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Accumulation and Chemical Forms of Cadmium in Tissues of Different Vegetable Crops

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Abstract: Large areas of arable lands in China have been contaminated by heavy metals, in which cadmium (Cd) contamination was the most prevalent. Cd accumulation in main food crops and leafy vegetables grown in Cd-contaminated fields has aroused considerable attention in recent years. The present study investigated the Cd pollution of farmland soils and vegetables in Qujing city of Yunnan Province, China. By comparing the Cd uptake capacities of different crops, this study aimed to provide guidance for agricultural production in Cd-contaminated farmland, and clarify the influence of Cd bioavailability in soil and chemical forms of Cd in plant roots on its migration. Results showed that soil Cd concentration was up to 37 mg kg^{-1}, which was 61-fold higher than the soil environmental quality standard in China. Concentration of Cd in 73% of the investigated vegetable samples, with the mean value of 5.43 mg Cd kg^{-1} (dry weight basis), exceeded the food safety standard of China. Leafy vegetables had the highest bioaccumulation factors (BF) and transfer factors (TF), with the mean values of 0.53 and 0.41, respectively. Water spinach (*Ipomoea aquatica* Forsk.), cole (*Brassica campestris* L.), and fennel (*Foeniculum dulce* Mill.) had the highest Cd TFs, with averages of 0.67, 0.66, and 0.64, respectively. On the contrary, garlic (*Allium sativum* L.), onions (*Allium fistulosum* L.), and pea (*Lathyrus odoratus* L.) had the lowest Cd TFs, with averages of 0.04, 0.03, and 0.04, respectively. The main chemical fraction of Cd in garlic root was insoluble phosphate (35–48%), whereas in water spinach root, it was pectate, protein binding or sorbed fraction (50–64%), resulting in a higher TF value of water spinach than garlic. These results indicate that there were significant differences in Cd uptake and accumulation between vegetables, and the Cd accumulation in leafy vegetable was significantly higher than that in alliums. Therefore, it is possible to reduce the uptake and accumulation of Cd in crop edible parts by the selection of vegetable species with low Cd accumulation capacity. The chemical fractions of Cd in crop roots, especially the proportions of more mobile fractions, might be an important reason for the root-to-shoot Cd transport and Cd accumulation in the aerial portions.

Keywords: vegetables; cadmium; bioaccumulation factors (BF); transfer factors (TF); chemical forms

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1. Introduction

In recent years, heavy metal pollution in farmland soils has raised increasing concerns, which can be caused by anthropogenic activities including mining, industrial waste discharge, sewage irrigation, and overuse of phosphorus fertilizer [1–4]. Among them, soil cadmium (Cd) and lead (Pb) contamination are considered more prevalent [5–9], which can trigger severe food safety problems [10–14], and hence received particular attention. It was often found that the contents of Cd and Pb in rice and vegetable crops exceeded the food safety standard [15,16]. These heavy metals are also phytotoxic and interfere with the assimilation of essential nutrients by plants, posing non-negligible risks to crop production and food safety [17,18].

There were obvious differences in the uptake and accumulation of heavy metals among different crop species [19,20]. These capacities were influenced by the growth characteristics of plants as well as the bioavailability of heavy metals in soil. Due to the complex dynamic interaction among heavy metals, soil, and organisms, only some of the heavy metals in the soil can be bio-absorbed and assimilated [21]. The migration and transformation of heavy metals in the soil varied with the available state or occurrence form [22], and their biological effectiveness on plants also differed [23]. However, previous research on heavy metals in soil mostly focused on the total content level, which do not accurately reflect the actual ecological risk of heavy metals pollution. The toxicity of heavy metals in plants varies greatly with their chemical forms. The migration capacity of Cd is stronger than that of Pb, thus Cd accumulated in plant roots more readily migrates to the aerial portion. Cd in ionic form is far more toxic than in an organic complex form because the more stable the complex, the lower the toxicity [24]. He et al. [25] found that in the cells of high accumulation rice (*Oryza sativa* L.) varieties, Cd mainly existed in the forms with higher migration potential. Fu et al. [26] found that Cd mainly existed in the root of *Phytolacca americana* L. as ethanol extract, and the potential to transport Cd from the root to the shoot is high.

Huize lead–zinc located in Qujing City, Yunnan Province was one of the typical mines of the large rich lead–zinc deposits in Sichuan, Yunnan, and Guizhou lead–zinc mineralization area in southwest China. Owing to the large scale, high grade, and many associated elements, it was one of the most famous nonferrous metal mines in China. Due to the backward production technique, the lead–zinc ores were smelted by indigenous methods for a long time. During the production process, a large amount of unrecycled heavy metals (Cd, Pb, Hg, etc.) were released into the atmosphere or kept in the slag. In Huize County, Yunnan Province, there were many abandoned sites derived from mining and smelting activities dating to the Ming and Qing Dynasties, which could not be used as agricultural land because of the high content of heavy metals in the soil. However, due to the sparse vegetation on these abandoned mine sites, heavy metals in the soil might spread through wind and rain, polluting the surrounding environment. Among them were about 31 local Pb smelting companies in Zhehai Town (103°03′–103°55′ E, 25°48′–27°04′ N). As these companies usually had a long production history and employed relatively backward production techniques, they discharged a large amount of Pb and Cd into the neighboring environment through waste gas and solid waste, producing lots of potential heavy metal pollution regions. Although some smelters had been shut down, the mine tailings still pose great hazards to surrounding farmland and crops.

Therefore, in this study, soil and nine main vegetable crops in polluted farmland near the waste site of the smelting plant in Zhehai town were selected to investigate the contents of toxic heavy metals (Cd and Pb) and mineral nutrients (zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn)) in crop edible parts and root zone soil. Due to the serious pollution of Cd in the local area, we further investigated the availability of Cd in soil and the chemical fractions of Cd in different tissues of crops. This study aimed to compare the Cd uptake capacities of different crops, provide guidance for agricultural production in Cd-contaminated farmland, and clarify the influence of Cd bioavailability in soil and chemical forms of Cd in plant roots on its migration. Through greenhouse experiments, the chemical forms of Cd in two crops (water spinach and garlic) with high- and low-Cd accumulation characteristics were compared over time to further explore the differences in Cd absorption, accumulation, and translocation between the two crops.

2. Materials and Methods

2.1. Experimental Design

In March 2021, crop plant and soil samples were collected in the Cd and Pb contaminated farmland around the waste smelter in Zhehai Town, Huize County, Qujing City, Yunnan Province (103°36′49″ E, 26°34′27″ N, 2191 m above sea level). The five sampling areas shown in Figure 1 are mainly located near Kongjia, Hujiapo, Fanjia, and Zhaojia villages.

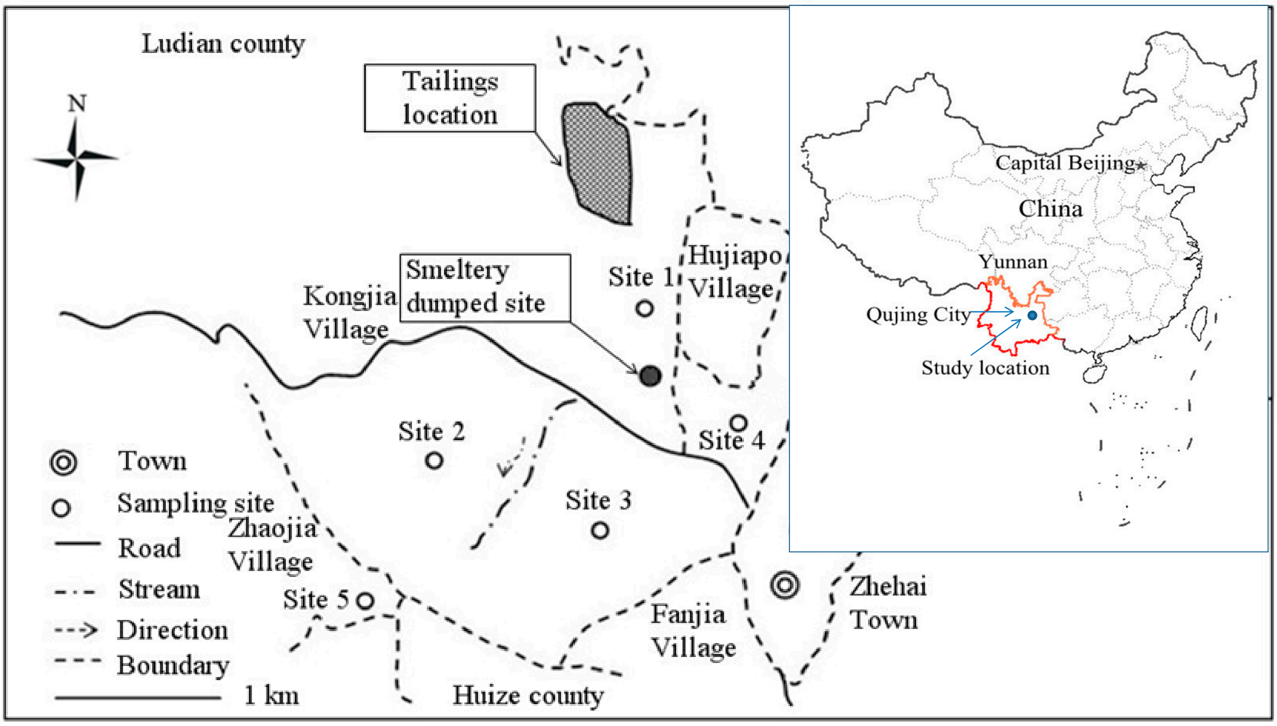

Figure 1. Sampling locations of the present study in Zhehai town, Qujing city in Yunnan Province, China.

A total of nine vegetable crops (Table 1) and their root zone soil were collected. Due to the limitation of the local crop planting season, when we arrived at the research site, only these nine kinds of vegetables could be collected from the farmland around the tailings pond. Sampling was conducted in four replicates, each of which was composed of three to five plants. The surface soil samples (0–20 cm) in the root zone were collected by the same method. The nine tested vegetable species could be divided into four types: (1) leafy vegetables (*Brassica campestris* L., *Brassica chinensis* L., *Foeniculum dulce* Mill., *Brassica juncea* L., *Ipomoea aquatica* Forsk), (2) allium vegetables (*Allium sativum* L., *Allium fistulosum* L.), (3) rootstalk vegetables (*Raphanus sativus* L.), and (4) legumes (*Lathyrus odoratus* L.). The basic physical and chemical properties of soil (pH, organic matter (OM), total nitrogen (TN), and total phosphorus (TP) and the background contents of Cd, Pb, Zn, Cu, Fe, Mn, and DTPA-extracted content are shown in Table 2. Compared with the secondary soil standard (Cd: 0.6 mg kg^{-1}; Pb: 120 mg kg^{-1}) in the Chinese Environmental Quality Standard for Soils (GB 15618-2018), the total concentrations of Cd and Pb exceeded the standard by 61 and 3.7 times, respectively, showing particularly serious soil Cd pollution in the tested area.

Table 1. Vegetable crop species collected from the farmland around the tailings pond.

Species	Category	Organ Sampled
Allium sativum L. (garlic)	allium vegetables	Leaf, Stem, Root
Allium fistulosum L. (scallion)	allium vegetables	Leaf, Stem, Root
Brassica campestris L. (cole)	leafy vegetables	Leaf, Stem, Root
Brassica chinensis L. (pakchoi)	leafy vegetables	Leaf, Root
Brassica juncea L. (senvy)	leafy vegetables	Leaf, Root
Foeniculum dulce Mill. (fennel)	leafy vegetables	Leaf, Stem, Root
Ipomoea aquatica Forsk. (water spinach)	leafy vegetables	Leaf, Stem, Root
Lathyrus odoratus L. (pea)	legumes	Leaf, Stem, Root
Raphanus sativus L. (radish)	rootstalk vegetables	Leaf, Root

Table 2. Physicochemical properties and metal concentrations of the soil samples collected from the farmland around the tailings pond (n = 36, DW).

	Properties					
	pH		**OM (%)**		**TN (%)**	**TP (%)**
Mean	6.82		6.25		0.11	0.33
Range	6.21–7.63		3.91–14.52		0.03–0.21	0.20–0.59
	Concentration of heavy metals in soil (mg kg^{-1}, DW)					
	Cd	Pb	Zn	Cu	Fe	Mn
Total conc.	37.52	565.70	1902.56	164.17	67,852.97	850.25
Range	4.71–199.06	257.56–1533.72	680.43–4734.01	80.42–282.09	44,675.13–99,025.15	388.09–1374.13
DTPA-extractable	16.14	89.25	748.26	7.76	20.07	135.67
Range	1.15–97.95	1.56–198.92	244.91–2271.17	0.31–19.03	0.93–102.13	86.07–235.17

Note: Range means the value from Minimum to Maximum of the soil samples.

2.2. Sample Pre-Treatment

Fresh plant samples collected in the field and soil samples in the root zone were packed into clean plastic bags and taken to the laboratory within 4 h. Plant samples were rinsed with (1) tap water, (2) deionized water, (3) 0.2% (v/v) HCl solution, and (4) deionized water twice [19]. Clean samples were then divided into edible and nonedible parts using a stainless-steel (Table 1), followed by the measurement of fresh weight. Plant tissues were oven-dried at 80–90 °C in an oven for 15–30 min, and then at 65 °C for 48 h before measuring the dry weight. Dry samples were then ground and sieved through a 0.5 mm stainless steel sieve. One portion of the samples was directly used to determine Cd chemical forms and the other portion was stored for the analysis of total Cd, Pb, and other metal elements.

Soil samples were first air-dried to constant weight. Then the samples were crushed with a rubber hammer to remove the stones, plant residues, and other foreign matters and then sieved through an 80-mesh stainless steel screen before use.

2.3. Indoor Experiment

According to the results of the field investigation, soil Cd pollution was particularly serious. In June 2021, an indoor trial was carried out to evaluate the chemical forms and potential toxicity of Cd in crop edible parts and their temporal change. A Cd high-accumulating vegetable (water spinach) and a low-accumulating vegetable (garlic) and soil materials containing 0 and 10 mg kg^{-1} Cd (in the form of $CdCl_2$, balanced for 20 days) were adopted in this trial. Uniform plant seedings with three true leaves were transplanted into the soils and grown for 10 (T1), 20 (T2), and 30 (T3) days, respectively. After plant harvest, the chemical fractions of Cd in plant tissues were determined over time series [26,27].

2.4. Sample Analysis

Plants and soil were digested with HNO_3-$HClO_4$ (5:1, v/v) and HNO_3-HCl-$HClO_4$ (3:1:1, v/v), respectively. Soil available Cd and Pb were extracted using diethylene triamine pentaacetic acid (DTPA) and determined following the method introduced by Wang et al. [15]. The Cd concentrations in the digests and extracts were determined using an atomic absorption spectrometer (Varian AA240FS, Palo Alto, CA, America), and Pb, Zn, Cu, Fe, and Mn were measured with inductively coupled plasma atomic emission spectrometer (ICP-OES, Perkin Elmer, Waltham, MA, USA). The plant and soil reference materials were GBW-07604 and GBW-07401 (China National Standards and Materials Center), respectively, and the recovery rates were 90–110%, meeting the quality control requirements of element analysis.

The pH value (soil water ratio 1:2 m/v) was determined with a pH meter [28]. OM was determined with the potassium dichromate dilution heat method [28]. TN and TP were determined with an automatic chemical analyzer (Smartchem 200, Alliance, France).

The chemical forms of Cd in crops were extracted following a sequential extraction procedure using the following extractants [24,26,29]: (1) 80% ethanol (ethanol) to extract inorganic fraction (nitrate, chloride, and amino acid salt) ($F_{ethanol}$), (2) deionized water (d-H_2O) to extract water-soluble fraction (organic acid, first generation phosphate) (F_{H2O}), (3) 1 M sodium chloride (NaCl) to extract pectinate, protein binding or adsorptive fraction (F_{NaCl}), (4) 2% acetic acid (HAc) to extract phosphate (second generation phosphate, orthophosphate) that is insoluble in water (F_{HAC}), (5) 6 M hydrochloric acid (HCl) to extract the insoluble fraction (mainly oxalate) (F_{HCl}), and (6) the residuals. The fresh plant samples were cut into small pieces of 1–2 mm^3 before extraction. In each step, 37.5 mL of the above extractants were added to PVC tubes containing plant samples. Tubes were then placed in a shaking table for full extraction for 18 h (120 rpm, 30 °C), and supernatant and plant residues were then separated by centrifugation. After collecting the supernatant, 37.5 mL of the same extractant was added to the plant residues and extracted for another 2 h under the same conditions. The extraction in each step was repeated twice and supernatants were pooled. Then the next extractant was added to the plant residue and extracted according to the above method [27]. The supernatant was evaporated on the hot plate and digested with 5 mL of HNO_3 and 1 mL of $HClO_4$. The residue is directly digested with HNO_3-$HClO_4$ (5:1, v/v). The concentration of Cd in the digestion was determined by a flame atomic absorption spectrometer (Varian AA240FS, Palo Alto, CA, America).

2.5. Statistical Analysis

Differences between different crop species and treatments were estimated using one-way and two-way analysis of variance (ANOVA). The mean square ratio between groups and within groups was estimated using the F-test. The means of different species and treatments were compared using Tukey's test. The relationship between soil Cd and plant Cd concentrations was analyzed using Pearson correlation analysis. All the statistical analyses were conducted using SPSS 22.0, and figures were drawn using Origin 8.5. The data are presented as mean or mean ± standard error (mean ± SE). In order to compare the enrichment and transport capacity of different vegetables for Cd, the bioaccumulation factor (BF) and transfer factor (TF) formulas are as follows [30]:

$$BF = \text{Cshoot (DW)}/\text{Csoil (DW)}$$

$$TF = \text{Cshoot (DW)}/\text{Croot (DW)}$$

where, Cshoot and Croot, respectively, represent the concentration of Cd in shoots and roots (dry weight basis, DW), and Csoil represents the content of Cd in soil (dry weight basis, DW).

3. Results

3.1. Concentrations of Cd, Pb, Zn, Cu, Fe, and Mn in Vegetable Crops

Concentrations of Cd, Pb, Zn, Cu, Fe, and Mn in the edible parts of different vegetable crops are shown in Figure 2. Compared with the Pollution Limits in Food (GB 2762-2022, China), 73% and 40% of the samples exceeded the threshold concentrations of Cd and Pb, respectively. The Cd concentration in edible parts of nine vegetable crops ranged from 0.06 to 20.77 mg kg^{-1}, with an average of 5.43 mg kg^{-1} (DW). Among these crops, the Cd concentrations in green vegetables, cole, water spinach, and pakchoi leaves were the highest, with average values of 14.61, 12.28, and 15.44 mg kg^{-1}, respectively, whereas the Cd concentrations in leaves and stems of scallion and garlic were the lowest, with averages of 0.51, 0.16, 2.10, and 1.98 mg kg^{-1}, respectively ($F = 28.3$, $p < 0.001$). The Pb concentrations in the edible part of the collected samples ranged from 0.03 to 19.40 mg kg^{-1}, with an average of 3.62 mg kg^{-1} (DW). The Pb concentration in pea stems, radish roots, and pakchoi leaves were 10.48, 10.35, and 7.78 mg kg^{-1}, respectively, which were significantly higher than that of other crops ($F = 13.6$, $p < 0.05$). It can be seen from the concentrations of micronutrients that Zn, Fe >> Mn, Cu ($p < 0.001$) in the edible part of crops collected. The average concentrations of Zn, Cu, Fe, and Mn in edible parts of vegetables collected were 344.78, 11.16, 326.05, and 22.44 mg kg^{-1}, respectively, and the concentration ranges were 8.45–2336.11 mg kg^{-1}, 0.97–29.78 mg kg^{-1}, 11.19–1454.29 mg kg^{-1}, and 0.95–120.07 mg kg^{-1} (DW), respectively (Figure 2).

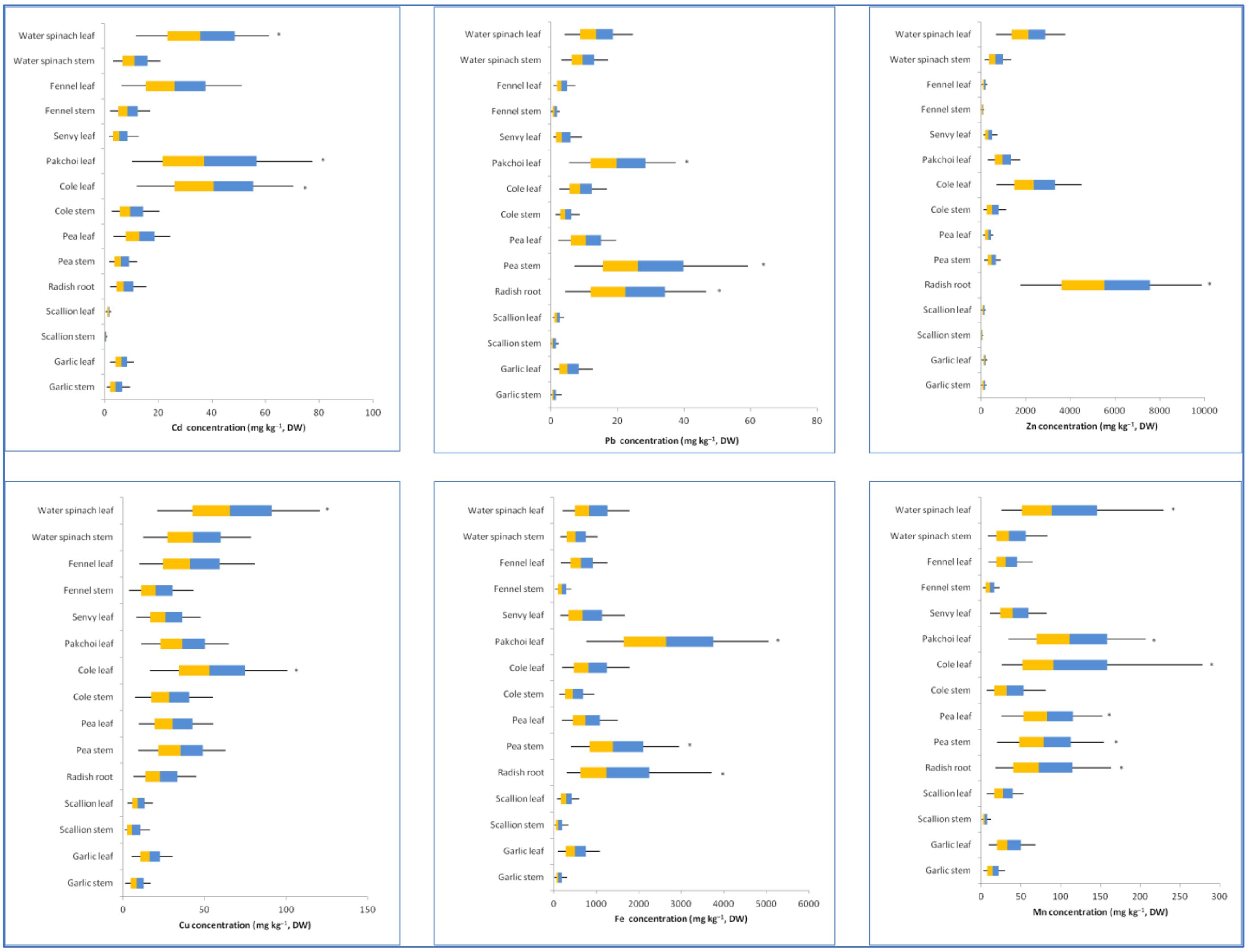

Figure 2. Concentrations of Cd, Pb, Zn, Cu, Fe, and Mn in the edible parts of nine vegetables ($n = 4$, DW). Box plots showing the range of Cd, Pb, Zn, Cu, Fe, and Mn in the edible parts of nine vegetables. Yellow box represents the range from 25% to the median, and blue box represents the range from the median to 75%. * indicates a significant difference between different edible parts of nine vegetables at $p < 0.05$ level.

3.2. Difference of BF and TFof Cd in Different Vegetables

The soil in the polluted area was particularly polluted by Cd (61 times the standard), and 73% of the crops exceeded the food safety standard for Cd. In view of the serious Cd pollution in the survey area, BF and TF of Cd in vegetables were investigated as shown in Figure 3. The Cd BF and TF of different vegetables and different tissues are shown in Figure 4. The Cd BF of cole and water spinach leaves, with an average of 1.22 and 1.14, respectively, were significantly higher than those of other vegetables ($F = 13.6$, $p < 0.001$). The Cd TF from root to leaf of water spinach, cole, and fennel leaves were significantly higher than those of from root to edible organs of other vegetables ($F = 33.4$, $p < 0.001$), with mean values of 0.67, 0.66, and 0.64, respectively. The BF and TF of Cd in garlic, onion, pea stem, and leaves were the lowest among the tested crop samples (Figure 3).

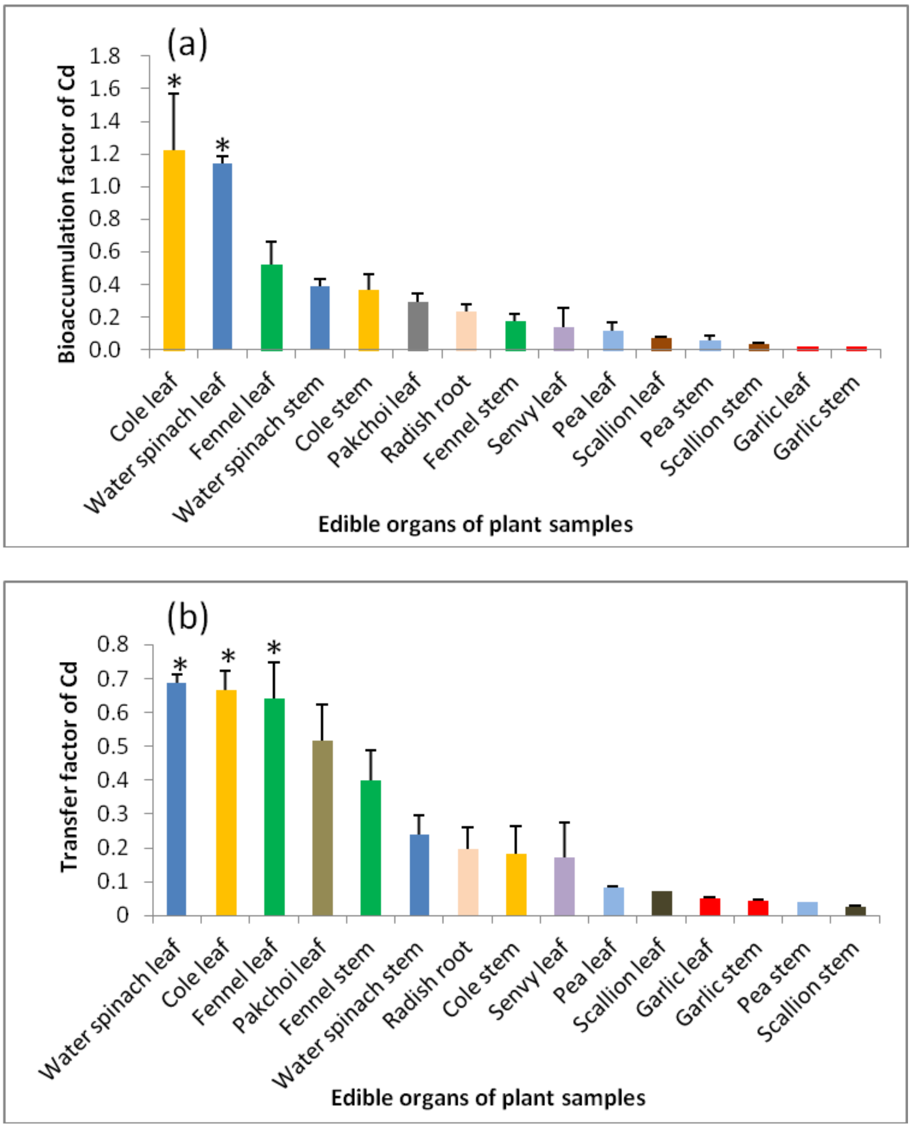

Figure 3. (**a**) Bioaccumulation factor (BF) and (**b**) transfer factor (TF) of Cd in edible parts of the vegetables (Mean ± SE, n = 4, DW). * indicates significant difference between different edible parts of nine vegetable at $p < 0.05$ level.

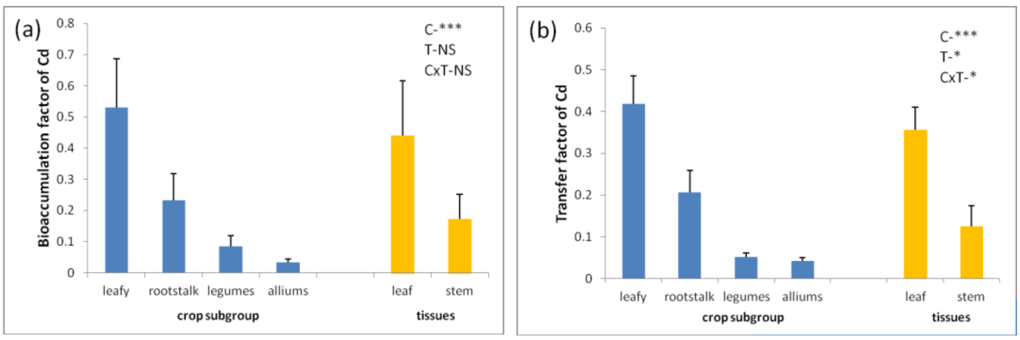

Figure 4. (**a**) Bioaccumulation factor (BF) and (**b**) transfer factor (TF) of Cd in vegetable subgroups and edible parts (Mean ± SE, DW). A two-way ANOVA was performed using as fixed factor crop subgroup (C), and tissues (T). The p-values of the two-way ANOVA for the effects of C, T and their interaction (C × T) on BF were ***, NS and NS, separately, and on TF were ***, * and *, respectively. *, $p \leq 0.05$; ***, $p \leq 0.001$; NS, not significant.

For different types of vegetables, the BF ($F = 11.6$, $p < 0.001$) and TF ($F = 19.3$, $p < 0.001$) of Cd in leafy vegetables were higher than those in other three types of vegetables (rootstalk, allium, legumes), which had average BF and TF of 0.53 and 0.41, respectively. For different edible parts, the TF of Cd from root to leaf was higher than that from root to stem ($F = 11.5$, $p < 0.05$) (Figure 4). The results of two-way ANOVA showed that the interaction of crop subgroup and tissues had a significant impact on TF ($F = 4.48$, $p < 0.05$), but no significant effect on BF ($F = 1.47$, $p > 0.05$).

3.3. Chemical form Distribution of Cd in Different Tissues of Vegetable Crops

The binding forms of Cd in different tissues of vegetable crops were compared, and the proportion of different forms is shown in Figure 5 (calculated from the concentration of each form, but the specific concentration is not listed). It can be seen that Cd in various tissues of vegetables was mainly in the NaCl-extracted fraction, followed by deionized-water-extracted, Hac-extracted, HCl-extracted, ethanol-extracted, and residue fractions. This showed that most of the Cd in these crops existed as pectinate, which was mainly bound to protein or in the form of adsorbed Cd. The inorganic salt form and residue form dominated by nitrate and chloride generally constituted the smallest proportions. This showed a way to alleviate the Cd toxicity in crop vegetables in the tested area with severe Cd contamination.

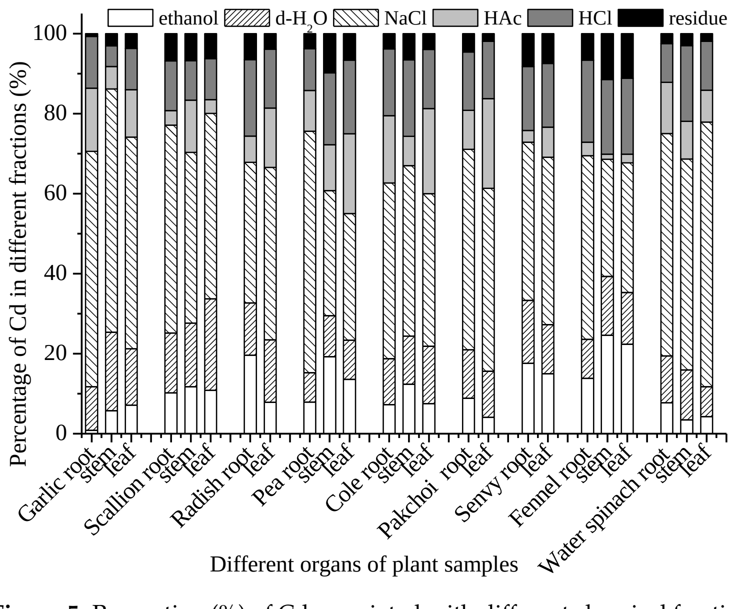

Figure 5. Proportion (%) of Cd associated with different chemical fractions (extracted by 80% ethanol, d-H_2O, 1M NaCl, 2% HAc, 0.6 M HCl, and residue) in different tissues (root, stem, and leaf) of the nine vegetables (mean, $n = 4$, DW).

From the perspective of different crop species, the proportion of ethanol-extracted fraction of garlic root was the lowest (0.8%, $F = 9.6$, $p < 0.001$) (Figure 5), enabling its strongest ability to restrict the root-to-shoot Cd translocation, which could be considered as a main reason for its low accumulation of Cd in the edible parts. The proportion of ethanol-extracted Cd in the roots of cruciferous crops (cole, Chinese cabbage, mustard, radish) was slightly larger than that of other crops, indicating that Cd could more easily migrate to the aerial parts of these crops.

3.4. Relationship between Cd Concentrations in Soil and Vegetable Tissues

The relationship between DTPA-extracted and total Cd concentrations in soil and Cd concentrations in different tissues of vegetable crops are shown in Figure 6. Results indicated that soil DTPA-extracted Cd concentration was significantly positively correlated with total soil Cd ($p < 0.0001$); There was a significant positive correlation between the concentration of Cd in roots and the concentration of DTPA-extracted Cd in root zone soil ($p < 0.0001$), but the relationship between the concentrations of Cd in stems and leaves and the concentrations of total and DTPA-extracted Cd in root zone soil did not reach a significant level ($p > 0.05$).

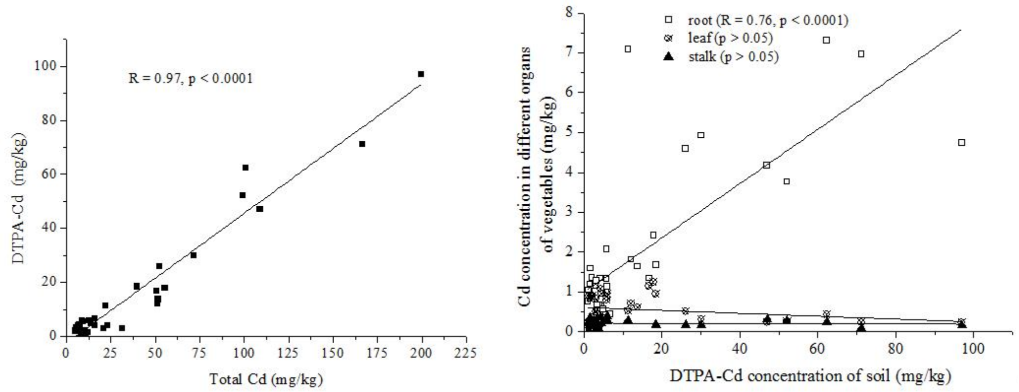

Figure 6. Correlations between DTPA-extracted Cd concentrations in soil (DW) and total Cd concentrations in soil (DW) and in different tissues of vegetables (DW).

3.5. Relationship between Chemical Forms and Transport of Cd in Vegetable Roots

The partial regression equation between the Cd form in the roots of vegetable crops and the aerial (stem and leaf) TF is shown in Table 3. The results show that the TF of Cd from the root to the leaf was positively correlated with the ethanol-extracted fraction of Cd in the root ($p < 0.01$). The TF of Cd from root to stem was negatively correlated with the NaCl-extracted ($p < 0.01$) and Hac-extracted ($p < 0.05$) fractions of Cd in the root.

Table 3. Stepwise regression between TF of Cd (from root to leaf/stem) and percentages of different Cd chemical forms in roots.

Transfer Factor	Stepwise Regression Formula	T and Sig.
Root to leaf	$Y = 0.142 + 0.075X_1$	$r_1 = 3.819$, $p_1 < 0.01$ ($n = 36$)
Root to stem	$Y = 1.304 - 0.014X_3 - 0.015X_4$	$r_3 = -3.786$, $p_3 < 0.01$; $r_4 = -2.249$, $p_4 < 0.05$ ($n = 24$)

Note: X_1, X_3, and X_4 indicate the proportion (%) of Cd associated with chemical forms extracted by 80% ethanol, 1M NaCl, and 2% HAc, respectively. Y indicates the transfer factor of Cd from root to stem/leaf.

3.6. Uptake, Accumulation, and Chemical Fraction Distribution of Cd in Different Tissues of Garlic and Water Spinach

Garlic (with weak Cd accumulation capacity) and water spinach (with strong Cd accumulation capacity) were planted based on contrasting Cd concentrations in their tissues and harvested at three time points (Figure 7). The total concentrations of Cd in roots, stems, and leaves and the Cd TF are shown in Table 4. The Cd concentrations in the roots of two crops increased gradually with time (from T1 to T3) ($p < 0.05$). In the second (T2) and third stage (T3), the Cd concentration in the roots of water spinach was significantly higher than that of garlic ($p < 0.05$). The Cd concentration in garlic stems decreased with time ($p < 0.05$), whereas that in water spinach stems did not change significantly ($p > 0.05$). In all three stages, the Cd concentration in water spinach stems was higher than that in garlic ($p < 0.05$). For leaves, the concentrations of Cd in water spinach increased significantly with time ($p < 0.05$), whereas that in garlic leaves did not change significantly ($p > 0.05$). Meanwhile, the concentrations of Cd in water spinach leaves in three stages were higher than those in garlic ($p < 0.05$). Besides, the Cd transfer coefficient from root to leaf in water spinach was significantly higher than that in garlic at all three stages ($p < 0.05$). The results of two-way ANOVA showed that crop varieties ($F = 49.94$, $p < 0.001$), tissues ($F = 7.27$, $p < 0.05$), and their interaction ($F = 26.26$, $p < 0.001$) had a significant impact on TF.

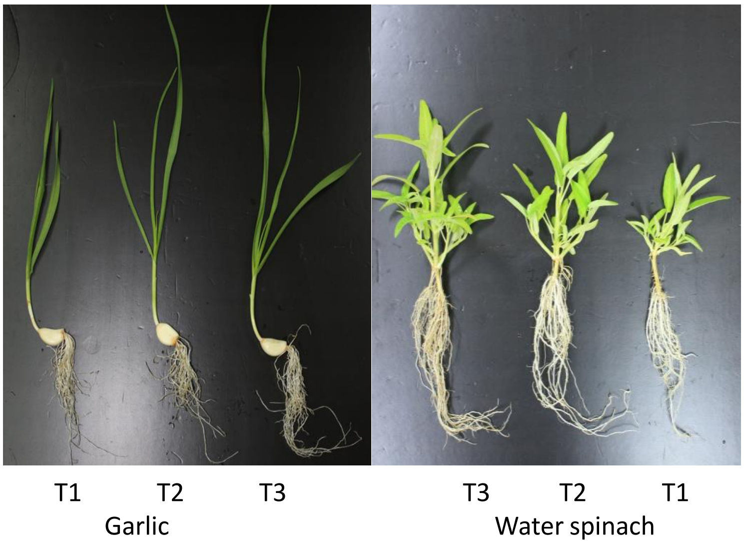

Figure 7. Garlic (with weak Cd accumulation capacity) and water spinach (with strong Cd accumulation capacity) were planted based on contrasting 10 mg Cd kg^{-1} concentrations in their tissues and harvested at three time points (T1-10 days, T2-20 days, T3-30 days).

Table 4. Cd concentrations in different tissues and transfer of Cd from root to stem/leaf in 10 mg Cd kg^{-1} treatment at three time stages (T1-10 days, T2-20 days, T3-30 days) in garlic and water spinach (n = 3, DW).

	Vegetables	**T1**	**T2**	**T3**
Root	garlic	48.44 ± 10.52 b	65.95 ± 12.23 ab	88.10 ± 3.32 a
	water spinach	150.83 ± 17.79 b*	203.75 ± 22.1 ab*	241.87 ± 11.93 a*
Stem	garlic	7.39 ± 0.35 a	5.04 ± 0.11 b	6.18 ± 0.88 ab
	water spinach	23.60 ± 5.85 a*	29.66 ± 0.65 a*	32.83 ± 0.65 a*
Leaf	garlic	2.41 ± 0.21 a	2.95 ± 1.26 a	3.26 ± 0.33 a
	water spinach	38.90 ± 4.48 b*	62.55 ± 3.32 ab*	89.05 ± 9.71 a*
TF(root-to-stem)	garlic	0.16 ± 0.14 a	0.08 ± 0.01 b	0.07 ± 0.01 b
	water spinach	0.16 ± 0.05 a	0.15 ± 0.02 a	0.16 ± 0.04 a
TF(root-to-leaf)	garlic	0.05 ± 0.01 a	0.05 ± 0.02 a	0.04 ± 0.003 a
	water spinach	0.28 ± 0.08 a*	0.31 ± 0.03 a*	0.41 ± 0.08 a*
Two-way ANOVA	C ***	T *	C × T ***	

Note: Different letters on the same row indicate a significant difference among three time stages at the level of $p < 0.05$, and the fixed factor was time stages. * behind letters indicates a significant difference between garlic and water spinach. A two-way ANOVA was performed using as fixed factor crop varieties (C), and tissues (T). The p-values of the two-way ANOVA for the effects of C, T and their interaction (C × T) on TF were ***, * and *, respectively. *, $p \leq 0.05$; ***, $p \leq 0.001$.

The chemical fraction distribution of Cd in different tissues (roots, stems, and leaves) of water spinach and garlic under Cd stress in three stages was analyzed and results are shown in Figure 8. The chemical fractions of Cd in garlic root were mainly Hac-extracted fraction, followed by the NaCl-extracted fraction. The chemical fractions of Cd in water spinach root were mainly the NaCl-extracted fraction, followed by the Hac-extracted fraction. With the increase of time, the Hac-extracted fraction of Cd increased in both crops. According to the transfer coefficient (Table 3), the root-to-shoot transport of Cd in these two crops was probably closely related to the proportion of Hac-extracted Cd in roots. The larger proportion of Hac-extracted Cd, the smaller TF, which was consistent with the results from our field trial. Moreover, the NaCl-extracted fraction was the predominant Cd fraction in garlic leaves. In water spinach leaves, NaCl-extracted Cd was the main fraction, followed by Hac-extracted fraction. However, the total concentration of Cd in garlic leaves was significantly lower than that in water spinach leaves ($p < 0.05$). Generally speaking, under high-Cd stress, water spinach showed a stronger ability to sequester Cd and mitigate Cd toxicity by adjusting the chemical fractions of Cd.

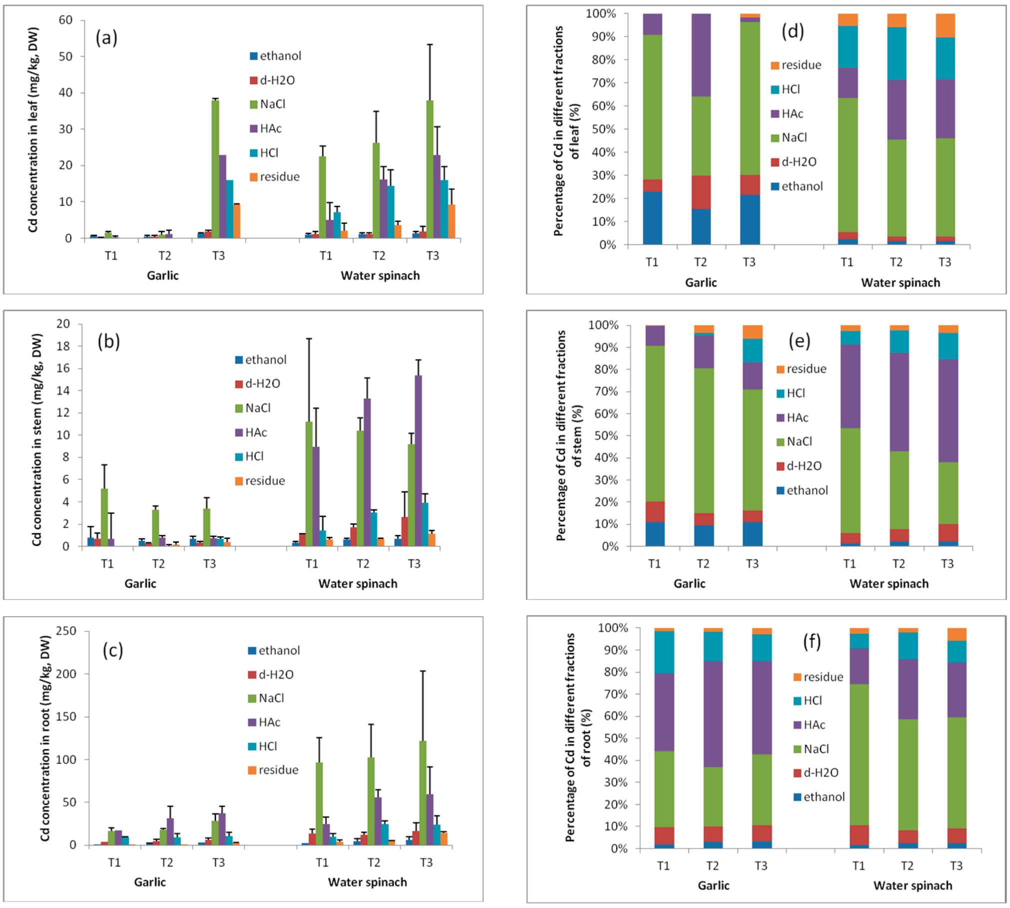

Figure 8. Concentrations and proportions of Cd associated with different chemical forms (extracted by 80% ethanol, d-H_2O, 1M NaCl, 2% HAc, 0.6 M HCl, and residue) in different tissues ((**a**,**d**): leaf; (**b**,**e**): stem; (**c**,**f**): root) of in garlic and water spinach at three time stages (T1-10 d, T2-20 d, T3-30 d) (n = 3, DW).

4. Discussion

4.1. Absorption and Accumulation of Cd and Pb by Different Vegetables

According to the field investigation, the soil and vegetable crops in the study area were contaminated by Cd and Pb to varying degrees, with particularly serious Cd pollution (the soil exceeded the environmental standard by 61 times, and the crops exceeded the standard by 73%) (Table 2, Figure 2). This showed that most of the vegetable crops harvested in the local area had potential food safety risks. Many studies showed that there was a large difference in the concentration of heavy metals among crop species in mine-impacted areas [15,31]. The highest concentration of Cd and Pb in the edible parts of vegetable crops in the present study were 250 and 583 times the lowest, respectively. It can be inferred that the variation of Cd and Pb concentrations in local vegetable crops was great, with the highest Cd concentration in vegetables, cole, water spinach, and fennel, and the highest Pb concentration in peas ($p < 0.05$). Local farmland might not be suitable for crop cultivation under such heavy metal pollution. However, there were still crop species with Cd and Pb concentrations within the safe range, which could thus be planted without food safety risks. For instance, the Cd and Pb concentrations in scallions both satisfied the food safety standard (Figure 2).

4.2. Differences in Cd Uptake and Accumulation Capacity between Different Types of Vegetable Crops

There were significant differences between different vegetables (types) in their ability to uptake and accumulate Cd. In recent years, a large number of studies have shown that screening crops with low Cd accumulation was a promising way to ensure safe agricultural production [18,32,33]. The BF was a parameter reflecting the ability of heavy metals to transfer from soil to plants [34]. There were significant differences in the ability of plants to assimilate heavy metals [20]. The present study showed that leafy vegetables had higher Cd enrichment capacity than the other three types of vegetables (rootstalk, allium, and legumes) (Figure 4). Some previous studies showed that leafy vegetables were more likely to accumulate Cd than non-leafy vegetables [31,35], which was consistent with the results of the present study. Among leafy vegetables, water spinach, and cole exhibited the highest BF, whereas garlic, onion, and pea had the lowest BF. Specifically, the BF of water spinach leaves was 0.08, which was 43 times higher than garlic leaves (Figure 4). This implies that leafy vegetables such as water spinach and cole could be regarded as high Cd accumulation vegetables, whereas garlic, onion, pea (legume) and others could be regarded as low Cd accumulation vegetables. This result was generally consistent with Kuboi et al.'s [36] classification of crop Cd accumulation, wherein cole of Cruciferae was defined as a high accumulator and pea (*Leguminosae*) as a low accumulator. In addition, garlic and onion were classified as stable accumulators by Kuboi et al. The difference in Cd uptake and accumulation capacity between different types of vegetables suggests that it is a feasible strategy to screen low accumulation crop species and types to alleviate the health risks posed by soil Cd contamination. At the same time, the difference in Cd uptake and accumulation between leafy vegetables and non-leafy vegetables might be due to their anatomical and physiological differences, which subsequently caused their different uptake, transport, accumulation, and sequestration abilities of Cd [37].

4.3. Cd Uptake and Accumulation in Different Tissues of Crops

Different tissues of crops had different capacities to accumulate Cd, which was considered an important way to cope with Cd stress. It was reported that soybeans could retain 98% of assimilated Cd in the root to prevent Cd from transferring to the aerial portions and disturbing the normal metabolism [38]. By contrast, lettuce had a high potential for Cd uptake and translocation, and around 50% of Cd would be distributed to the aerial portion [39]. It can thus be seen that the distribution of Cd in different crops was different. The present research found that most of the collected vegetables could effectively sequester most of the Cd in the roots, especially peas and garlic, in which retained Cd in the roots accounted for 81.2% and 79.6% of the whole plant, respectively (the data involved in Figure 5 are not listed). This implies that for most vegetable crops, the root acts as a dominant pool for Cd accumulation and a defending system against Cd translocation into the aerial part, suggesting that the toxicity of Cd to plants and its internal transport can be hindered by the structural and physiological characteristics of the root. The accumulation of heavy metals in different tissues of vegetable crops was generally root > stem > leaf [40], but there were also cases (such as *Brassica juncea* and *Brassica chinensis*) in which root > leaf > stem [31,41]. In the present study, the distribution of Cd in different tissues of the nine vegetable crops collected was root > leaf > stem, which indicated that the health risk of edible stems of local vegetable crops was lower than that of leaves.

4.4. Biological Availability of Cd in Soil

DTPA extractant was widely used to extract metal soluble, exchangeable, organically bound, and some metal oxides from the soil, and to distinguish soil metal mobility through solubility [42]. Our research data shows that there was a significant positive correlation between DTPA-extracted Cd and total Cd concentration in soil (Figure 6), indicating that bioavailable Cd concentration was generally controlled by total Cd in local farmland soils. The availability of soil Cd, which averaged 43% (the ratio of DTPA-extracted form of soil

Cd to the total content), was considerably high and thus easily migrated into plant and surrounding areas.

Studies have shown that there was a linear relationship between trace elements in soil and crops. In this study, the concentration of DTPA-extracted Cd in the root zone soil of tested crops was positively correlated with the concentration of Cd in the root zone of crops. However, the relationships of soil DTPA-extracted Cd with the Cd concentration in crop stems and leaves did not reach significance. This is similar to the results obtained by Egwu and Agbenin [43], which might be due to the different morphological and physiological characteristics of different crops. A series of defense mechanisms, including the adsorption, sequestration, chelation, and excretion of heavy metals, restricted the distribution of toxic elements by sensitive tissues and inhibited their root-to-shoot translocation [44,45]. It can be seen that the Cd accumulation in crop aerial parts not only depended on the available concentration of Cd in soil but was also affected by the mechanism of root uptake, retention, and translocation.

4.5. Effects of Crop Root Chemical Forms on Cd Transport

The biological effects of heavy metals were closely related to their chemical forms. Different chemical forms varied in phytotoxicity and migration capacity. For example, an inorganic Cd fraction ($F_{ethanol}$) and a water-soluble fraction (F_{H2O}) had higher migration capacity and were more toxic to plant cells than the insoluble phosphate fraction (F_{HAc}) and the oxalate fraction (F_{HCl}) [46]. In this study, it was found that Cd in the collected plant samples in roots, stems, and leaves mainly existed in the form of F_{NaCl}. Zhou et al. [47] believed that the proportion of F_{NaCl} might be one of the key factors affecting the accumulation of Cd in different crops because the protein-bound Cd might affect Cd transport. Weng et al. [48] also pointed out that the chemical form of Cd is related to its transport capacity. $F_{ethanol}$ and F_{H2O} possessed the strongest migration capacity, followed by F_{NaCl}, and F_{HAC}, and F_{HCl} had the weakest migration capacity. Our research showed that the proportion of $F_{ethanol}$ in the roots of crops was positively correlated with the transport rate of Cd from roots to leaves. The proportions of F_{NaCl} and F_{HAc} were negatively correlated with the transport rate of Cd from roots to stems. This suggests that in the present study, the Cd translocation to the aerial part increased with an increasing proportion of F_{NaCl} and F_{HAc} but decreased with an increasing proportion of $F_{ethanol}$ in crop roots. Fu et al. [26] considered that the separation of Cd from organic ligands in vacuoles or the combination of Cd with pectin and protein (F_{NaCl}) in cell walls might be the reason for phytolacca's adaptation to Cd stress. Compared with Cd fractions in other crops' roots, the proportion of $F_{ethanol}$ in garlic was the smallest, whereas the proportions of $_{FNaCl}$ and F_{HAc} were the largest, leading to its low Cd translocation rate. Weng et al. [48] found that the Cd in the roots and leaves of *Kandelia obovata* was mainly composed of F_{NaCl} and F_{HAC}, which manifested its enhanced Cd tolerance. In the present study, the chemical forms of Cd in garlic (low-Cd accumulator) and water spinach (high-Cd accumulator) were investigated in greenhouse experiments.

It was found that in roots, the chemical fractions of Cd in garlic roots were mainly F_{HAc}, followed by F_{NaCl}. By contrast, the chemical fractions of Cd in the root of water spinach were mainly F_{NaCl}, followed by F_{Hac}-extracted (Figure 8). Because the migration capacity of F_{HAc} was weaker than that of F_{NaCl} [47], the ability of water spinach to transport Cd from root to shoot was clearly higher than that of garlic, and this property was maintained over time (Table 4).

5. Conclusions

Results of the present study indicated that the heavy metal pollution in soils and crops in the study area was serious, especially Cd. The Cd and Pb concentrations in local farmland exceeded the environmental standard by 61 and 3.7 times, respectively. The Cd availability of the soil was 43% on average, which was closely related to the Cd concentration in plant roots. However, there was no clear interaction between soil-available Cd and the Cd concentrations in crop stems and leaves. Up to 73% of collected crop samples exhibited Cd concentrations beyond the food safety standard in the edible parts. The predominant Cd fraction in crop roots was F_{NaCl}, which along with F_{Hac} showed a negative correlation with the root-to-shoot Cd translocation rate. By contrast, the proportion of $F_{ethanol}$ in crop roots was positively correlated with the rate of Cd transport from roots to leaves. Leaf vegetables more readily accumulated Cd than onion, garlic, rhizome, and leguminous plants. Therefore, it is suggested that the cultivation of leaf vegetables should be reduced or even prohibited in this area. In view of different crop species, cole and water spinach had the highest Cd accumulation capacity, whereas garlic, onion, and pea had the lowest Cd accumulation capacity. Results of the greenhouse experiment showed that the chemical fraction of Cd in the root was mainly F_{HAc} in garlic and F_{NaCl} in water spinach. Owing to the different transport capacities of different chemical fractions, the ability of water spinach to transport Cd from root to shoot was higher than that of garlic, and this property was maintained with the increase of time. Overall, different vegetable crops had significant differences in the accumulation capacity of Cd. The Cd uptake, transport, and accumulation in different crop species were determined not only by the bioavailability of soil Cd, but also by the difference in the distribution of Cd chemical fraction of Cd.

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