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Distribution of cadmium in oilseed rape and Indian mustard grown on cadmium contaminated soil

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Abstract: Heavy metal distribution in the specialized accumulating plants was important for phytoextraction technique. Hydroponic and pot experiment were conducted to investigated Cd phytoextraction ability and Cd distribution in the plant of oilseed rape species. The results showed that oilseed rape Chuanyou Ⅱ-10 was more effective in phytoexetraction Cd among 21 varieties of oilseed rape and indicator plant Indian mustard. Cd concentration in the shoot of Chuanyou Ⅱ-10 and Indian mustard gradually decreased with an increase in growth period, while the amount of Cd uptake increased with the increase of growth period. There was constantly decrease in Cd concentration from the base leaves to the top leaves of Chuanyou Ⅱ-10 and Indian mustard, the percentages of Cd uptake in older leaves were higher than those of younger leaves. Older leaves of Chuanyou Ⅱ-10 and Indian mustard extracted more Cd for the Cd contamination soil, leaves should as far as possible develop before they reached the soil and the older leaves were harvested in priority.

Keywords: cadmium; oilseed rape; Indian mustard; distribution

Introduction

Phytoremediation that uses some specialized plants to assimilate or detoxify cadmium contaminated soil is a promising new technology (Robinson, 1998; Lasat, 2002). *Thlaspi caerulescens* and Indian mustard (*Brassica juncea*) were used as Cd accumulator for research all along (Escarre, 2000; Lombi, 2000; Salt, 1995; Ebbs, 1997), while the small size, slow growth of *Thlaspi caerulescens* and geographical growth requirement of Indian mustard may limit their utility for phytoremediation (Black, 1995). Mustard oilseed rape (*Brassica juncea* L.) is one of the staple crops in China and is also a member of the Brassicaceae family, some of them have been succeeded to select to be potential for phytoremediation of Cd contamination soil (Su, 2004). Cd accumulation and tolerance inside plant tissue is expressed by the compartmentalization of Cd: Cd appears mostly to be localized in the apoplast of *Thlaspi caenclescens*, whereas little in vacuoles (Vázquez, 1992), Cd is associated with organic acid in leaf cell of *Thlaspi caenclescens* (Brown, 1995). Cd is associated with organic acid in xylem, with alga silk in leaf of *Brassica junica*, Cd concentration in pidermal cell of leaf is 43 time higher than other leaf tissues (Salt, 1995), it has been demonstrated that Cd is associated with phytochelatins in *Brassica juncea* (Salt, 1995), however contray conclusion was also reported (Robinson, 1994).

The localization of Cd in Cd accumulators has been mostly studied, but Cd distribution information of these specialized plants which have the ability to accumulate Cd has not been understood, so it should be known that Cd distribution within the plant, what position and what period of plant harvested can be obtained maximal Cd extraction. The objectives of the present study were to select Cd tolerant and accumulative oilseed rape genotype for Cd distribution experiment with Indian mustard used as comparing plant, then a series of pot experiments were conducted to study Cd distribution characterics of Indian mustard and oilseed rape in all growth period, temporal changes in Cd concentration of shoot and root was recorded, at the same time leaves were grouped according to their ages and respective concentration of Cd was measured, percentages of shoot Cd uptake of oilseed rape in total plant were investigated with comparison to that of Indian mustard in pot trails.

1 Materials and methods

Twenty-one varieties of oilseed rape were obtained from different provinces of China and Indian mustard seeds were obtained from the National Herbarium of the USA (accessions 426308). The soil used in the study was collected from experiment section of China Agriculture University, physicochemical characteristics of the soil are summarized here as: pH(5:1) 7.66, CEC 20.1 cmol/kg, organic carbon 1.26%, total Cd 0.06 mg/kg, DTPA-Cd 0.02 mg/kg.

1.1 Selection of higher Cd accumulating oilseed rape for Cd distribution experiment

Oilseed rapes and Indian mustard were planted in sand washed with deionized water, then were cultured in 25—28℃ dark environment, after germination, the seedlings were cultured in ray greenhouse until they had 3 veritable leaves, then were transplanted to 32 L plastic basin filled with basal nutrition solution. The composition of nutrition solution was as follows (mol/L): 0.5×10^{-3} K_2SO_4, 0.25×10^{-3} KH_2PO_4, 0.325×10^{-3} $MgSO_4$, 5.0×10^{-4} NaCl, 8.0×10^{-6} H_3BO_3, 1.0×10^{-6} $MnSO_4$, 0.4×10^{-6} $ZnSO_4$, 0.4×10^{-6} $CuSO_4$, 0.1×10^{-6} Na_2MoO_4, 4.0×10^{-6} Fe-EDTA, 1.0×10^{-3} $CaSO_4$, 1.0×10^{-3} NH_4NO_3. Some $CaCO_3$ dust was launched in order to keep pH about 6.5. There were two plants per hole and three replications for each treatment, the solutions were continuous aerated with an aquarium air pump and changed every 2 d. the 1/4, 1/2 concentration nutrition solution was used in turn, in the end total nutrition solution was used. The seedlings were treated with 3 mg/L $CdSO_4$ after 4 weeks positive growth, at the end of 42 d growth period, plants were harvested by cutting at the shoots base to separate the shoots and roots.

1.2 Cd distribution of oilseed rape and Indian mustard in different growth period

13 × 12 cm plastic pots were contained 400 g loamy calcareous soil, the soil was fertilized with 0.30 g N in the

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form of $(NH_4)_2SO_4$, 0.20 g P_2O_5 and 0.30 g K_2O in the form of KH_2PO_4 and K_2SO_4 respectively, then spiked with Cd at a concentration of 100 mg/kg as $CdSO_4$. All pots were arranged in a completely randomized design with four replications for each treatment. After germination, the seedlings were thinned to 4 plants per pot, with pots being watered daily using deionized water. Plants were harvested by cutting the shoots at the soil surface, and the harvest period is the 28, 35, 42, 49, 56, 63 d respectively.

1.3 Cd distribution of oilseed rape and Indian mustard with different age

The soil, plant and experiment methods used were as above. Porcelain pots containing 3 kg air dried soil were used in the trail, different treatments consisted of higher Cd accumulating oilseed rape Chuanyou Ⅱ-10 and Indian mustard with 2 different Cd levels (20, 40 mg/kg soil) as $CdSO_4$. At the end of growth period, plants were harvested by cutting the shoots at the soil surface and the leaves and stem were separated. Leaf number was from the base of the plant, counting the first identifiable leaf as No.1, No.2, to core leaf and stem.

1.4 Sample analysis and data statistic

Harvested plants were washed with tap water and rinsed with double deionized water before oven dried at 70℃ for 48 h, and the dry weight yields were measured. Oven-dried plant tissues were ground using a stainless steel mill. Subsamples of ground materials were digested with a mixture of concentration HNO_3-$HClO_4$, and total Cd concentration were determined using atomic absorption spectrometry (AAS). The amounts of plant Cd uptake selected by Cd tolerant genotype of oilseed rape and Indian mustard was calculated.

All the data were analyzed with a SAS statistical package through an IBM personal computer. One way ANOVA was carried out to compare the means of different treatments; where significant F values were tested with the least significance different test.

2 Results and discussion

2.1 Selection of higher Cd accumulating oilseed rape for Cd distribution experiment

There are two preconditions about phytoextraction Cd technique application. On one hand, Cd concentration of plant tissue is higher, on the other hand, plant can grow well and has a higher dry weight yield (Ebbs, 1997), whereas the amount of shoot Cd uptake is calculated from shoot dry weight yield and shoot Cd concentration, so it is ultimately requisite for phytoextraction Cd from Cd contamination soil and can be used as an indicator of shoot Cd uptake and accumulation capability. The shoot Cd uptake of 21 different varieties of oilseed rape and Indian mustard is given in Table 1. Among the 21 varieties, shoot Cd uptake of 12 varieties of oilseed rape was higher than that of Indian mustard, but shoot Cd uptake of 5 varieties was significant higher than that of Indian mustard, number 13 oilseed rape (Chuanyou Ⅱ-10) had the highest Cd uptake amount, so mustard oilseed rape Chuanyou Ⅱ-10 was selected as higher Cd accumulating oilseed rape and researched for Cd distribution experiments.

Table 1 Shoot Cd uptake by 21 different varieties of mustard oilseed rape (*Brassica juncea* L.) (number 1 to 21) and Indian mustard (number 22) grown in 3 mg/L Cd culture solution

Plant code	Cd uptake amount, μg/plant	Plant code	Cd uptake amount, μg/plant
1	8.98 ± 3.89	12	278.39 ± 34.75
2	107.99 ± 12.00	13	1005.42 ± 145.12*
3	496.37 ± 34.57*	14	471.05 ± 83.60*
4	340.04 ± 42.34	15	189.33 ± 11.53
5	255.80 ± 42.15	16	211.91 ± 27.67
6	39.86 ± 4.17	17	308.96 ± 23.99
7	146.72 ± 40.23	18	301.41 ± 47.99
8	295.11 ± 11.94	19	677.07 ± 28.34*
9	263.60 ± 45.89	20	350.66 ± 33.42
10	196.81 ± 12.77	21	105.01 ± 9.55
11	396.48 ± 10.53*	22	261.59 ± 49.38

Notes: Values are means ± *SD* (n = 3); *. significantly different from Cd uptake amount of oilseed rape and Indian mustard at $p < 0.05$ according to *LSD*-test

2.2 Cd distribution of oilseed rape and Indian mustard in different growth period

Fig. 1 shows shoot and root Cd concentration with variational trend of oilseed rape and Indian mustard in 63 d growth period, with an increase of growth time, shoot and root Cd concentration of oilseed rape and Indian mustard markedly decreased. The changing rules of shoot and root Cd concentration kept accordance with toxicity symptoms of plants: at the beginning of the experiment, etiolation of shoot manifested in evidence, plant growth got restrained, along with the extend of growth period, etiolation of shoot gradually weaken, and the plant could relatively grow healthy.

The results (Fig. 1) depicted that oilseed rape and Indian mustard could highly absorb Cd from Cd contaminated soil in the growth prophase, but at this time, dry weight

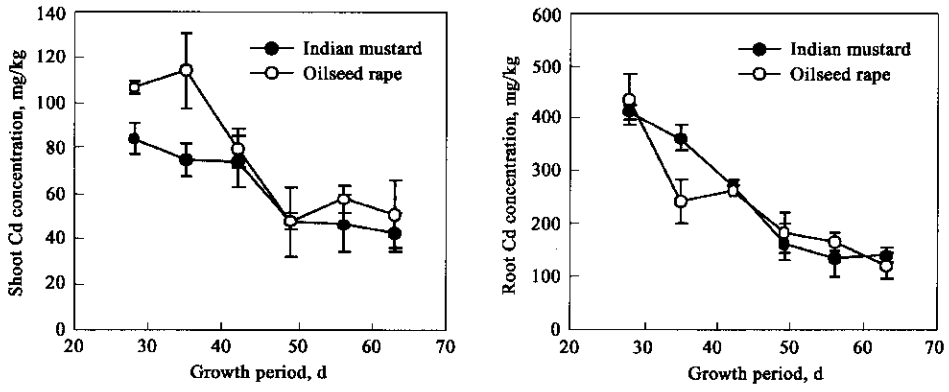

Fig. 1 Shoot and root Cd concentration of Indian mustard and oilseed rape grown in different growth period
Bars represent standard errors, n = 4

yields of shoot and root were relatively lower, Cd uptake of shoot and root was concentrated. After this, Cd uptake of shoot and root was gradually diluted with growth period prolongation, so Cd concentration of shoot and root gradually decreased.

From Fig. 1 we can also know that shoot Cd concentration of oilseed rape was higher than that of Indian mustard in the whole growth period, it appears that Cd accumulation capacity of oilseed rape Chuanyou Ⅱ-10 was better than that of Indian mustard.

Table 2 shows shoot dry weight, ratio of shoot and root of oilseed rape and Indian mustard in different growth period. Shoot dry weight yields of oilseed rape and Indian mustard all along increased with time varied, at the same time dry weight yields of shoot were higher than that of the root, the ratio of shoot and root of oilseed rape and Indian mustard was 3.75—5.70, and this is the base condition in which oilseed rape and Indian mustard can uptake and accumulate Cd from Cd contaminated soil(Su, 2002). From Table 2 we can also get the result that shoot dry weight yields of oilseed rape were higher than that of Indian mustard, so the growth ability of oilseed rape was stronger than that of Indian mustard at the same growth period.

Table 2 Shoot dry weight, ratio of shoot and root and shoot Cd uptake of Indian mustard and oilseed rape grown in different growth period

Days	Indian mustard			Oilseed rape		
	Shoot, g/pot	Ratio(S/R)	Shoot Cd uptake, μg/pot	Shoot, g/pot	Ratio(S/R)	Shoot Cd uptake, μg/pot
28	0.77 ± 0.16	5.70	64.75 ± 14.71	1.05 ± 0.09	4.92	111.61 ± 13.04
35	1.49 ± 0.07	5.04	111.31 ± 14.53	1.67 ± 0.15	4.46	191.09 ± 33.96
42	2.21 ± 0.12	4.77	164.09 ± 31.28	2.53 ± 0.12	5.08	201.17 ± 22.59
49	3.35 ± 0.44	3.75	153.85 ± 30.61	4.00 ± 0.18	4.90	190.11 ± 9.25
56	4.84 ± 0.26	4.30	223.02 ± 53.49	5.29 ± 0.44	4.86	303.31 ± 35.26
63	5.74 ± 0.29	4.21	270.93 ± 24.56	7.22 ± 0.69	5.39	390.99 ± 62.12

Note: Values are means ± *SD*($n = 4$)

Table 2 also shows that shoot Cd uptake of oilseed rape and Indian mustard markedly increase with increase of gowth period, this could be because that the amount of shoot Cd uptake were calculated from shoot dry weight yield and shoot Cd concentration, despite shoot Cd concentration gradually decrease(Fig. 2), but dry weight yield of shoot obviously mounted up(Table 2), so shoot Cd uptake of oilseed rape and Indian mustard gradually increase in 63 d growth period.

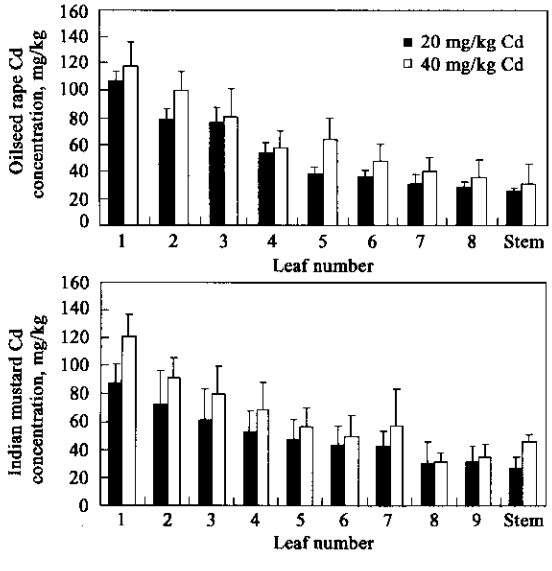

Fig.2 Different leaf and stem Cd concentration of oilseed rape and Indian mustard grown on Cd contaminated soil
Bars represent standard errors, $n = 3$

2.3 Cd distribution of oilseed rape and Indian mustard with different age

It has been demonstrated that Cd uptake and accumulation appeared to be more in the younger leaves of both *Thlaspi caerulescens* and *Brassica juncea* exposed to 0.1 μg/ml Cd for 24 h (Salt, 1995), Perronnet *et al*. (Perronnet, 2003) similarly reported that in the Viver population of *Thlaspi caerulescens* grown on contamination soil younger leaves exhibited higher Cd concentration than older ones. But the contrary result in the experiment was also reported, Cd concentration of older leaves was higher than younger leaves in two cultivars of potato(*Solanum tuberosum* L.)(Dunbar, 2003). Mckenna *et al*. (Mckenna, 1993) investigated the effects of cadmium on the accumulation and tissue distribution of cadmium in lettuce and spinach, showed that plants were grown in nutrient solutions containing 0.010—0.316 μmol/L Cd, Cd accumulated more in old than in young leaves of both crops at any solutions Cd level. Distribution of the cation in leaf segments of maize changed with their ages and Cd concentration in the growth medium, in leaves seedlings treated with 100, 200 μmol/L Cd, the amounts of Cd increased with the age of segments, higher Cd concentration in the oldest leaves (Drazkiewicz, 2003). Different leaf and stem Cd concentration of oilseed rape and Indian mustard on two different Cd concentration soil is given in Fig.2. In general, there was constantly decrease from the base leaves of oilseed rape and Indian mustard to the top leaves at soil Cd concentration of 20 mg/kg or 40 mg/kg, the older leaves showed a markedly higher Cd concentration than younger leaves. This suggests that Cd is one of the less mobile elements, distribution of Cd in plant might thus be related to growth, more active metabolism, medium concentration, plant species, and so on.

Fig.2 shows that shoot Cd concentration of oilseed rape and Indian mustard at soil Cd concentration of 40 mg/kg was higher than that of soil Cd concentration of 20 mg/kg. This suggested that with increase of soil Cd concentration, Cd extraction capacity of oilseed rape and Indian mustard increased.

Table 3 shows percentages of different Leaf and stem Cd uptake of oilseed rape and Indian mustard in total shoot grown at two different Cd added soil, the Cd uptake amount in total shoot(%) varied according to the leaf age, compared to other groups, No.2—5 older leaves showed higher percentages, for

oilseed rape, even total percentages of four leaves Cd uptake in total shoot exceed to 60%.

Table 3 Different leaf and stem Cd uptake of Indian mustard and oilseed rape in total shoot(%) grown at different Cd added soil

Leaf number	Oilseed rape		Indian mustard	
	20 mg/kg Cd	40 mg/kg Cd	20 mg/kg Cd	40 mg/kg Cd
1	8.87	6.98	8.63	9.01
2	14.18	13.08	11.40	10.38
3	23.18	18.58	12.26	12.30
4	20.01	17.85	14.17	13.01
5	13.51	19.12	13.30	12.92
6	9.36	10.64	11.30	9.76
7	4.37	5.32	9.63	7.86
8	1.70	3.51	4.22	4.15
9			3.21	5.53
Stem	4.81	4.91	11.87	15.08

Notes: Number 1—9 represent from the No. 1 leaf of bottom of the plant to the No. 9 leaf of top of the plant

3 Conclusions

Mustard oilseed rape(*Brassica juncea* L.) Chuanyou Ⅱ-10 was more effective in phytoextraction Cd among 21 oilseed rape varieties and indicator plant Indian mustard at hydroponic culture experiment condition. Shoot and root Cd concentration of oilseed rape and Indian mustard decreased with time, but shoot Cd uptake markedly increased with increase of growth period. At soil Cd concentration of 20 mg/kg or 40 mg/kg, Cd concentration of the older leaves was markedly higher than that of younger leaves, percentages of older leaves Cd uptake in total shoot were markedly higher than those of younger leaves. Considering that phytoremediation application of Cd distribution characteristics of oilseed rape and Indian mustard in different growth period and with different ages, shoot should as far as possible develop before they reached the soil and the older leaves were harvested in priority.

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