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Chemical fractionations and bioavailability of cadmium and zinc to cole (*Brassica campestris* L.) grown in the multi-metals contaminated oasis soil, northwest of China

Yiming Yang[1,2], Zhongren Nan[1,*], Zhuanjun Zhao[1], Shengli Wang[1], Zhaowei Wang[1], Xia Wang[1]

1. Key Laboratory of Western China' s Environmental System, Ministry of Education, College of Resource and Environment, Lanzhou University, Lanzhou 730000, China. E-mail: yyiming2004@126.com
2. School of Chemical Engineering, North West University for Nationalities, Lanzhou 730030, China

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Abstract

A pot experiment was conducted to study the relationship between distribution of cadmium (Cd) and zinc (Zn) and their availability to cole (*Brassica campestris* L.) grown in the multi-metal contaminated oasis soil in northwest of China. The results showed that Cd and Zn in the unpolluted oasis soil was mainly found in the residual fractionation, however, with increasing contents of Cd and Zn in the oasis soil, the distribution of Cd and Zn changed significantly. The growth of cole could be promoted by low Cd and Zn concentration, but significantly restrained by high concentrations. There was antagonistic effect among Cd and Zn in the multi-metals contaminated oasis soil. Stepwise regression analysis between fractionations distribution coefficients of the two meals in the soil and their contents in cole showed that both Cd and Zn in the exchangeable fractionation in the oasis soil made the most contribution on the uptake of Cd and Zn in cole. The bio-concentration factor (BCF) of Cd was greater than Zn in cole, and BCFs of the two metals in leaves were greater than those in roots. The translocation factors of the two metals in cole were greater than 1, and the two metals mainly accumulated in the edible parts in cole. Therefore, cole is not a suitable vegetable for the oasis soil because of the plants notable contamination by heavy metals.

Key words: fractionation; bioavailability; cadmium and zinc; cole; oasis soil

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Introduction

Heavy metal contamination in soils is now a global environmental problem. Heavy metals in soils can not be bio-degraded, but can be bio-accumulated by plants, and pose toxicity beyond certain limit. Excessive heavy metals may cause greater toxicity, and pose a threat to human health through food chains. Many studies have found that the toxicity and mobility of heavy metals depended not only on their total amounts but also on their chemical fractionations (Greenway and Song, 2002; Tandy et al., 2009; Wang et al., 2009; Chojnacka et al., 2005; Gupta and Sinha, 2006; Li et al., 2007; Silveira et al., 2006; Rodríguez et al., 2009; Ma and Rao, 1997; Li and Thornton, 2001; Kartal et al., 2006).

There are many methods for classifying heavy metal fractionations (Tessier et al., 1979; Ahnstrom and Parker, 1999; Qiao et al., 2003; Silveira et al., 2006). Tessier's five-step sequential extraction procedure (Tessier et al., 1979) has been widely used, in which heavy metals in soils were categorized in five chemical fractionations including exchangeable fractionation (F1), carbonate-bound fractionation (F2), Fe-Mn oxide bound fractionation (F3), organic matter-bound fractionation (F4) and residual fractionation (F5) (Tessier et al., 1979; Lucho-Constantino et al., 2005; Silveira et al., 2006; Rodríguez et al., 2009). F1 is bioavailable fractionation, F2, F3 and F4 are potential bioavailable fractionation, and F5 is unbioavailable fractionation (Ma and Rao, 1997; He et al., 2005; Rodríguez et al., 2009).

The oasis soil is a major agricultural soil and mainly distributes in the oasis in the arid desert region including Xinjiang Autonomous Region and the Hexi Corridor, northwest of China. There is a long history to irrigate oasis soils with wastewater due to drought and water shortage in this region. The oasis soil has been seriously contaminated, especially polluted from heavy metals (Nan and Zhao, 2000; Ding et al., 2008), which caused a serious threat to human and the environmental health in the oasis region.

* Corresponding author. E-mail: nanzhongren@lzu.edu.cn

Many studies have found that the uptake of heavy metals by plants was related to the physical and chemical properties of soils, soil types, plant species, and the interaction between heavy metals (McBride, 2003; Sun et al., 2003; Kidd et al., 2007). Usually, there are synergy, antagonistic, or additive effects among different heavy metals (Zhou et al., 1994; Zhu et al., 1997).

Cole (*Brassica campestris* L.) is grown and eaten widely throughout China. But the bioavailability of heavy metals to cole in the oasis soil in northwest of China has not been reported. Therefore, there is significance to research the uptake of heavy metals by cole under multi-metal contamination. The present study revealed the bioavailability of heavy metals to cole under Cd-Zn contamination based on a pot experiment in the oasis soil in northwest of China to assess health risks.

1 Materials and methods

Experimental reagents used were selected with superior grade (Tianjin Kermel Chemical Reagents Corpration, China) and certified reference samples, GSS-1 (GBW07401) and GSB-6 (GBW10015) were used.

1.1 Experimental design

Pot experiments were conducted under open air conditions at Linze County, Hexi Corridor, Northwest China in 2008. The irrigation-silting soils are derived from oasis/desert soils, and textures mainly sandy. Soil pH in water (1:5, W/W) was measured by a combined glass calomel electrode; carbonate content was determined using the carbon dioxide volume method; OM content was measured using the potassium dichromate oxidation method; and CEC was determined using ammonium acetate method. Soil properties were pH (H_2O) 8.47, 1.54% organic mater (OM) content, 7.39% $CaCO_3$ content, and cation exchange capacity (CEC) 8.11 cmol/kg. The background concentration levels of heavy metals in agrarian land are 0.118 mg/kg for Cd and 70.5 mg/kg for Zn dry weight (dw).

The soil used was an irrigation-silt sandy soil, excavated from an oasis farm, mixed several times into one large heap, then passed through a 10 mm sieve to remove large stones and grass debris. Nine treatments (one control and eight amendments) were replicated three times in a randomized block design. The control was not amended with inorganic Cd and Zn. The others, one by one, had been spiked with different solutions of $Cd(NO_3)_2$ and $Zn(NO_3)_2$ to elevate soil metal concentrations as shown in Table 1, which were designed based on the ratio of Cd to Zn and their maximum concentrations values in the multi-metals contaminated oasis soil in our field pollution investigation. The heavy metals were added by spraying a solution of each metal salt over relatively dry soil spread out on a large tray. The soil was turned over and sprayed several times then watered and left to equilibrate outdoors for two weeks before planting vegetables. The plant was seeded and taken care of according to cultivation system.

The whole vegetables were harvested after 60 days of growth. The vegetable was cut to separate parts grown under and above the soil. Leaves were washed three times using de-ionized water. Roots were abundantly washed with tap water to eliminate soil particles, and rinsed three times with de-ionized water. All plant samples were dried in an oven at 70°C passed through 2 mm sieve for further experiment.

Soil samples were taken from the pots after harvesting the vegetables. After removing crop debris, soil samples were air-dried at room temperature, passed through 100 mesh (ϕ = 0.149 mm) sieve.

1.2 Determination of Cd and Zn in soils and cole

Total Cd and Zn concentrations in soils were determined according to standards (GB/T17139-1997, GB/T17141-1997). Total Cd and Zn in cole were extracted using the acid digestion mixture (HNO_3-$HClO_4$-HF) (EPA3010A). The supernatant was obtained from the digestion, filtered, reconstituted to the desired volume, and analyzed by an atomic absorption spectrometer (AAS, M6MK2, Thermo Electron Corporation, USA) for Cd and Zn concentration. Cd and Zn in soil solutions obtained from Tessier's sequential extraction procedure were also determined by AAS. The solutions were acidified with HNO_3 and stored at 4°C until Cd and Zn concentrations were determined.

1.3 Tessier's sequential extraction procedure

Tessier's sequential extraction procedure was used. Cd and Zn were extracted by $MgCl_2$ solution in F1, by HAc-NaAc solution in F2, by $NH_2OH{\cdot}HCl$ solution in F3, by HNO_3-H_2O_2-NH_4Ac solution in F4, and by HNO_3-$HClO_4$-HF solution in F5.

1.4 Data analysis

Total Cd and Zn concentrations in soils, cole, and sequential extractions were determined in triplicates. SPSS16.0 and EXCEL2003 software were used for data analysis.

2 Results and discussion

2.1 Chemical fractionations distribution of Cd and Zn in the soil

All fractionations contents of Cd and Zn increased with increasing Cd and Zn contents in soil (Fig. 1), especially, in F1, F2, F3 for Cd and in F2 and F3 for Zn. The parameter

Table 1 Concentrations of Cd and Zn in tested soil (unit: mg/kg)

Treatment	CK	I	II	III	IV	V	VI	VII	VIII
Cd	0	0.35	0.75	1.25	1.80	2.50	3.50	5.00	7.50
Zn	0	50	100	180	300	450	600	800	1000

CK: control.

fractionation distribution coefficient (FDC) was usually defined as the one metal fractionation content accounting for the percentage by total amount of the same metal. The results (Fig. 2) showed that with the increase in Cd and Zn contents, FDCs of Cd in F1 and F2 increased significantly; and those in F3 increased firstly, then decreased; those in F4 little changed; those in F5 dramatically decreased. FDCs of Zn in F1, F2 and F3 increased significantly; those in F4 little changed; those in F5 dramatically decreased. FDCs of Cd in F1 and F2 were more than Zn; while those of Cd in F3, F4 and F5 were less than Zn.

Previous studies showed that in the uncontaminated soil, F5 was mainly heavy metal fractionation, which was unbioavailable for the plants (Gao et al., 2001; Wang and Zhou, 2003; Guo and Zhou, 2005), but the percentage of heavy metals associated with available fractionation increased with the increase of total amount of heavy metals (Gao et al., 2001; Zhou et al., 2003; Wang and Zhou, 2003; Guo and Zhou, 2005). Similar results were found in the present study. The results showed that Cd and Zn in uncontaminated oasis soil mainly was in unbioavailable fractionation, with the increase in amounts of two metals in the soil, contents of Cd and Zn in bioavailable fractionation increased significantly, while the Cd content of the bioavailable fractionation was much higher than Zn, which showed that the activity of Cd was stronger than Zn, and its toxicity was correspondingly greater than Zn.

2.2 Growth status of cole in the soil

The dry weight (dw) of roots and leaves increased, and reached peak value, and then decreased with the increasing Cd and Zn in the soil (Table 2). When the treated level of Cd and Zn was V and IV, the dry weight of roots and leaves reached the peak value, respectively, which indicated that those values were critical concentrations to roots and leaves, and beyond it, the roots and leaves would be poisoned, then the growth would be restrained. The critical values of Cd and Zn in leaves were lower than those in roots, and the accumulated amounts of Cd and Zn in leaves were higher than those in roots.

These results indicated that low concentrations of Cd and Zn could promote cole growth, while high concentrations of those restrain cole growth. This conclusion was the same as the results of previous studies (Song et al., 1996; Li et al., 2002; Zong et al., 2007). The high concentrations of Cd and Zn may damage the roots of cole, thereby restrained uptake of nutrient elements in roots.

2.3 Bioavailability of Cd and Zn

2.3.1 Accumulation of Cd and Zn in cole

Contents of Cd and Zn in cole increased with increasing Cd and Zn concentrations in the soil (Table 2).

However, contents of both Cd and Zn in cole under Cd-Zn compound contamination were lower than those under single Cd or Zn contamination (Tables 2 and 3), which

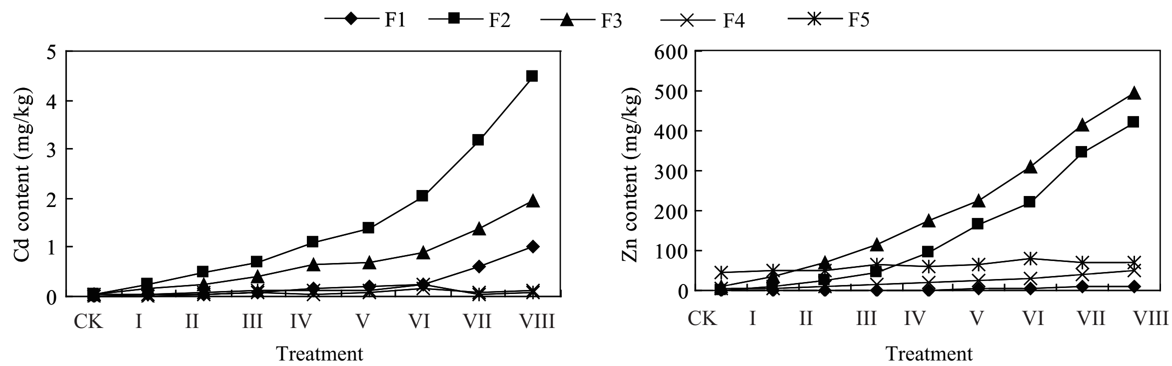

Fig. 1 Distribution of Cd and Zn fractionations content in the soil with different treatments. F1: exchangeable fractionation, F2: carbonate-bound fractionation, F3: Fe-Mn oxide bound fractionation, F4: organic matter-bound fractionation, F5: residual fractionation. Values were means of three replications.

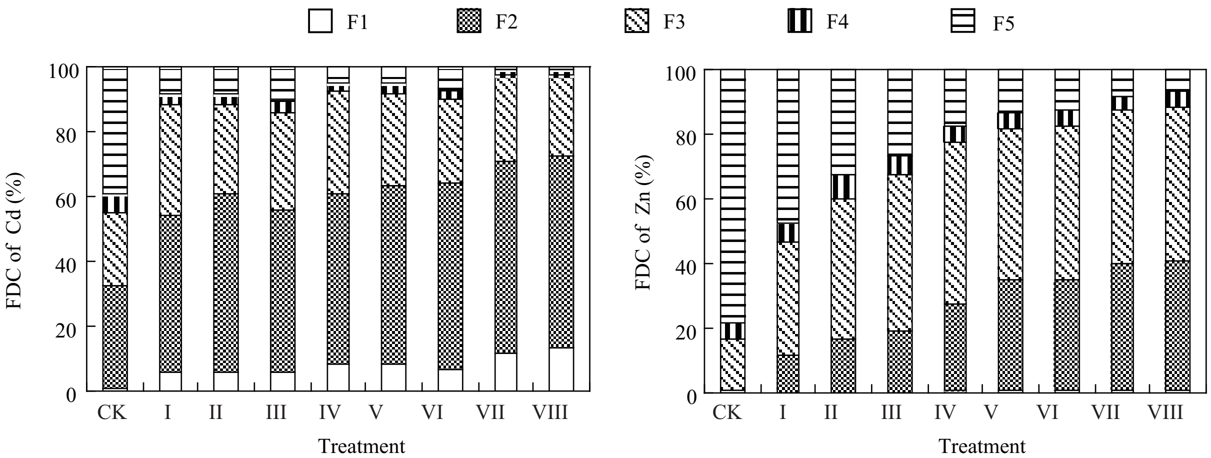

Fig. 2 Change of the fractionation distribution coefficient (FDC) of Cd and Zn in soil with different treatments.

Table 2 Change of dry weight of roots and leaves and heavy metals content in cole with different treatments

Treatment	Dry weight (g/pot)		Cd content (mg/kg)		Zn content (mg/kg)	
	Root	Leaf	Root	Leaf	Root	Leaf
CK	0.09	0.52	0.63	0.36	91.005	56.350
I	0.16	0.91	0.48	1.64	124.985	168.455
II	0.15	0.81	0.54	4.46	187.100	277.015
III	0.19	0.92	2.62	4.07	257.160	354.790
IV	0.19	1.35	2.36	7.52	294.705	358.355
V	0.21	1.15	2.92	11.98	264.190	462.000
VI	0.14	0.81	1.75	15.60	470.105	594.735
VII	0.11	0.77	5.79	19.03	592.495	715.255
VIII	0.06	0.42	6.59	20.86	531.335	769.485

Values were means of three replication samples.

Table 3 Change of heavy metals content in cole under single metal treatment

Treatment	Cd content (mg/kg)		Zn content (mg/kg)	
	Root	Leaf	Root	Leaf
CK	0.15	0.86	106.290	100.390
I	1.94	4.20	154.582	192.820
II	2.61	8.38	218.610	267.260
III	11.40	15.27	270.515	330.620
IV	14.23	17.60	327.528	371.040
V	22.78	33.22	401.972	438.160
VI	23.38	33.51	656.390	757.510
VII	30.80	36.37	867.252	907.930
VIII	46.99	45.35	1218.182	1211.780

Values were means of three replication samples.

indicated that there was antagonistic effect among Cd and Zn in the multi-metal contaminated oasis soil.

Zhu et al. (1997) found that Cd-Zn pollution had a synergistic effect on Zn, and low content of Cd could promote Zn uptake in wheat, lettuce, tomato and cabbage on purple soil. However, Cd-Zn pollution had an antagonistic effect on Cd, and high content of Zn could restrain Cd uptake in those on purple soil. Zhou et al. (1994) found the influence of compound pollution of Cd and Zn on rice plant was not unitary additive or antagonistic effect. The interaction of Cd and Zn resulted in a decrease in Zn uptake and an increase in Cd accumulation in rice plant. The present study is inconsistent with previous reports probably due to the specific soil type and plant species.

In order to compare the uptake and translocation capacity of the two metals in soil-cole system, two parameters, bio-concentration factor (BCF) and translocation factor (TF) were introduced. BCF was the ratio between the metal concentration in plant tissues and that in their rooted soils, and TF was the ratio between metal concentration in other plant parts and that in roots.

Song et al. (1996) found that the tissue accumulation rate of heavy metals in the edible parts of spinach is Cd > Zn. Similarly, Li et al. (2002) found that the remove rate of heavy metals from soils to celery is Cd > Zn. The present study observed similar phenomenon. BCFs of Cd and Zn under different treated levels in roots and leaves of cole are shown in Fig. 3. In general, BCF of Cd in cole was more than Zn, and BCFs of both Cd and Zn in leaves were greater than those in roots. With the increasing Cd and Zn concentrations, BCFs in roots and leaves increased and reached peak value, and then decreased. Sun et al. (2003) found that the cumulative coefficient of heavy metal were higher under low heavy metal doses, on the contrary, the cumulative coefficient of heavy metal were low under high doses, but the absolute amount of heavy metals accumulated increased with the increasing heavy metals dose. Similar results were found in the present study.

TFs of Cd and Zn in leaves were more than 1, and the TF of Cd was greater than Zn (Fig. 3).

The above results showed that BCFs of Cd and Zn in cole had critical values, beyond them, roots would be damaged by Cd and Zn, although the uptake and accumulation amounts of Cd and Zn in cole increased with increasing heavy metals dose, but their uptake rates gradually decreased. Hence, BCFs of two heavy metals in cole increased at first, and then decreased. The above results also showed that Cd and Zn mainly accumulated in the edible part of cole above the ground.

Many studies indicated that cole had high accumulation

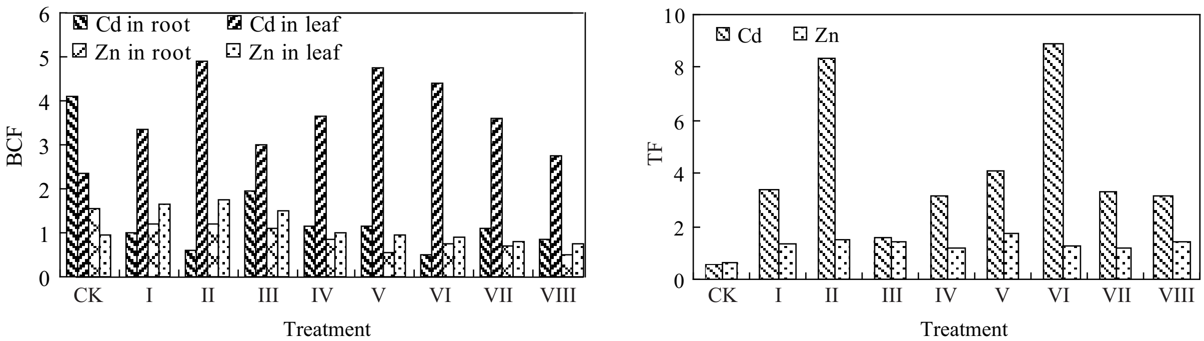

Fig. 3 Change of bio-concentration factor (BCF) and the translocation factor (TF) of Cd and Zn with different treatments.

Table 4 BCFs of Cd and Zn in cole planted in various soils

Soil type	pH	BCF of Cd		BCF of Zn		Reference
		Shoot	Root	Shoot	Root	
Red soil	5.05	6.05–284.83	6.62–472.15	0.88–2.94	1.50–2.45[a]	Xu et al., 2007
Dystric purplish soil	5.57	–	–	2.03–6.13 (shoot + root)	–	Chen et al., 1990
Eutric purplish soil	7.09	–	–	1.14–2.58 (shoot + root)	–	Chen et al., 1990
Calcaric purplish soil	8.14	–	–	1.31–1.88 (shoot + root)	–	Chen et al., 1990
Lou soil	7.90	1.29–1.26	1.98–5.95	–	–	Zhang et al., 2009
Cinnamon soil	8.24	1.03–2.90	2.12–4.09	–	–	Liu et al., 2002
Oasis soil	8.47	2.35–4.92	0.50–4.09	0.73–1.76	0.5–1.53	This study

for Cd and Zn, and even could be regarded as a hyperaccumulator for Cd (Chen et al., 1990; Su and Wong, 2002; Wang et al., 2003, 2005; Liu et al., 2002; Ru et al., 2005; Xu et al., 2007; Xiang *et al*., 2009; Wu and Su, 2009; Zhang et al., 2009).

BCFs of Cd and Zn in cole in various soils are given in Table 4. BCFs of Cd and Zn in cole in oasis soil were less than those in red soil which probably due to the higher pH value in the oasis soil. Because of higher accumulations of Cd and Zn in cole in the oasis soil, they may make greater risk for human health through food chains. Therefore, cole is not a suitable crop for the oasis soil because of the plants notable contamination.

2.3.2 Relationship between the uptake of Cd and Zn in cole and their chemical fractionations in soil

Significant positive relationship between Cd content in cole and FDC of Cd in F1 in soil were found, the corresponding correlation coefficients (R) were 0.895 for roots and 0.857 for leaves; significant positive relationship between Zn content in cole and the FDC of Zn in F1 in soil were also found with corresponding correlation coefficients 0.877 for roots and 0.891 for leaves (Table 5).

Zhu et al. (2002) found exchangeable Cd and Zn were highest available and made the most contribution to the lettuce plant in purple soil, and Cui et al. (2005) found that exchangeable Cd and Zn were extractable by reed plant, Chen et al. (1990) found that exchangeable Zn was highest available to cole in the purple soil. Similar results were observed in the present study due to the both exchangeable (F1) Cd and Zn were available to cole.

Table 5 Correlation coefficient (R) between FDC of Cd and Zn in the soil and Cd and Zn contents in cole

Chemical fractionation	R (Cd)		R (Zn)	
	Leaf	Root	Leaf	Root
F1	0.857**	0.895**	0.891**	0.877**
F2	0.582	0.761*	0.899**	0.957**
F3	–0.256	–0.330	0.633	0.710*
F4	–0.777*	–0.758*	–0.522	–0.435
F5	–0.539	–0.639	–0.809**	–0.883**

* Significance at level $p < 0.05$; ** significance at level $p < 0.01$.

Stepwise regression analysis (Fig. 4) between contents of Cd and Zn in roots and leaves of cole and FDCs of Cd and Zn in the oasis soil showed that both Cd and Zn in F1made the most contributions on the accumulation of Cd and Zn in roots and leaves of cole.

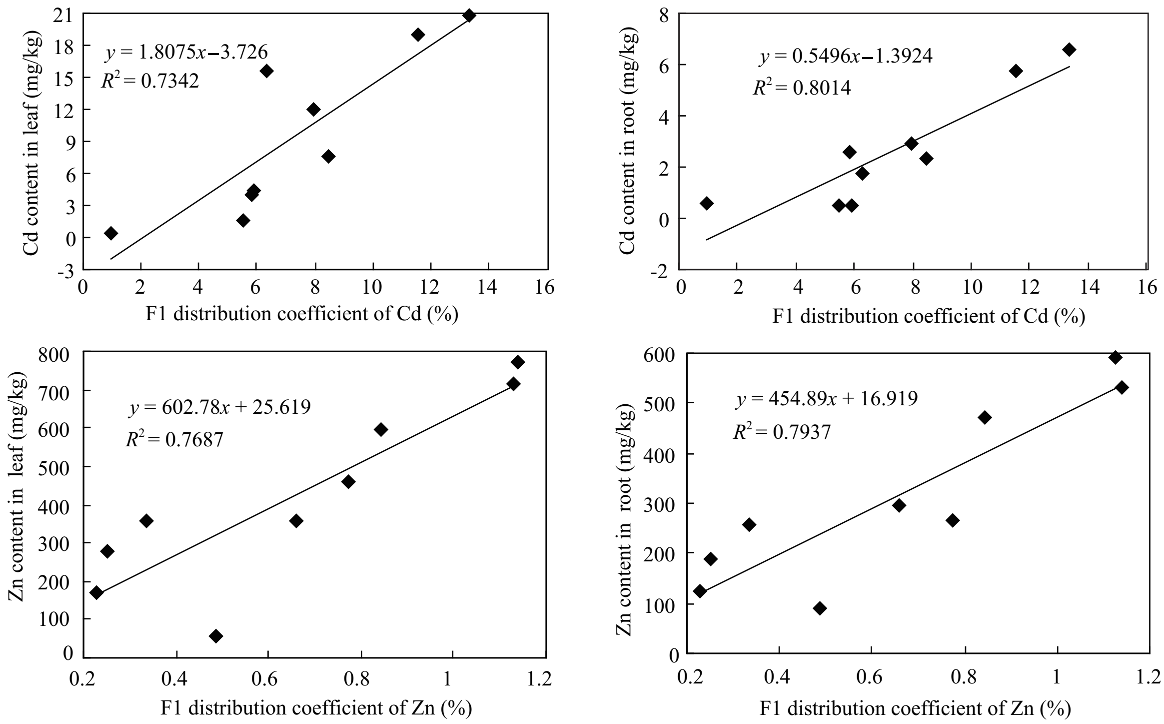

Fig. 4 Stepwise regression analysis between the fractionation distribution coefficient (FDC) of Cd and Zn in soil and Cd and Zn contents in cole root and leaf.

3 Conclusions

Cd and Zn in the unpolluted oasis soil was mainly found in the residual fractionation (F5), however, with the increasing contents of two metals in the oasis soil, the fractionations distribution of Cd and Zn significantly changed. FDCs of Cd and Zn in the soil increased significantly in F1 and F2, changed little in F4 and decreased dramatically in F5.

Low concentrations of Cd and Zn could promote the growth of cole, but high concentrations of Cd and Zn significantly restrained the growth of cole. There was antagonistic effect among Cd and Zn in the multi-metals contaminated oasis soil.

Both Cd and Zn in F1 made the most contributions on uptake of Cd and Zn in roots and leaves of cole. BCF of Cd in cole was greater than Zn, and BCFs of both Cd and Zn in leaves were greater than those in roots. TFs of Cd and Zn in leaves were more than 1, and the TF of Cd was greater than Zn. The two metals mainly accumulated in the edible part of cole, therefore, they had potential risk to human health through food chains. Cole is not a suitable vegetable to be grown in the oasis soil contaminated by Cd and Zn.

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