DIFFERENCE OF CD ENRICHMENT AND TRANSPORT IN ALFALFA (*MEDICAGO SATIVA* L.) AND INDIAN MUSTARD (*BRASSICA JUNCEA* L.) AND CD CHEMICAL FORMS IN SOIL

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Abstract. In order to compare the application potential of alfalfa (*Medicago sativa* L.) and Indian mustard (*Brassica juncea* L.) on cadmium contaminated soil, difference in dry weight, Cd content and accumulation, transport and enrichment coefficient between alfalfa and Indian mustard were studied under different Cd levels (0, 75, 150, 300 and 600 $mg \cdot kg^{-1}$) using soil culture test. Also, Cd forms in soil were analyzed. The results showed that the dry weight of shoots and roots of alfalfa and Indian mustard decreased significantly ($P < 0.05$) with the increase of soil Cd levels, decreasing by 50.00-71.90% and 29.60-59.30% (alfalfa), 59.60-89.00% and 64.30-74.80% (Indian mustard) compared with the control. With the increase of soil Cd level, Cd concentration and Cd accumulation in shoots and roots of alfalfa and Indian mustard increased significantly ($P < 0.05$). At same soil Cd level, Cd concentration in shoots and roots was in order of alfalfa > Indian mustard, while Cd accumulation was in order of Indian mustard > alfalfa. At the soil Cd level of 75 $mg \cdot kg^{-1}$, Cd concentration in shoots of alfalfa and Indian mustard exceeded the critical value of Cd hyperaccumulator (100 $mg \cdot kg^{-1}$), 356.463 $mg \cdot kg^{-1}$ and 308.735 $mg \cdot kg^{-1}$ respectively. The enrichment coefficient (BCF) of alfalfa and Indian mustard was above 1 under all Cd levels, and the transport coefficient (TF) and BCF was in order of Indian mustard > alfalfa. Based on comprehensive consideration of biomass, Cd accumulation, transport coefficient and enrichment coefficient, Indian mustard is more suitable as repair material for soil Cd pollution.
Keywords: *soil Cd pollution, Cd hyperaccumulator, transfer coefficient, enrichment coefficient, soil Cd forms*

Introduction

Cadmium (Cd) is the top soil heavy metal pollutant. According to statistics, about 30,000 tons of Cd is released into the environment each year in the world, and of which Cd released into the soils reaches about 82-94% (Xiong et al., 2015). The total amount of Cd emitted into the environment from industrial waste is 680 tons per year in China (Ning et al., 2015). Cadmium pollution in farmland is 280,000 hm^2, and annual output of agricultural products with excessive Cd is nearly 1.5 million tons in China (Li et al., 2017b; Liu et al., 2017). Cadmium can be absorbed by plants and can be enriched in the human body through the food chain, endangering human health. Therefore, soil Cd pollution control and remediation has drawn wide attention from researchers (Li et al., 2017b; Marzban et al., 2017).

Indian mustard (*Brassica juncea* L.) is cruciferous *Brassica* with short growth cycle and great aboveground biomass. Capable of enriching a variety of heavy metals. When Cd was added at 0-200 $mg \cdot kg^{-1}$, the biomass of Indian mustard was unaffected, and

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shoot Cd concentration was up to 7.824-102.672 mg·kg^{-1}, and root Cd concentration was up to 0.374-191.910 mg·kg^{-1} (Yang et al., 2011). Alfalfa (*Medicago sativa* L.) a leguminous perennial herb, is a high-yielding cash crop. Sun and Song (2015) reported that Cd concentration below 25 mg·kg^{-1} can promote the growth of alfalfa shoot and root. With the increase of Cd level in soil, Cd concentrations in alfalfa shoot and root increase, and also has a higher enrichment effect on Cd ions. At present, there is little research on comparison of Cd uptake by alfalfa and Indian mustard. Therefore, in this study, the differences in biomass, Cd concentration and accumulation in alfalfa and Indian mustard, as well as Cd forms in soil were studied using soil culture test with different Cd concentrations, and plant suitable for soil Cd pollution remediation was screened out.

Materials and methods

Plant material, soil and Cd treatments

The seeds of alfalfa (*Medicago sativa* L.) were purchased from Jiangxi Scarecrow Agricultural Park, and the seeds of Indian mustard (*Brassica juncea* L.) were purchased from Angoo Plant Science and Technology, China. The soil was collected from the purple soil base of Southwest University in Beibei District of Chongqing, China. The soil pH was 5.23, the content of organic matter was 8.87 g·kg^{-1}, that of available phosphorus was 38.59 mg·kg^{-1}, that of available potassium was 65.07 mg·kg^{-1}, that of total nitrogen was 1.54 g·kg^{-1}, that of available nitrogen was 74.60 mg·kg^{-1}, that of total Cd was 0.110 mg·kg^{-1}, and that of available Cd < 0.005 mg·kg^{-1}.

The soil culture experiment was conducted in the glass greenhouse of College of Resources and Environment of Southwest University, China from March to May in 2015. An opaque black plastic basin (25 cm in diameter and 17 cm in height) was used in the experiment. The 5 kg of air-dried soil screened by 5 mm sieve was loaded in each basin. The $CdCl_2 \cdot 2.5\ H_2O$ solution was added to the soil and mixed well. The soil Cd^{2+} pollution levels were simulated at 0, 75, 150, 300 and 600 mg·kg^{-1}, and kept balance for two weeks. The seedlings of Indian mustard and alfalfa with 5 cm high were then transplanted 6 and 10 plants for each pot. The moisture content in soil was kept 70-75% of the maximum moisture in the fields with deionized water. Fast- measurement of Soil Moisture (TZS-IW, Zhejiang Tuopu Instrument Co., Ltd., China) was used to determine the moisture content in soil. In the basic fertilizer the used amount of P ($NH_4H_2PO_4$) and K (KCl) were 100 and 150 mg·L^{-1} respectively and Nitrogen content ($NH_4\ H_2\ PO_4$ and urea) was 180 mg·L^{-1}. N fertilizer was applied in three installments: 40% for basal and 60% each for seedling stages which applied with 15 days' interval after transplanting, each time 30%. All experiments were performed in triplicate and arranged at random. After 60 days, 6 (Indian mustard) and 10 (alfalfa) plants for each pot were harvested, and were separated into shoots and roots, washed free of soil with tap water and then rinsed with 0.1 M HCl followed by several rinses with deionized water (Kachenko and Singh, 2006). The harvested plants were oven-dried at 105 °C for 15 min, and oven drying to constant weight in 60 °C.

Analysis of soil physicochemical properties

The soil pH was ascertained in 1:5 (soil:water), and available phosphorus, available potassium and total nitrogen in soil was determined in term of a previously report

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(Rayment and Higginson, 1992). The soil organic matter content was determined on the basis of a previously published method (McCleod, 1975).

Analysis of Cd concentration in soil and plants

Soil containing Cd was first boiled with HNO_3–$HClO_4$ (*v*:*v* = 4:1), and its concentration was determined using an atomic absorption spectrophotometer (SIMMA 6000; PerkinElmer, Norwalk, CT, USA). The plant samples were first air-dried and ground and the Cd concentration in the plants was determined using a similar method to that used to quantify the levels of Cd in the soil. The results were monitored for quality control in following with plant standard reference material (GBW08513) and soil (GBW08303) obtained from the National Institute of Standards and Technology, China. The recovery rates of all the plants and soils were higher than 95%, and the relative standard deviation (RSD) for the precision of the tests was less than 10%.

1. Transport coefficient (TF) = Cd concentration in shoot ($mg \cdot kg^{-1}$) / Cd concentration in root ($mg \cdot kg^{-1}$) (Sun et al., 2016).
2. Enrichment coefficient (BCF) = Cd concentration in shoot (root) ($mg \cdot kg^{-1}$) / soil Cd ($mg \cdot kg^{-1}$) (Feng et al., 2016).

Soil Cd form determination

Method proposed by Tessier et al. (1979) was used to extract Cd from soil. The Cd concentration of each form was determined by atomic absorption spectrophotometry (Perkin Elmer SIMMA 6000, Norwalk, USA). The detection limit of atomic absorption spectrophotometer was 0.005 $mg \cdot kg^{-1}$. Soil standard substance (GBW # 08303) from the National Institute of Standards and Technology was used to monitor the quality of the results. The Cd recovery of all soil samples was higher than 95%, and the accuracy of relative standard deviation (RSD) was within 10%.

Statistical analysis

Three-way analysis of univariate ANOVA and correlation analysis were performed using SPSS version 21.0 package (SPSS, 2009). The variables analyzed separately were Cd concentration and Cd accumulation in alfalfa and Indian mustard, and Cd chemical forms in soil. The level of significance was 0.05.

Results

Biomass

As shown in *Table 1*, with the increase of soil Cd level, shoot and root dry weight of alfalfa and Indian mustard decreased significantly ($P < 0.05$), decreasing by 50.00-71.90% and 29.60-59.30% (alfalfa), 59.60-89.00% and 64.30-74.80% (Indian mustard) respectively. At same soil Cd levels, biomass of Indian mustard shoot and root was significantly higher than that of alfalfa. The tolerance coefficients of alfalfa shoot and root were 28.21-50.00% and 40.74-70.37% when exposed to Cd, respectively. When the Cd pollution level was above 150 $mg \cdot kg^{-1}$, the tolerance coefficients of each part were lower than 50%. The tolerance coefficients of Indian mustard shoot and root were 10.97-40.37% and 25.22-35.65% when exposed to Cd, respectively, both lower than 50%.

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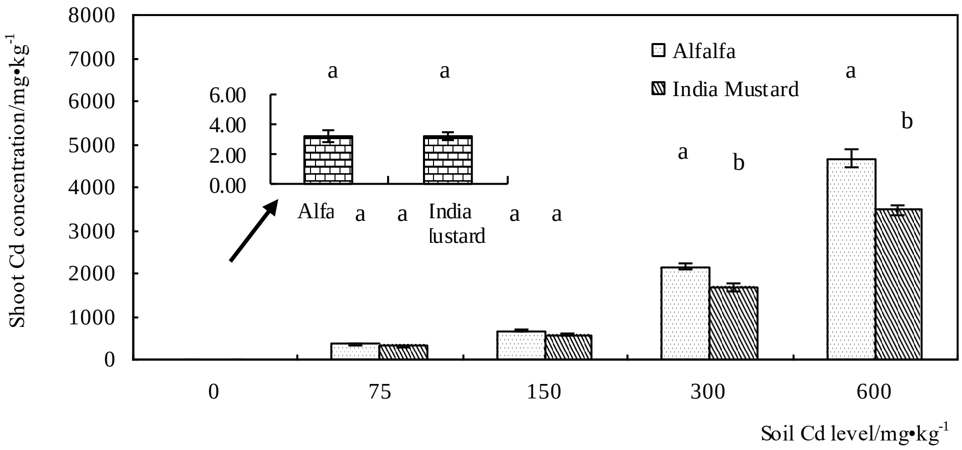
***Table 1.** Dry weights and resistance coefficient of two plants in different soil Cd levels*

Soil Cd levels ($mg \cdot kg^{-1}$)	Dry weight ($g \cdot plant^{-1}$)				Resistance coefficient (%)			
	Shoot		Root		Shoot		Root	
	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard
0	0.078±0.007a	0.857±0.033a	0.027±0.002	0.115±0.013a	—	—	—	—
75	0.039±0.008a	0.346±0.041a	0.019±0.001b	0.041±0.001a	50.00	40.37	70.37	35.65
150	0.023±0.003b	0.157±0.020a	0.013±0.002b	0.032±0.003a	29.49	18.32	48.15	27.83
300	0.022±0.001b	0.140±0.013a	0.013±0.002b	0.030±0.002a	28.21	16.34	48.15	26.09
600	0.022±0.001b	0.094±0.017a	0.011±0.001b	0.029±0.001a	28.21	10.97	40.74	25.22

Note: tolerance coefficient = Cd treatment plant biomass / control plant biomass × 100. The results listed are mean ± standard deviation. Different lowercase letters indicate the difference between different plants under the same Cd level ($P < 0.05$). The same below

Cd concentration in shoot and root

As shown in *Figure 1*, with the increase of soil Cd level, the Cd concentrations in shoots and roots of the two plants increased significantly ($P < 0.05$).



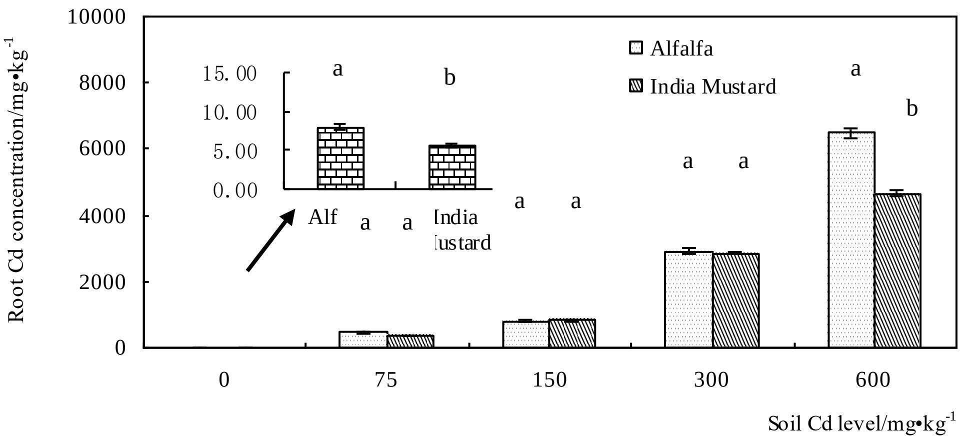


***Figure 1.** Effect of different soil Cd levels on Cd concentration of shoots and roots in two plants. Note: Different lowercase letters indicate the difference between different plants under the same Cd pollution level (P < 0.05). The same below*

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Under the same Cd level, the Cd concentration in roots of the two plants was higher than that in shoots, and Cd concentrations in shoot and root were in order of alfalfa > Indian mustard. At 75 mg·kg^{-1} soil Cd level, shoot Cd concentrations in alfalfa and Indian mustard passed the critical level of Cd hyperaccumulator (100 mg·kg^{-1}) which were 356.463 mg·kg^{-1} and 308.735 mg·kg^{-1} respectively.

Cd accumulation in shoot and root

As can be seen from *Figure 2*, with the increase of soil Cd levels, Cd accumulation in shoots and roots of the two plants increased with the increase of soil Cd level. At the 600 mg·kg^{-1} soil Cd level, Cd accumulations of shoot and root in alfalfa and Indian mustard reach the peak by 175.540, 101.632 and 324.70 μg·plant^{-1} respectively. Under the same Cd level in soil, Cd accumulations of shoot and root was in order of alfalfa < Indian mustard.

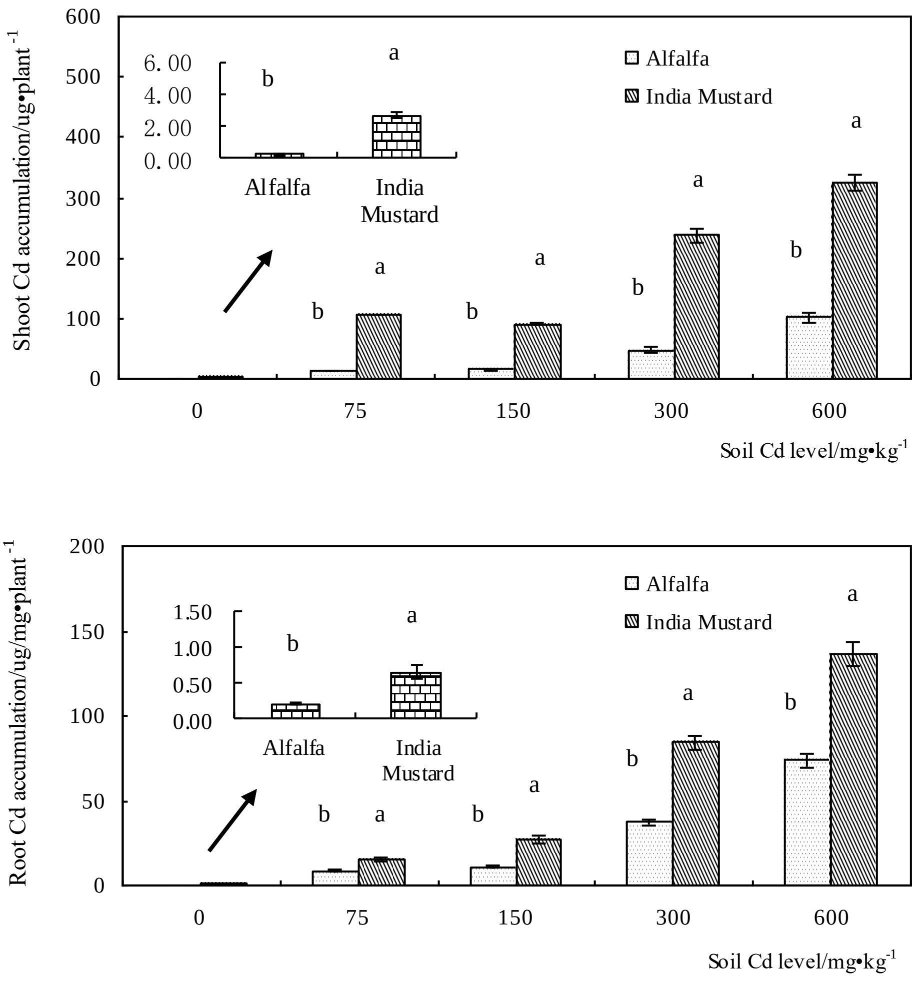

Figure 2. *Effect of different soil Cd levels on Cd accumulation in two plants*

Comparison of enrichment and transport capacity

As shown in *Table 2*, BCF of shoots of alfalfa and Indian mustard decreased firstly, reaching the lowest level at 150 mg·kg^{-1} Cd level, and then increased. Compared to that

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at 150 mg·kg^{-1} Cd level, the BCF of shoots of alfalfa and Indian mustard increases by 62.94% and 76.16% (alfalfa), 47.30% and 50.85% (Indian mustard) at the 300 and 600 mg·kg^{-1} Cd levels. With the increase of soil Cd level, the $BCF_{root/soil}$ of alfalfa decreased first, reaching the lowest at 150 mg·kg^{-1} Cd level, down by 10.48% compared with that at 75 mg·kg^{-1} Cd level, and then increased. For Indian mustard, the $BCF_{root/soil}$ first increased and then decreased, reaching the peak at 300 mg·kg^{-1} Cd. The BCF of the two plants was above 1, with that of Indian mustard > alfalfa. The TF of alfalfa and Indian mustard decreased with the increase of soil Cd levels, and the TF value was below 1. Under the same Cd levels, the TF was in order of Indian alfalfa > alfalfa.

Table 2. *The values of BCF and TF in two plants*

Soil Cd levels mg·kg^{-1}	$BCF_{Shoot/Soil}$		$BCF_{Root/Soil}$		$BCF_{Plant/Soil}$		TF	
	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard
0	-	-	-	-	-	-	-	-
75	4.753±0.274a	4.116±0.023b	6.042±0.278a	4.917±0.163b	10.795±0.005a	9.034±0.186b	0.789±0.082b	0.838±0.023a
150	4.421±0.159a	3.837±0.068b	5.409±0.006a	5.541±0.026a	9.830±0.165a	9.379±0.041a	0.817±0.028a	0.693±0.0150b
300	7.204±0.052a	5.652±0.305b	9.714±0.237a	9.521±0.116a	16.918±0.185a	15.173±0.421b	0.742±0.023a	0.593±0.025b
600	7.788±0.200a	5.788±0.219b	10.803±0.243a	7.780±0.105b	18.591±0.443a	13.568±0.324b	0.721±0.002a	0.744±0.018a

Note: BCF (Enrichment coefficient) = Cd concentration in shoot (root) (mg·kg^{-1}) / soil Cd (mg·kg^{-1}) (Feng et al., 2016); TF (Transport coefficient) = Cd concentration in shoot (mg·kg^{-1}) / Cd concentration in root (mg·kg^{-1}) (Sun et al., 2016)

Soil Cd chemical forms

As shown in *Table 3* and *Figure 3*, the Cd content in different forms of soil (EXC, CAB, FeMn, OM and RES) increased significantly with the increase of soil Cd level ($P < 0.05$). Cadmium fraction distribution coefficient (FDC) also demonstrated a corresponding trend. At the same Cd level, soil Cd of the two plants mainly exists as EXC-Cd. Cadmium fraction distribution coefficient (FDC) of EXC-Cd in soil of alfalfa and Indian mustard were 63.92-81.87% and 57.97-80.71% respectively. The OM-Cd content had the lowest distribution proportion under the same soil Cd, with FDC at only 0.87-13.33%. At the 300 mg·kg^{-1} and 600 mg·kg^{-1} Cd levels, exchangeable Cd content in the soil planted with Indian mustard was significantly lower than that planted with alfalfa.

Discussion

Earlier reports suggested that Cd had no significant effect on plant growth at a low Cd level (Liu et al., 2013; Yu et al., 2017) and even stimulated and promoted plant growth (Wei et al., 2004). In this experiment, the biomass of alfalfa was significantly lower than that of the control at the 75 mg·kg^{-1} Cd level, indicating that 75 mg·kg^{-1} Cd had a serious injury to alfalfa growth. The results are consistent with those of Lou et al. (2015). In this study, with the increase of soil Cd level. It may be probable that growth and development of plants were directly related to Cd level, soil physicochemical property, fertility and soil type, but the specific reason remains to be further studied.

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Table 3. *Effect of different Cd levels on Cd chemical forms in soil*

Soil Cd levels ($mg\cdot kg^{-1}$)	EXC-Cd		CAB-Cd		FeMn-Cd		OM-Cd		RES-Cd	
	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard
0	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
75	46.093±0.521a	40.141±0.551b	9.093±0.834a	10.437±2.011a	8.694±0.328b	10.088±2.509a	2.522±0.499b	5.540±1.491a	5.708±0.667a	3.033±0.308b
150	95.442±0.090a	66.357±1.262b	14.273±1.675a	11.933±1.001b	10.879±0.840a	10.586±1.999a	3.525±1.494b	8.288±1.247ba	6.221±0.161a	5.385±0.339b
300	142.382±22.228a	117.148±7.278b	20.886±0.359a	19.150±0.242a	14.508±1.807a	8.578±0.003b	3.528±0.170b	12.283±0.758a	7.702±0.654a	7.890±0.167a
600	330.700±3.299a	291.113±18.349b	37.603±0.311a	36.098±0.705a	19.884±0.488a	15.802±3.222b	4.863±0.164	7.776±2.234a	10.892±0.160a	9.888±0.178a

Note: EXC-Cd, CAB-Cd, FeMn-Cd, OM-Cd and RES-Cd indicate exchangeable Cd, carbonate-bound Cd, Fe-Mn oxides-bound Cd, Organic Cd and residuals Cd, respectively

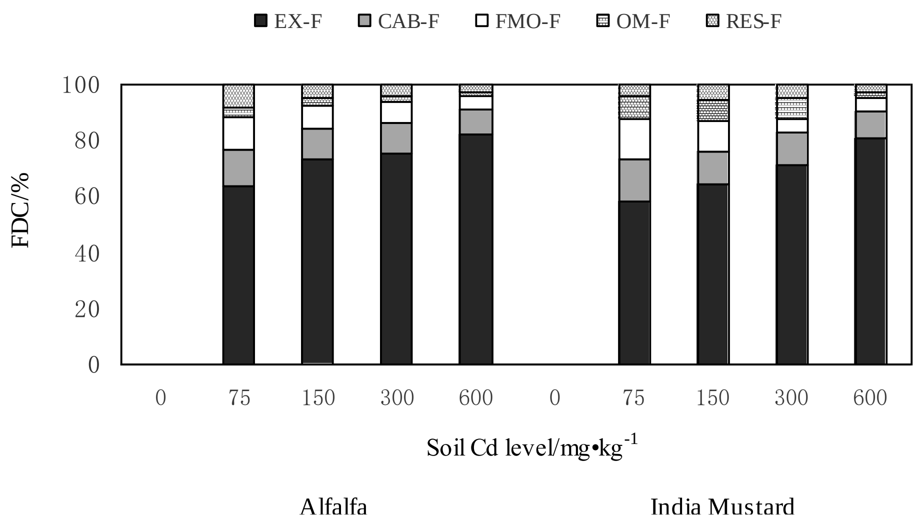

Figure 3. *Effect of different soil Cd levels on Cd fraction distribution coefficient (FDC) in soils. Note: EX-F, CAB-F, FMO-F, OM-F and RES-F indicate Cd distribution coefficient (FDC) of exchangeable Cd, carbonate-bound Cd, Fe-Mn oxides-bound Cd, Organic Cd and residuals Cd in soil, respectively*

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For Cd hyperaccumulator plants, Cd concentration in shoot should be 100 times higher than that of common plants (the critical value of Cd is 100 $mg \cdot kg^{-1}$); Cd concentration of plant root should be lower that of shoot; the plant growth does not appear obvious toxicity symptoms and has strong resistance to Cd (Li et al., 2017a; Lin and Feng, 2017; Sun and Song, 2015;). The results of this study showed that, Cd concentration in shoots of alfalfa and Indian mustard exceeded the 100 $mg \cdot kg^{-1}$ threshold of hyperaccumulator plant at the 75 $mg \cdot kg^{-1}$ Cd level. This result was similar to that reported by Yang et al. (2011). In comparison of Cd concentration in shoots and roots of the two plants under the same soil Cd level, that of alfalfa > Indian mustard. However, in the analysis of biomass and Cd accumulation, the biomass of Indian mustard was much higher than that of alfalfa, therefore, its Cd accumulation was also significantly higher than that of alfalfa. Different plant organs have different levels of heavy metals (Li et al., 2017b). In this experiment, it was found that, for both alfalfa and Indian mustard, that in root > shoot at different soil Cd levels, and their roots had strong Cd enrichment ability, but their ability to transport Cd to shoots was weak. Similar results were reported by Chen et al. (2014), Jiao et al. (2014), Hattab et al. (2013), and Sun et al. (2016), but different from the report by Yang et al. (2011). In this experiment, with the increase of Cd concentration, Cd transport index of Indian mustard first increased and then decreased, while alfalfa Cd transport index also increased. This result was different from earlier reports (Li and Song, 2016). In addition, BCF of alfalfa and Indian mustard were above 1 when exposed to Cd, while TF was lower than 1. The higher root BCF value of Indian mustard suggests that Indian mustard has the strongest Cd enrichment ability.

It has been reported that there is a significant difference in soil Cd form among different crops (Zhang et al., 2017). In this experiment, EXC-Cd, CAB-Cd and FeMn-Cd of alfalfa were significantly higher than that of Indian mustard under high concentration of Cd, while RES-Cd and OM-Cd were lower than that of Indian mustard, which was similar to the results of previous studies (Huang et al., 2000). It may be because different root exudates produced by different crops change Cd forms in the soil and mitigate Cd damage to the crop. In this experiment, the Cd in the two plants at different Cd levels both existed in the form of highly active EXC-Cd, and Cd concentration in different forms increased with the increase of Cd added concentration, which may be because Cd entered soil in the form of Cd solution with the highest activity (Xiong et al., 2015).

Conclusion

With the increase of soil Cd level, the biomass of shoots and roots of alfalfa and Indian mustard decreased significantly. The EXC-Cd was the dominating form of soil Cd. With the increase of Cd level in soil, the percentage of EXC-Cd in total Cd also increased continuously. At 75 and 150 $mg \cdot kg^{-1}$ Cd levels, Cd concentrations in shoots of alfalfa and Indian mustard exceeded the critical value of hyperaccumulators, The BCFs of both alfalfa and Indian mustard were above 1. At the same soil Cd level, for BCF and TF values, those of Indian mustard > alfalfa. Based on comprehensive consideration of biomass, Cd accumulation, transport coefficient and enrichment coefficient, Indian mustard has stronger Cd accumulation ability, thus more suitable as repair material for soil Cd pollution.

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