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Characteristics and Mechanisms of Soil Co-Contamination Affecting the Transfer of Cadmium and Arsenic in Peanut (*Arachis hypogaea* L.)

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Abstract: Soil co-contamination with cadmium (Cd) and arsenic (As) occurs frequently and has caused increasing concern. This study aimed to explore the transfer characteristics and the chemical forms, subcellular distribution of Cd and As, as well as the synthesis of phytochelatins (PCs) and other chelates in peanut (*Arachis hypogaea* L.) plants grown in a Cd and As co-contaminated soil, shedding light on the mechanisms involved. Compared with the single Cd contamination, Cd–As co-contamination led to a higher accumulation of Cd in peanut plants. Conversely, compared to the single As contamination, the As content increased in peanut shoots but decreased in roots and grains under Cd–As co-contamination. Furthermore, the Cd–As interaction resulted in notable changes in peanut plants' physiological and biochemical responses. In the roots and shoots, there was an 81.8% and 60.0% increase in water-soluble Cd. In the roots, metallothioneins (MTs) content increased by 50%, while PCs increased by 6.4% in the shoots. These changes promoted the translocation of Cd from roots to grains. The Cd–As interaction also influenced the synthesis of MTs in the roots, showing a 41.2% increase, and facilitated the transfer of As to the shoots. In peanut shoots, Cd increased the cell wall fraction of As by 34.5%, decreased the proportion of water-soluble As by 31.8%, and increased PCs content by 6.9%. These changes inhibited the migration of As from shoots to grains. Overall, Cd–As co-contamination increased Cd in peanut grains by increasing water-soluble forms and MTs in roots, while Cd–As co-contamination decreased As in peanut grains by increasing cell wall fractions and PCs in shoots. These findings provide a theoretical basis for understanding Cd–As interactions in soil–peanut systems.

Keywords: cadmium; arsenic; peanut; subcellular distribution; chemical forms

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1. Introduction

Heavy metal pollution has become a serious environmental issue globally, posing significant threats to living organisms, including humans [1,2]. Industrialization and urbanization have led to the release of toxic metals such as cadmium (Cd), arsenic (As), and others, resulting in ecological destruction [2]. In particular, Cd has emerged as a major concern, with contamination exceeding national standards in a significant portion of China's agricultural land [3]. As a metalloid, As is known to have severe health consequences, including cancer and various other disorders [4]. The most serious combined pollution of toxic elements in Chinese soil is the co-contamination of Cd and As, both geological and anthropogenic sources [5]. It is reported that the Cd and As concentrations in 7% and 2.7% of the soil samples exceed the national soil quality standards for these elements,

respectively [6]. The Cd hyperaccumulator *Sedum plumbizincicola* and As hyperaccumulator *Pteris vittata* have been identified and applied for the phytoremediation of Cd and As-contaminated soils [7]. Both Cd and As can enter the food chain through the uptake and accumulation by crops, posing significant risks to consumers [7]. Therefore, it is essential to investigate the transfer and accumulation characteristics of Cd and As in crops to better understand their behavior in contaminated environments and develop strategies to manage co-contaminated soils.

Previous studies have examined the individual effects of Cd or As on plants, providing valuable insights into their uptake, translocation, and sequestration mechanisms [8]. However, there is a controversial understanding of their combined effects and their interactions in soil-crop systems, as well as the underlying physiological and biochemical mechanisms. Some studies have reported synergistic effects, while others have shown contrasting results [9,10]. Furthermore, the subcellular distribution and chemical speciation of heavy metals play crucial roles in their translocation and accumulation in plants. However, research on the chemical speciation and subcellular distribution of heavy metals, particularly under low concentrations (non-toxic levels) of Cd and As co-contamination, is still lacking. Therefore, understanding the specific interactions between Cd and As in plants will enable the development of effective strategies for managing Cd–As co-contaminated soil.

Peanuts (*Arachis hypogaea* L.) are widely cultivated and consumed globally, making them an important crop for food production. Peanut is one of the three major oil crops in China. According to Food and Agriculture Organization (FAO) statistics, the global production of peanuts was about 48.76 million tons in 2019, and 35.93% was in China, ranking first in the world [11]. Peanuts are susceptible to heavy metal contamination, and their cultivation in Cd and As-co-contaminated soils raises concerns about the safety of peanut-derived products [12]. The maximum allowable limits of Cd (0.50 mg/kg) and As (0.50 mg/kg) in peanuts have been set in China (GB 2762-2022) [13]. It has been observed that peanuts have a high capacity to accumulate Cd, which significantly affects their yield and quality [14,15]. However, limited studies have focused on the uptake and translocation of Cd and As in peanuts in the context of Cd–As co-contamination. The underlying mechanisms of Cd–As interactions within peanut plants are still unknown. Therefore, it is crucial to investigate the transfer and accumulation characteristics of Cd and As in peanut plants and the associated mechanisms to understand their behavior and potential risks.

In this study, we hypothesized that there would be notable changes in the transfer characteristics and physiological and biochemical responses of peanuts grown in Cd and As-co-contaminated soil compared with single Cd or As contaminated soil. Therefore, the goal of this study was to explore the characteristics and mechanisms of soil co-contamination affecting the transfer of Cd and As in peanut plants grown in a Cd and As co-contaminated soil, providing mechanistic insights into Cd–As interactions in the soil–peanut system.

2. Materials and Methods

2.1. Materials Description

Peanut cultivar Ganhua No. 5 was selected for the experiment. Ganhua No. 5 is the main cultivated peanut cultivar in the study region and was derived from the parental peanut cultivar Yueyou551-11 by radiation. The soil type widely distributed in the region is Udic Ferrosol [FAO (1998) classification], known as the red soil in Chinese. The soil used was obtained from the upper layer (0–20 cm) of the cultivated soil. It was air-dried and sieved through a 10 mm sieve to eliminate plant material and ensure homogeneity. The principal characteristics of the soil were as follows: pH (H_2O), 4.96; organic matter (OM), 15.48 g/kg; cation exchange capacity (CEC), 11.73 cmol/kg; total nitrogen, 1.02 g/kg; total phosphorus, 0.62 g/kg; total Cd, 0.14 mg/kg; total As, 11.78 mg/kg.

2.2. Experimental Design

The outdoor pot experiment took place at the Ecological Experimental Station of Red Soil, Chinese Academy of Sciences, which was free from any sources of heavy metal pollution

in its vicinity. This site has a temperate monsoon climate with an annual average temperature and precipitation of 17.6 °C and 1750 mm, respectively; the main rainfall period is between April and June. Three Cd and As treatments were applied to the experimental soils based on the Risk Control Standard for Soil Contamination of Agricultural Land in China (GB 15618-2018) [16] and the prevailing conditions of soil pollution by Cd and As in China. To serve as sources of As and Cd, $Na_3AsO_4 \cdot 12H_2O$ and $3CdSO_4 \cdot 8H_2O$ were respectively added in appropriate quantities after thoroughly mixing them with soil samples (8 kg). Each plastic pot used for the experiment had dimensions of 30 cm in upper diameter and 26 cm in height. In total, four treatments were applied, including the control (CK, no Cd or As added to the soil), single Cd contamination (Cd, 0.6 mg/kg), single As contamination (As, 40 mg/kg), and Cd–As co-contamination (Cd + As, 0.6 mg/kg Cd + 40 mg/kg As).

The soil was then left for aging (under outdoor natural conditions) for about twelve months. During the aging, soil water contents were maintained at 80% of the maximum water-holding capacity by adding deionized water and weighing the pots every week. These pots were arranged in a randomized complete block design with a total of 4 treatments, each with 4 replicates.

After the completion of the aging period, soils were sampled and analyzed for total and available Cd and As. Through the determination of soil available Cd and As, we found that the contents of available Cd under Cd + As and Cd treatments were 0.29 ± 0.03 mg/kg and 0.21 ± 0.06 mg/kg, respectively. And the content of available As under Cd + As and As treatments was 9.76 ± 0.02 mg/kg and 8.65 ± 0.03 mg/kg, respectively.

Peanut seeds were sown in early April 2021 through the broadcast method. N (0.15 g/kg), P (0.05 g/kg), and K (0.1 g/kg) in the form of urea, calcium magnesium phosphate, and potassium sulphate were added as base fertilizers to maintain proper growth and development of the plants. The soils were watered to maintain normal growth during the peanut growing period. The peanut plants were harvested at the end of August after getting fully mature.

2.3. Soil and Plant Analysis

2.3.1. Sample Collection and Pretreatment

After harvest, peanut plants were divided into roots, shoots, shells, and grains. The roots were first soaked in 5 mmol/L $CaCl_2$ solution for 20 min and then rinsed in both tap and deionized water. The other plant parts were washed first with running tap water and then rinsed with deionized water three times. The fresh plant samples were divided into two parts; one was immediately used for physiological and biochemical analysis, and the other was dried at 105 °C for 30 min and then dried at 70 °C until a constant weight was attained, followed by the determination of the dry matter.

2.3.2. Determination of Plant Cd and As

Approximately 0.25 g dried plant samples in powder form were digested with 5 mL of nitric acid (GR) and 3 mL of hydrogen peroxide (GR) in a system of high-pressure sealed digestion vessels (HTLAB, HR-25, Shanghai, China); for detailed information, refer to our previous description [17]. The plant-certified reference materials (GSB-23a for Cd, GSB-6a for As, National Research Center for Certified Reference Materials, Langfang, China) were used to control the determination quality. The recovery rate of Cd and As ranged from 95% to 103% and 94–106%.

The distribution characteristics of Cd and As in peanuts were characterized by the bioconcentration factor (BCF) and translocation factor (TF). The bioaccumulation factor (BCF) was calculated as follows:

BCF = [Cd or As content (mg/kg) in a certain part of peanut]/[total Cd or As content (mg/kg) in soil];

The translocation factor (TF) was calculated as follows (take roots-grains as an example):

$TF_{roots\text{-}grains}$ = [Cd or As content in grain (mg/kg)]/[Cd or As content in root (mg/kg)].

Total accumulation amount of Cd or As in peanut = the content of Cd or As in each organ × the biomass of each organ (dry weight).

2.3.3. Subcellular Fractionation

The subcellular fractions of Cd and As in plant roots and shoots were extracted by differential centrifugation [18]. The frozen plant tissue (0.5 g) was added to a 10 mL extraction solution containing 0.25 mM sucrose, 50 mM Tris-HCl buffer solution (pH 7.5), and 1.0 mM DL-dithioerythritol to grind into a homogenate. The homogenate was centrifuged at the speed of 3000 r/min for 15 min and precipitated into "cell wall fraction", which was mainly composed of the cell wall and cell wall debris. The supernatant was further centrifuged at the rotational speed of 15,000 r/min for 30 min. The resulting precipitate and supernatant are called "organelle fraction" and "soluble fraction," respectively. All operations were carried out at 4 °C. The cell walls and organelle components were transferred into 100 mL conical flasks with deionized water, evaporated to dryness, and digested with 5 mL HNO_3 and 3 mL H_2O_2.

2.3.4. Extraction of Cd and As in Different Chemical Forms

The chemical forms of Cd and As in peanut shoots and roots were determined according to the method described by Wang et al. [19]. The chemical forms extracted according to the extraction order are:

(1) F_E: 80% ethanol to extract inorganic form;
(2) F_W: de-ionized water (d-H_2O) to extract water-soluble form;
(3) F_{NaCl}: 1 M NaCl to extract pectates and protein-integrated form;
(4) F_{HAc}: 2% acetic acid (HAc) to extract insoluble form;
(5) F_{HCl}: 0.6 M hydrochloric acid (HCl) to extract oxalate acid-bound form; and
(6) F_R: the last remaining residue form.

A total of 20 mL of extract solution (w/v, 1:10) was added into 2.0 g of the fresh sample in small quantities several times to grind and homogenize and then transfer to a 50 mL plastic centrifuge tube. After constant temperature shaking at 25 °C for 22 h, centrifuge at 5000× *g* for 10 min, pour out the supernatant, add 10 mL of extractant, shake at 25 °C for 2 h, centrifuge at 5000× *g* for 10 min, pour out the supernatant, and combine the two supernatants in 50 mL in a triangular flask. The above-mentioned supernatant and precipitation components separated by differential centrifugation and chemical reagent step-by-step extraction were put into a conical flask, placed on an electric hot plate (70 °C), evaporated to near dryness, transferred to a plant digestion tube, and 5 mL nitric acid and 3 mL of hydrogen peroxide was added to digest. After the digestion, the lid was opened to drive the acid to about 1 mL of liquid on an electric hot plate. Then, make up to 14 mL with 5% nitric acid (prepared with ultrapure water).

2.3.5. Determination of Soil Available Cd and As

Available Cd in soil was extracted with 0.1 M $CaCl_2$ solution. First, 5 g air-dried soil was sifted with 2 mm, then 25 mL extractant (soil-liquid ratio 1:5) was added, then oscillated at 210 r/min at 25 °C for 1 h, filtered by 0.45 μm filter. Available As in soil was extracted by 0.05 M $NH_4H_2PO_4$, air-dried soil (2 g) was equilibrated with 25 mL of 0.05 M $NH_4H_2PO_4$ at 250 r/min for 16 h, and the suspensions were centrifuged at 3500× *g* for 15 min, then the supernatants were filtered through 0.45 μm filters. The contents of Cd in digested samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (NexION 300, PerkinElmer, Shelton, CT, USA). And the contents of As were measured by hydride generation atomic fluorescence spectrometry (HG-AFS, AFS-610D2, Beijing Ruili Instrumental Company, Beijing, China).

2.3.6. Determination of Phytochelatins (PCs), Glutathione (GSH), Phytochelatins Synthetase (PCSase), and Metallothioneins (MTs)

A 0.1 g fresh sample was homogenized with 2 mL of pre-chilled phosphate buffer solution (PBS, 0.1 M, pH = 7.4) and then centrifuged at 12,000× *g* for 20 min at 4 °C to collect the supernatant for further analyses. Subsequently, the concentrations in the extracts were measured with corresponding reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Statistical Analysis

SPSS 25.0 was applied for statistical analyses. At the 95% confidence level, one-way ANOVA was conducted to compare the significant differences under different treatments. The data are drawn and generated by the OriginPro 2021 software.

3. Results

3.1. Accumulation and Translocation of Cd in Peanuts

The results presented in Table 1 demonstrate that the application of either a single contaminant or a combination of both did not have a significant impact on the dry weight of the different parts of the peanut. However, it was observed that the presence of As stimulated the accumulation of Cd in the roots, shoots, shells, and grains of the peanut plant (Table 2). Under the combined application of Cd and As, there was a substantial increase in the Cd content in the roots, shoots, shells, and grains by 33.0%, 42.9%, 27.8%, and 63.6%, respectively. Moreover, the total accumulation amount of Cd in peanuts increased by 41.0% compared to the single Cd contamination treatment.

Table 1. Dry weight of different peanut parts.

Treatments	Root	Shoot	Shell	Grain
CK	3.93 ± 0.50	82.5 ± 5.92	10.4 ± 1.41	45.5 ± 4.98
Cd	3.53 ± 0.35	88.6 ± 6.54	11.5 ± 2.00	46.3 ± 8.36
As	4.26 ± 0.32	85.7 ± 6.50	12.6 ± 2.48	38.3 ± 3.24
Cd + As	4.41 ± 0.28	85.9 ± 3.98	14.8 ± 1.23	47.6 ± 4.22

Mean values and standard deviations (n = 4) are presented. CK, Cd, As, Cd + As represent control, single soil Cd contamination, single soil As contamination, and Cd and As co-contamination treatment, respectively.

Table 2. Cd and As content, and total Cd and As accumulation amount in peanut.

Treatments	Root	Shoot	Shell	Grain	Total Accumulation Amount (μg)
		Cd Content (mg/kg)			Cd
CK	1.38 ± 0.30 c	1.76 ± 0.25 c	0.84 ± 0.09 c	0.13 ± 0.04 c	166 ± 25.0 c
Cd	8.81 ± 0.71 b	11.32 ± 1.81 b	4.74 ± 0.42 b	0.77 ± 0.14 b	1126 ± 189 b
Cd + As	11.72 ± 1.74 a	16.18 ± 3.96 a	6.06 ± 0.50 a	1.26 ± 0.30 a	1588 ± 334 a
		As Content (mg/kg)			As
CK	0.63 ± 0.06 c	0.48 ± 0.06 c	0.11 ± 0.02 c	0.13 ± 0.03 c	49.6 ± 8.11 c
As	4.06 ± 0.22 a	1.27 ± 0.18 b	0.79 ± 0.06 a	0.44 ± 0.09 a	153 ± 17.3 b
Cd + As	3.15 ± 0.09 b	2.05 ± 0.27 a	0.61 ± 0.08 b	0.34 ± 0.04 b	215 ± 23.0 a

Values (mean ± SD, n = 4) with different letters in the same column indicate significant differences between treatments at the 0.05 level. CK, Cd, As, Cd + As represent control, single soil Cd contamination, single soil As contamination, and Cd and As co-contamination treatment, respectively.

Based on the findings presented in Table 3, it is evident that there was no significant difference in the translocation factor between the addition of Cd alone and the co-contamination of Cd and As. The translocation factor, which represents the movement of Cd from roots to shoots, roots to shells, roots to grains, shoots to shells, shoots to grains, and shells to grains, remained relatively stable and unaffected by the environmental conditions.

Table 3. Translocation factor of Cd and As in different parts of peanut.

Treatments	Root-Shoot	Root-Shell	Root-Grain	Shoot-Shell	Shoot-Grain	Shell-Grain
			Cd			
CK	1.30 ± 0.14 a	0.62 ± 0.08 a	0.10 ± 0.03 a	0.48 ± 0.03 a	0.08 ± 0.02 a	0.16 ± 0.04 a
Cd	1.37 ± 0.04 a	0.54 ± 0.03 a	0.08 ± 0.00 a	0.39 ± 0.03 a	0.06 ± 0.00 a	0.15 ± 0.02 a
Cd + As	1.37 ± 0.17 a	0.53 ± 0.12 a	0.11 ± 0.01 a	0.40 ± 0.12 a	0.08 ± 0.01 a	0.21 ± 0.05 a
			As			
CK	0.77 ± 0.06 a	0.18 ± 0.03 a	0.20 ± 0.03 a	0.23 ± 0.04 c	0.26 ± 0.03 b	1.16 ± 0.26 a
As	0.31 ± 0.05 c	0.19 ± 0.02 a	0.11 ± 0.02 b	0.63 ± 0.11 a	0.35 ± 0.07 a	0.57 ± 0.15 b
Cd + As	0.65 ± 0.07 b	0.19 ± 0.02 a	0.11 ± 0.01 b	0.30 ± 0.05 b	0.17 ± 0.03 c	0.57 ± 0.08 b

Values (mean ± SD, n = 4) with different letters in the same column indicate significant differences between treatments at the 0.05 level. CK, Cd, As, Cd + As represent control, single soil Cd contamination, single soil As contamination, Cd and As co-contamination treatment, respectively.

3.2. Accumulation and Translocation of As in Peanut

The results presented in Table 2 indicate that the presence of Cd decreased As content in roots, shells, and grains while it increased the As content in shoots. Under the co-contamination of Cd and As, there was a noticeable decrease in the As content in roots, shells, and grains by 22.4%, 22.8%, and 22.7%, respectively. Conversely, the As content in shoots exhibited a significant increase of 61.4%. Moreover, the total accumulation amount of As in peanuts increased by 40.5% compared to the single As contamination treatment.

Based on the findings presented in Table 3, it is evident that under the co-contamination of Cd and As, there were notable changes in the translocation factors of root-shoot, shoot-shell, and shoot-grain. Specifically, the translocation factor of root-shoot showed a substantial increase of 109.7% compared to the single As contamination treatment. In contrast, the translocation factor of the shoot-grain displayed a significant decrease of 51.4% compared to the single As contamination treatment. This indicates that under the combined application, a lower proportion of As is translocated from the shoots to the grains of the peanut plant.

3.3. Subcellular Distribution of Cd and As in Peanut

The distribution of Cd in different subcellular components of peanut roots and shoots is shown in Figure 1. The results indicate the following hierarchy: cell wall fraction (F1) > soluble fraction (F3) > organelle fraction (F2). Under both single Cd contamination and Cd–As co-contamination, the cell wall components of Cd in root cells accounted for 72% and 66% of the total subcellular Cd in roots, respectively. In contrast, lower Cd levels were observed in the organelles (9–10%) and soluble components (18–24%) of peanut roots. Notably, under co-contamination, the proportion of Cd in soluble components in roots increased by 33.3% compared to single Cd contamination, indicating a significant increase in Cd transfer from roots to shoots. Similarly, in shoot cells, Cd was predominantly distributed in the cell wall components, accounting for 50% and 45% of the total subcellular Cd under single Cd contamination and Cd–As co-contamination, respectively. The soluble components accounted for 37–38% of Cd in shoot cells. Although lower Cd levels were observed in the organelles of peanut shoots (13–17%), the organelle component content of Cd increased by 30.8% under co-contamination compared to single contamination. Additionally, the proportion of Cd in cell wall components in shoots decreased by 10.0% compared to single Cd contamination, indicating an increased transfer of Cd from shoots to grains.

In root cells under As alone and Cd–As co-contamination, the distribution of As was predominantly observed in the cell wall components, accounting for 51% and 53% of the total subcellular As in roots, respectively (Figure 2). This was followed by the organelles (29–31%) and soluble components (18%). Similarly, in shoot cells under As single contamination and Cd–As co-contamination, As was primarily distributed in the cell wall components, accounting for 55% and 74% of the total subcellular As in roots, respectively. This was followed by the soluble

components (17–29%) and organelle components (9–16%). Notably, the cell wall components content of As in shoots with co-contamination increased by 34.5%, while the proportion of soluble components As decreased by 41.4% compared to single As contamination.

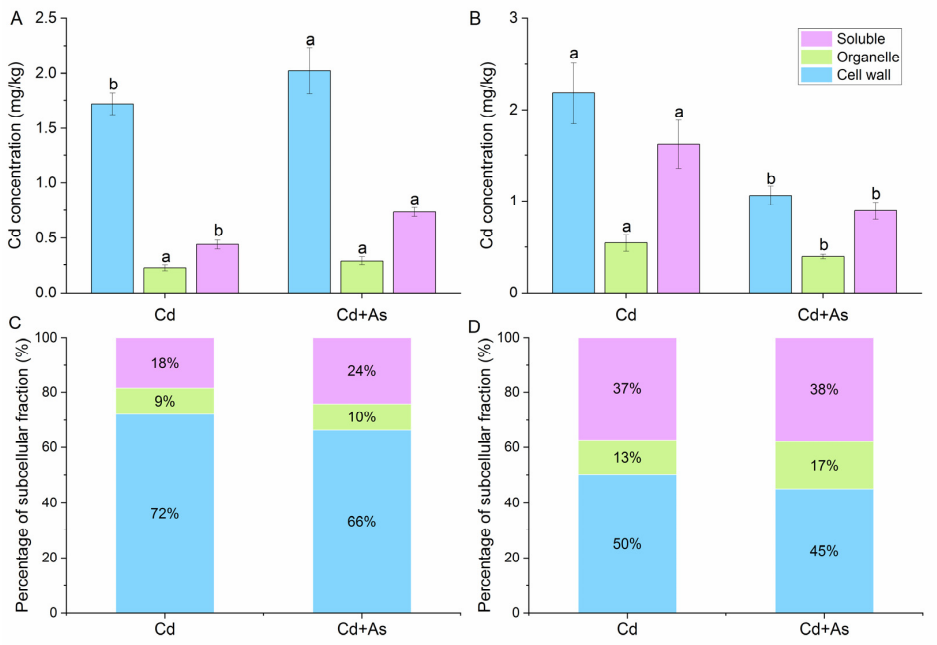

Figure 1. Concentration and percentage of subcellular distribution of Cd in roots (**A**,**C**) and shoots (**B**,**D**) of peanut. Cd and Cd + As represent single soil Cd contamination and Cd and As co-contamination treatment, respectively. Values are means of four replicates. Different letters in the same subcellular fraction indicate significant differences between the treatments at the 0.05 level.

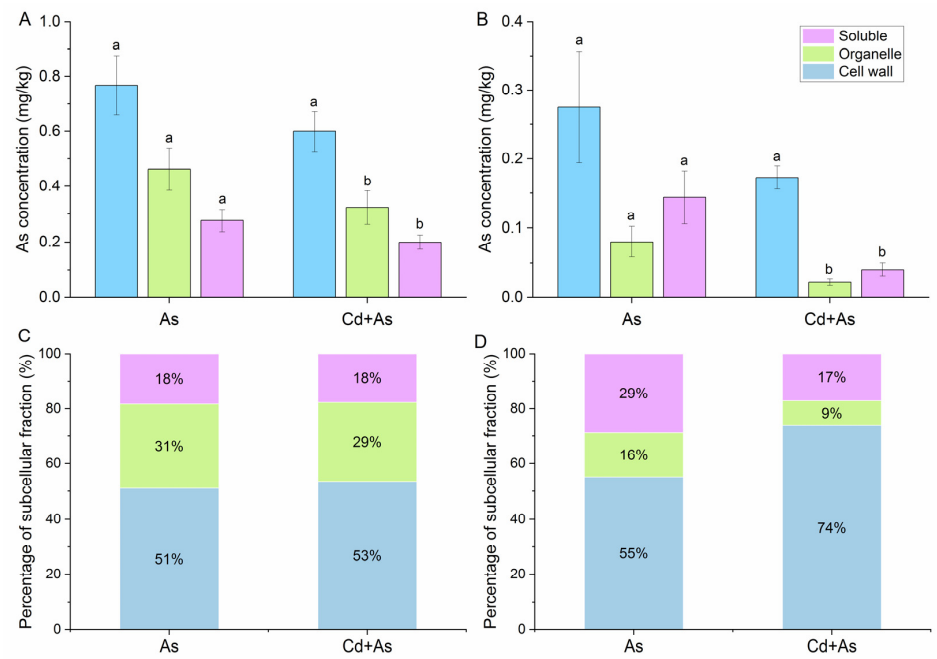

Figure 2. Concentration and percentage of subcellular distribution of As in roots (**A**,**C**) and shoots (**B**,**D**) of peanut. As and Cd + As represent single soil As contamination and Cd and As co-contamination treatment, respectively. Values are means of four replicates. Different letters in the same subcellular fraction indicate significant differences between the treatments at the 0.05 level.

3.4. Chemical Speciation of Cd and As in Peanut

In both roots and shoots, the NaCl extractable Cd (F_{NaCl}) was found to play a dominant role in the overall Cd distribution. Under both Cd single contamination and Cd + As co-contamination, pectate and protein-integrated Cd (F_{NaCl}) accounted for 71% and 62% of the total Cd species in roots, respectively (Figure 3). Similarly, in shoots, pectate and protein-integrated Cd (F_{NaCl}) accounted for 69% and 61% of the total Cd species, respectively. Furthermore, in roots and shoots, the co-contamination significantly increased the Cd concentrations extracted using d-H_2O (F_W) while it decreased the HCl extractable Cd (F_{HCl}) concentration. The d-H_2O extractable Cd (F_W) in roots and shoots showed an increase of 60.0% to 81.8%, whereas the HCl extractable Cd (F_{HCl}) in roots and shoots decreased by 11.6% to 12.7% compared to single Cd contamination.

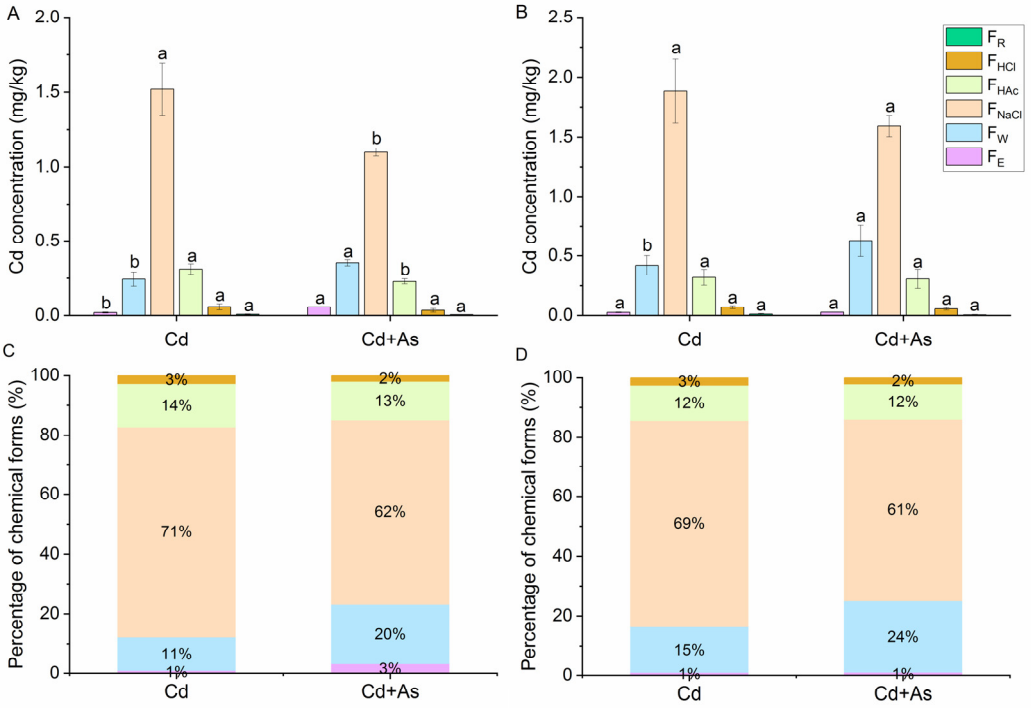

Figure 3. Concentration and percentage of chemical speciation of Cd in roots (**A**,**C**) and shoots (**B**,**D**) of peanut. The chemical forms of Cd extracted by 80% ethanol, distilled water, 1 M NaCl, 2% acetic acid, and 0.6 M HCl were named as F_E, F_w, F_{NaCl}, F_{HAc}, and F_{HCl}, respectively, and the residual form was defined as F_R. Cd and Cd + As represent single soil Cd contamination and Cd and As co-contamination treatment, respectively. Values are means of four replicates. Different letters in the same chemical form indicate significant differences between the treatments at the 0.05 level.

In both roots and shoots, the dominant form of As was found to be the residual fraction (F_R). Under both single As and Cd–As co-contamination, the residual As (F_R) accounted for 52% and 55% of the total root As species and 48% and 54% of the total shoot As species, respectively (Figure 4). The co-contamination significantly decreased the As concentrations extracted using d-H_2O (F_W) in roots while it increased the HAc extractable As (F_{HCl}) and residual As (F_R) concentrations in shoots.

3.5. Contents of PCs, GSH, PCSase, and MTs in Peanut

The co-contamination resulted in a significant increase in the synthesis of PCs in shoots and MTs in roots (Table 4). In roots, the MTs increased by 41% to 50%, while the PCs in shoots increased by 6.4% to 6.9% compared to the levels observed under single Cd or As contamination. The observed increase in root MTs played a crucial role in promoting the transport of Cd and As from roots to shoots. However, GSH and PCSase were not significantly affected by the co-contamination in both roots and shoots.

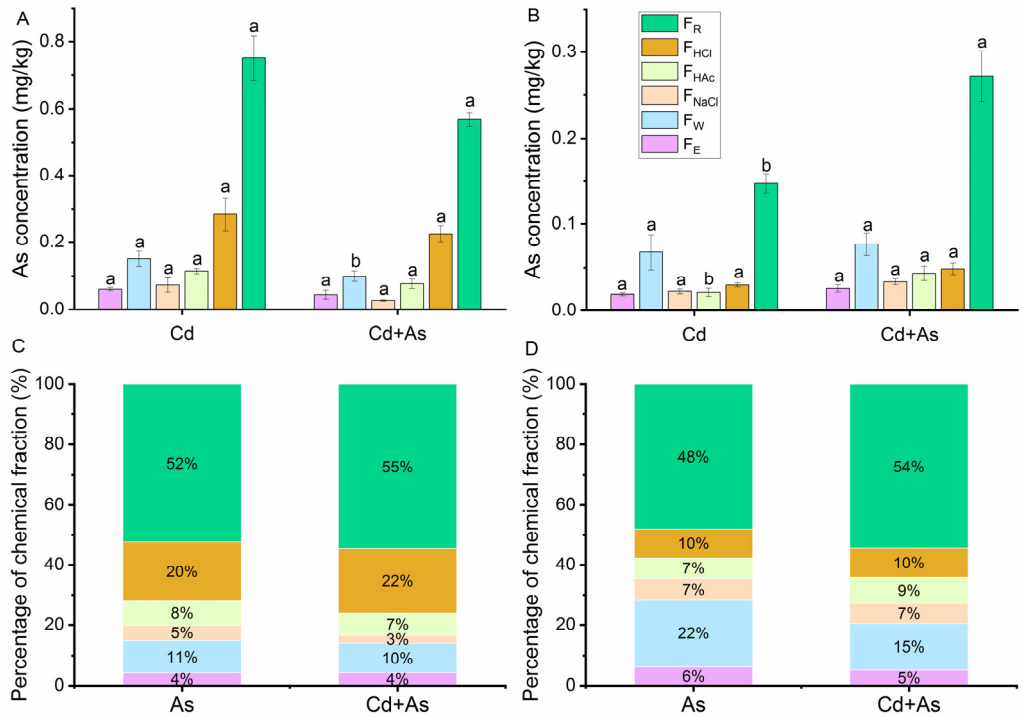

Figure 4. Concentration and percentage of chemical speciation of As in roots (**A**,**C**) and shoots (**B**,**D**) of peanut. The chemical forms of As extracted by 80% ethanol, distilled water, 1 M NaCl, 2% acetic acid, and 0.6 M HCl were named as F_E, F_w, F_{NaCl}, F_{HAc}, and F_{HCl}, respectively, and the residual form was defined as F_R. As and Cd + As represent single soil As contamination and Cd and As co-contamination treatment, respectively. Values are means of four replicates. Different letters in the same chemical form indicate significant differences between the treatments at the 0.05 level.

Table 4. Contents of PCs, GSH, PCSase, and MTs in peanut roots and shoots.

Treatments	Roots				Shoots			
	PCs (ng/g)	GSH (ng/g)	PCSase (U/g)	MTs (ng/g)	PCs (ng/g)	GSH (ng/g)	PCSase (U/g)	MTs (ng/g)
Cd	3.46 ± 0.01 a	1.24 ± 0.06 a	6.15 ± 0.22 a	0.16 ± 0.02 b	3.77 ± 0.13 b	1.26 ± 0.02 a	6.39 ± 0.07 a	0.28 ± 0.02 a
As	3.30 ± 0.16 a	1.24 ± 0.03 a	6.04 ± 0.06 a	0.17 ± 0.02 b	3.75 ± 0.03 b	1.28 ± 0.03 a	6.36 ± 0.09 a	0.25 ± 0.05 a
Cd + As	3.52 ± 0.14 a	1.30 ± 0.05 a	6.20 ± 0.10 a	0.24 ± 0.04 a	4.01 ± 0.09 a	1.17 ± 0.04 a	6.46 ± 0.10 a	0.26 ± 0.04 a

Values (mean ± SD, n = 4) with different letters in the same column indicate significant differences between treatments at the 0.05 level. Cd, As, and Cd + As represent single soil Cd contamination, single soil As contamination, and Cd and As co-contamination treatment, respectively.

4. Discussion

4.1. Accumulation and Translocation of Cd in Peanut under Cd and Cd + As Treatments

Peanuts are known for their strong ability to accumulate heavy metals, surpassing other legumes such as beans and soybeans under similar growing conditions [14]. In our study, we observed that the distribution of Cd content in different organs of the Ganhua 5 peanut variety followed the order of shoots > roots > shells > grains (Table 2). This finding is consistent with previous research, which has shown that Cd is readily transported to the shoots in peanuts [20].

The subcellular distribution of heavy metals plays a significant role in their migration and accumulation within plants. Cellular partitioning, specifically the cell wall and vacuolar compartmentalization, can influence the level of intracellular free heavy metals [21]. The plant cell wall, composed of polysaccharides and proteins, contains functional groups like carboxyl, hydroxyl, amino, and aldehyde, which have a high affinity for Cd ions. These substances can bind with Cd ions, impeding their transmembrane transport

and limiting the entry of Cd into the symplastic pathway [22]. The soluble components of plants, comprising cytoplasm and vacuoles, play a crucial role in heavy metal chelation. Sulfhydryl compounds, metallothioneins, organic acids, and amino acids within plants can form complexes with heavy metal ions, transforming them into low-toxic or non-toxic substances and sequestering them within vacuoles, thus reducing heavy metal stress [23]. In our experiment, we found that the subcellular distribution of Cd in peanuts was primarily in the cell wall and soluble components (Figure 1), which helps mitigate the stress caused by Cd in plants. Similar studies by Su et al. [24] and Shi et al. [25] also observed that Cd primarily accumulated in the soluble components of peanut roots under low Cd exposure levels. Furthermore, the concentration of Cd can significantly influence the subcellular distribution in peanut roots. At high Cd concentrations, Cd is more immobilized in the cell wall, reducing its entry into the symplast and hindering its transfer to the shoots. This finding aligns with our research results, where we observed that Cd was predominantly distributed in the cell wall (F1), followed by the soluble component (F3), and the organelle (F2) in lower peanut roots and shoots under different treatments (Figure 1). The binding of Cd to the cell wall restricts its migration, confirming the influence of cell wall binding on Cd transport, as suggested by Shi et al. [25].

The chemical speciation of heavy metals in plants also affects their transport and accumulation. These chemical forms can be categorized as active or inactive. Active forms include water-soluble states, while other forms are considered inactive [19]. Previous studies have shown that the distribution of Cd chemical speciation in the aboveground parts of the Huayu 22 peanut variety was highest in F_{NaCl}, followed by F_E, and lowest in F_{HAC} and F_{HCl} [26]. In our study, we observed a similar trend, except that the F_{HCl} component of aboveground Cd in Ganhua 5 peanut was higher than F_W (Figure 3), which may be attributed to varietal differences. The distribution of Cd forms in the root system followed the same pattern as the aboveground parts, with the highest content observed in the F_{NaCl} fraction (Figure 3).

Under Cd–As co-contamination, the concentrations of Cd in roots, shoots, and grains were higher compared to single Cd contamination (Table 1). The addition of As significantly increased the Cd concentration in grains, which aligns with the findings of Sun et al. [27]. Measurement of the available Cd content in the soil revealed no significant difference between co-contamination and single contamination treatments. This indicates that the synergistic effect on Cd accumulation in peanut plants was not achieved by altering the available Cd content in the soil but rather through peanut uptake.

The promotion of Cd accumulation by As is attributed to multiple mechanisms. Firstly, it was demonstrated that the adsorption of As could increase the negative charge on the root surface, enhancing the adsorption and absorption of Hg^{2+} by roots [26]. Given the similar chemical properties of Cd, Pb, and Hg, the promoting effect of As on Cd absorption could be explained by this perspective. The migration of heavy metals in plants is often influenced by their subcellular distribution and chemical speciation. Soluble and active heavy metal ions are more prone to translocation. Studies have also shown that the regionalization of root cells is an effective mechanism affecting the long-distance transport of Cd [28]. Organic acids in plants can form complexes with heavy metals, facilitating their transport and assisting in their transfer to leaves. Research has found that the form of organic acid-metal chelates is advantageous for transport in the xylem of plants such as corn (*Zea mays* L.) due to the competition between ions during xylem [29]. Once inside the root cell, Cd is absorbed and fixed by cellulose, pectin, and glycoprotein in the cell wall, reducing further transport [30]. In the case of Cd–As co-contamination, the levels of Cd bound to cell wall components, pectin, and proteins in roots and shoots decreased, explaining the promoting effect of As on Cd transport through subcellular and chemical morphological changes.

Inorganic As added to the soil can activate the phytochelatin synthase gene PCSase, leading to increased synthesis of PCs [31]. Zhang et al. [32] discovered that soluble Cd-PCs complexes are the main form of Cd transport from roots to shoots in plants. The greater the

synthesis of PCs in the roots, the stronger the migration ability of Cd to shoots. PCs are also involved in mediating Cd's long-distance transport in the phloem of *Brassica napus* [33]. Wang et al. [34] found that plants can reduce the transport of Cd from roots to shoots by reducing the synthesis of PCs in roots. In rape plants, an increase in GSH content was found to significantly inhibit the transport of Cd from roots to the aboveground parts [35]. This is because GSH serves as a substrate for PCs synthesis, and an increase in PCs synthesis is often accompanied by a decrease in GSH content. Based on these research findings, it can be concluded that the transport of Cd to shoots is negatively correlated with GSH in the root system and positively correlated with PCs in the root system.

In our experiment, peanut shoots showed a significant increase in the content of PCs under Cd–As co-contamination (Table 4). Meanwhile, the content of GSH decreased with the increase in PCs synthesis. These results indicate that Cd–As co-contamination promoted the transport of Cd from roots to shoots, which was associated with the overall Cd accumulation and concentration in different parts. Apart from sulfhydryl compounds, MTs are also involved in the chelation and transport of heavy metals. Zhang et al. [36] observed an increased accumulation of Cd in *Arabidopsis thaliana* expressing the garlic AsMT2b gene. This indicates that the upregulation of metallothionein expression also promotes the transport of Cd to the shoots to some extent. In our experiment, the MT content in peanut roots under Cd–As co-contamination was significantly higher than in the single Cd or As contamination group (Table 4), suggesting that MTs also facilitated the transport of Cd from roots to aboveground parts.

4.2. Accumulation and Translocation of As in Peanut under As and Cd + As Treatments

Previous research has shown that under aerobic conditions, the primary form of As present in soil and absorbed by plants is As (V). Once taken up by plants, As (V) undergoes rapid reduction to As (III). A portion of the As (III) binds to GSH or forms complexes with PCs, becoming sequestered in vacuoles. This sequestration serves as a major barrier to the translocation of As to aboveground tissues. The remaining fraction is transported through the xylem to the aboveground parts of the plant [37]. In our experiment, we found that under single As contamination, As in both root and shoot cells of peanuts was predominantly distributed in cell wall components, accounting for more than half of the subcellular proportion (Figure 2). Similarly, in cabbage roots treated with 5 and 10 mg/L As, As was mainly stored in the cell wall components, representing more than 50% of the total As content [38].

The influence of Cd–As co-contamination on the uptake of As in different organs of peanuts exhibited distinct patterns. The addition of Cd hindered the accumulation of As in peanut grains and roots but promoted its accumulation in shoots (Table 2). Changes in the translocation factor revealed a significant increase in the transport of As from roots to shoots, while the translocation factor from shoots to grains decreased significantly (Table 3). Consequently, the total As accumulation amount in peanut plants was higher under Cd–As co-contamination compared to single As contamination. These findings highlight the importance of considering not only the concentration or enrichment coefficient but also the total accumulation amount when investigating the interactive effects of heavy metals. A comprehensive assessment of the total accumulation amount provides a more accurate characterization of the interactions of heavy metals in co-contaminated soil.

Research by Xu [39], analyzing the membrane surface potential of root cells, revealed that an increase in Cd concentration in the soil solution led to a reduction in the electronegativity of the membrane surface. This, in turn, decreased the exclusion of As from the membrane surface, thereby promoting plant As uptake. This phenomenon explains the promoting effect of Cd on the overall accumulation of As in peanuts. Earlier studies have also suggested that the addition of lead can stimulate the production of phosphates (P) and sulfhydryl compounds (SH) in plants. As shares transporters with P, which enhances the uptake of As [40]. Similarly, it was found that high concentrations of Hg (2.5 mg/L) can impede the conversion of arsenate to arsenite, enabling As to be transported via the phosphate pathway,

thereby increasing its accumulation in rice shoots [41]. Since Cd and Hg are bivalent heavy metal ions, Cd may also influence the reduction process of As and subsequently impact As uptake. Based on our previous conclusions, the addition of Cd significantly inhibited the accumulation of As in peanut grains and roots while promoting its accumulation in shoots. The change in the translocation factor indicated that Cd enhanced the transport of As from roots to shoots and inhibited its transport from shoots to grains. Considering this phenomenon in conjunction with the transport mechanism of As in plants, it can be speculated that Cd may affect the reduction process of As (V) to As (III). This is because Cd binds to GSH, which serves as the reductant in the As (V) reduction process, thereby influencing both the reduction of As (V) and its subsequent transport [42]. Li et al. [43] also discovered that high concentrations of Cd treatment (50 mg/L) can activate PCs synthetase, promoting the synthesis of PCs and reducing the content of the substrate GSH.

Cd may also compete with As (III) for chelation, thereby reducing the chelation of As (III) by PCs and other compounds and increasing the transport of As from roots to shoots. In addition to chelation competition, Cd's combination with GSH, which is the reductant in As reduction, further affects the reduction of As (V) and its transport process [23]. Sulfur (S) serves as a precursor for the synthesis of GSH and PCs. Numerous studies have demonstrated that plants under Cd stress can enhance the uptake and reduction of sulfate, leading to an increased synthesis rate of GSH [44,45]. Furthermore, it was observed that S significantly increased the uptake and transport of As in *Pteris vittata* [46]. Based on this, it can be speculated that Cd may promote the uptake and transport of As from roots to shoots by facilitating S uptake.

Regarding the decrease in shoot-to-grain transport, it is possible that the addition of Cd stimulated the production of PCs in the phloem, which inhibited the accumulation of As in peanut grains. Under Cd–As co-contamination, the proportion of As bound to cell walls and residual components in shoots increased, while the proportion of As complexed with soluble organic acids decreased from 22% to 15%, thereby limiting the transport of As to grains. In this experiment, the aboveground PCs in peanuts under Cd–As co-contamination were significantly higher than those under single As contamination (Table 4). This finding can explain why Cd–As co-contamination reduced the transport of As from shoots to grains, as more As was sequestered by the shoots. The results indicated that the complex formed by PCs and As was fixed in the vacuole, limiting the further transport of As and reducing the amount of As transported from shoots to grains [37,47]. This is different from the observation that PCs promote Cd transport. Uraguchi et al. [48] have demonstrated that phytochelatin synthase has contrasting effects on Cd and As accumulation in rice grains, suggesting the existence of at least partially distinct PC-dependent pathways for As and Cd. Grispen et al. [49] discovered that tobacco transformed with the AtMT2b gene exhibited increased transport of As from roots to shoots. This suggests that enhanced expression of MTs also contributes to the transport of As to shoots to some extent.

Therefore, the impact of Cd on As accumulation can be attributed to its influence on the reduction of As in roots, chelation by PCs, S absorption in roots, and transporter expression. The significant increase in As transport from roots to shoots under Cd–As co-contamination underscores the importance of studying the molecular mechanisms that regulate As (V) reduction to As (III) in different plant species.

5. Conclusions

In conclusion, the results of the pot experiment indicated that soil Cd–As co-contamination increased the food safety risk of Cd while decreasing the risk of As in peanuts. Total plant accumulation amount provides a more accurate characterization of the interactions of Cd and As in co-contaminated soil. The observed changes in subcellular distribution, chemical forms, and contents of PCs and MTs in peanut roots and shoots provide insights into the mechanisms underlying Cd and As accumulation and translocation under Cd–As co-contamination. The complex interactions between Cd and As and their impact on various cellular processes highlight the need for a holistic approach to studying heavy metal contamination in peanuts.

Future research should focus on elucidating the cross-talk between different metal transporters and chelation mechanisms to better understand how co-contamination affects heavy metal uptake and distribution in peanuts.

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