translocation in a peanut plant. To verify this inference, the relationships between plant Cd accumulation (the aerial parts and kernels) and root Cd uptake were further analyzed, as described in the following section.

Fig. 2 Percentage distribution of cadmium accumulation among different tissues of the plant for the two peanut cultivars under different treatments (DW)



Relationships between plant cadmium accumulation and root cadmium uptake

Figure 3 illustrates the relationships between plant Cd accumulation (the aerial parts and kernels) and root Cd uptake. The correlation coefficients of the fitting lines all exceeded 0.6. The Cd accumulation contents (μg of Cd in a plant) of either kernels or aerial parts had a close relationship with those of roots for each peanut cultivar. Interestingly, the significance of the fitting relationship between root Cd uptake and Cd accumulation in the aerial parts decreased when the data from the two cultivars were pooled. The correlation coefficient of the fitting relationship between root Cd uptake and Cd accumulation in the aerial parts for the pooled cultivars between was observed to decrease for *Huayu-20* and *Huayu-23* separately.

Discussion

Previous studies have demonstrated that Cd accumulation and translocation in peanuts vary strongly with peanut cultivars (Shi et al. 2014; Wang et al. 2016b). Consistent findings were obtained in this study, with high cultivar variations in biomass, Cd re-distribution, and accumulation in plant tissues, especially in kernels for peanuts. This study investigated the responses of two peanut cultivars that differ in Cd translocation patterns. Cd uptake by plants (either via roots or through pegs and pods) and internal Cd translocation are the two important mechanisms that determine Cd concentration in the kernels of peanuts (Angelova et al. 2004; Wang et al. 2016b). This difference in Cd translocation explains why *Huayu-20* accumulated more Cd in plant tissues than did *Huayu-23*, indicating that the high Cd translocation cultivar (*Huayu-20*) is more sensitive to Cd stress. Cultivar differences, especially in kernel

Cd accumulation, therefore highlight the importance of screening for high- or low-efficiency internal Cd translocation cultivars (Lu et al. 2013; Wang and Li 2014). Moreover, previous research has indicated that the differences in seed composition may be related to differences in Cd accumulation and partitioning into kernels among peanut cultivars (Vogel-Mikuš et al. 2010). Approximately 80% of Cd in peanut kernels is mainly bound to proteins and little Cd is found in oil (Carrin and Carelli 2010; Wang and Li 2014). In this research, *Huayu-20* represents a high-protein peanut cultivar, and it had higher Cd concentration in kernels than did *Huayu-23*, which supports the view that the protein proportion is related to kernel Cd level. Therefore, cultivars, with a low-efficiency of internal Cd translocation or that are used only as a source of cooking oil, are more appropriate than are other cultivars for cultivation in Cd-contaminated fields.

Our previous research found that internal Cd translocation and root uptake are potentially the predominant mechanisms underlying cultivar variation in Cd accumulation in peanut kernels (Wang et al. 2016b). Peanut roots can very effectively extract Cd from growth medium and transfer it to kernels (Angelova et al. 2004).

These previous results are consistent with the findings of the present study that the Cd accumulation contents of either the kernels or aerial parts were closely related to those of roots in each peanut cultivar. The strong ability of peanut roots to extract Cd is evident from the variation in the percentage distribution of Cd uptake by roots among the different Cd treatments. Our findings are consistent with the important effects of crop roots, which affect Cd accumulation and translocation (Daud et al. 2013; Uraguchi et al. 2009). The difference between cultivars is also evidenced by the change in the significance of the fitting relationship between root Cd uptake and Cd accumulation in the peanut plants when the data from the two cultivars were pooled.

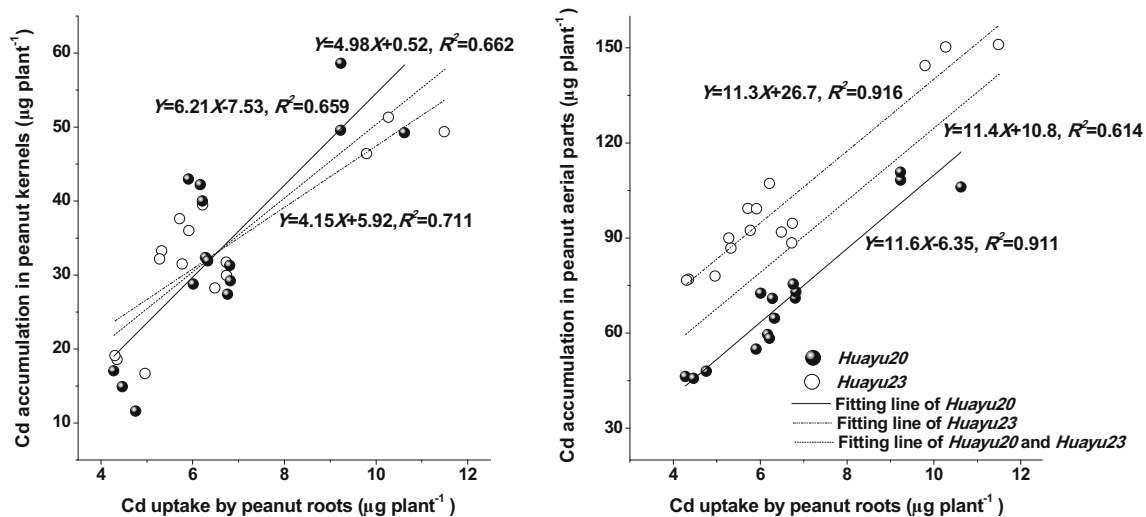


Fig. 3 Relationships between plant cadmium accumulation and root cadmium uptake of the two peanut cultivars under different treatments

A previous study suggested that Cd accumulation by peanuts occurs mainly via the true root system in the deeper soil layer (McLaughlin et al. 2000). However, the relationships between Cd uptake by roots and Cd accumulation and translocation in peanut plants, as well as the mechanisms to cultivar variation, remain unclear. This research gap provided the incentive for the present research. In addition to investigating the responses of two peanut cultivars that differ in Cd translocation patterns, the present study also aimed to investigate the role that the depth of the Cd-polluted soil plays in these processes. The growth medium was divided into four layers, and of which only one received added Cd; this was performed to identify the layer that is most important for Cd accumulation in the various tissues of peanut. The present study found that compared with applying Cd to the cultivated layer, applying Cd to deeper layers significantly altered Cd concentrations in roots by 25.8–35.0 and 20.0–79.5% for *Huayu-20* and *Huayu-23*, respectively, and altered the concentration in kernels by 39.9–46.6 and 22.6–39.6% for *Huayu-20* and *Huayu-23*, respectively. When applying Cd to the deeper layers, significant differences in plant Cd uptake coefficients were observed between the TD (layer 45–60 cm) and TB/TC (layer 15–30 or 30–45 cm) treatments; whereas, the percentage distribution of Cd accumulation in kernels was significantly higher in the TC treatment (a deeper layer) than in the TA (cultivated layer) treatment for both peanut cultivars. These findings are consistent with the observation that Cd uptake by peanuts occurs mainly from via the true root system in the deeper soil layer, with weaker effects in the cultivated layers (McLaughlin et al. 2000). This implies that the pathway of Cd translocation from the main roots to kernels is more efficient than is that from the pod shells and pegs. Therefore, higher concentrations of Cd in the deeper layers (the main layer for the true root system) might directly lead a higher concentration of Cd in the peanut kernels. Accordingly, Cd applied to the deeper layers rather than to the cultivated layer might be responsible for Cd accumulation in the roots of peanuts. This phenomenon is principally due to the larger biomass distribution when applying Cd to the cultivated layer. Lu et al. (2013) indicated that root morphology (especially of the fine roots) has great importance in determining Cd accumulation in peanuts. They found that excess Cd considerably decreased root lengths, root surface area, specific root length, and the number of root tips but increased the root diameters in peanut. Root-to-shoot Cd translocation via the xylem and into the transpiration stream is the process that largely determines shoot Cd accumulation in many crops (Xin et al. 2017). However, the Cd compartmentalization in the roots, including cell wall binding and vacuolar sequestration, limits the Cd translocation to the shoots via the xylem, which may be a critical mechanism in low Cd accumulation cultivars (Xin and Huang 2014). Moreover, actual field conditions are much more complicated than the conditions in the laboratory due to the influences of

numerous environmental factors, e.g., soil pH, temperature, water condition, Eh values, cation exchangeable capacity, and soil activity. These natural factors, which also may vary with soil depth, and their interactions strongly affect root Cd uptake, accumulation, and translocation (Wang et al. 2014; Ouyang et al. 2014). The actual mechanisms remain to be further investigated by designing more experiments and by employing more techniques (e.g., isotopic tracing and root morphology scanning), which have shown good performance in studying successive Cd movement from soils to crops (Lu et al. 2013; Salmanzadeh et al. 2017). Accordingly, soil modification should take the full depth of root exploration into account when using agronomic techniques to manage Cd uptake.

The results also showed non-significant and significant differences in the percentage distribution of Cd accumulation in roots among the different treatments for *Huayu-20* and *Huayu-23*, respectively. The two peanut cultivars differ with respect to the importance of the different Cd uptake routes of Cd and their impacts on Cd accumulation and distribution within the peanut plant (Wang et al. 2016b). Therefore, in addition to screening more appropriate peanut cultivars, agronomic techniques to manage Cd uptake will require modification of the soil to the full depth of root exploration rather than only to the surface strata where the pods develop.

Conclusions

The two peanut cultivars showed high cultivar variation in biomass, Cd accumulation, and percentage Cd distribution among various plant tissues, especially kernels. *Huayu-23* had significantly higher biomass production (3.28–94.0%) than did *Huayu-20*, especially in the aerial parts (stems and leaves) and kernels, leading to a metal dilution phenomenon. The Cd concentrations in peanut tissues were increased on average by 28.9–172 and 28.3–111% when Cd was added to the soil for *Huayu-20* and *Huayu-23*, respectively. The largest amount of Cd in a peanut plant was accumulated by the aerial parts, followed by the kernels. *Huayu 20* accumulated more Cd in plant tissues than did *Huayu-23* due to the former's high Cd translocation efficiency. Different Cd treatments in the full depth of the root zone induced significant alterations in the Cd accumulation of peanut tissues, especially kernels, in both cultivars. Cd accumulation contents of either kernels or aerial parts had a close relationship with those of roots for each peanut cultivar. The percentage distribution of Cd accumulation by kernels was significantly higher in the deeper layer than in the top layer of the root zone for the both peanut cultivars. Accordingly, this study suggests that agronomic techniques to manage Cd uptake should consider the peanut cultivars as well as the full depth of root exploration.

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