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Journal of Environmental Sciences 21(2009) 1125–1128

JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Cadmium uptake and speciation changes in the rhizosphere of cadmium accumulator and non-accumulator oilseed rape varieties

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Received 15 October 2008; revised 15 December 2008; accepted 31 December 2008

Abstract

Characteristics of cadmium (Cd) uptake kinetics and distribution of Cd speciation in the rhizosphere for Cd accumulator and non-accumulator oilseed rape varieties were investigated under nutrient solution and rhizobox soil culture conditions. The results showed that the maximal influx (V_{max}) for Cd^{2+} and K_m were significantly different for the two oilseed rape varieties. The value of V_{max} for Cd accumulator oilseed rape Zhucang Huazi was two-fold greater than that for oilseed rape Chuan you II-93. The exchangeable Cd concentration in the rhizosphere was significantly lower than in non-rhizospheric soils supplemented with $CdSO_4$ for both the varieties. Carbonate-bound Cd in the rhizosphere of Cd accumulator oilseed rape was significantly higher than that in the rhizosphere of non-accumulator oilseed rape and non-rhizospheric soil. Cd accumulator oilseed rape had a higher Cd^{2+} affinity and more ability to uptake insoluble Cd in the soil than the non-accumulator oilseed rape.

Key words: cadmium; oilseed rape; rhizosphere; uptake kinetics

DOI: 10.1016/S1001-0742(08)62391-8

Introduction

Cadmium is a toxic element without any known physiological function in plants (Wang *et al*., 2007). Special consideration should be paid to Cd pollution in soil-plant systems because of its high mobility and low toxic concentrations in organisms (Moreno *et al*., 2000). About 14000 hm^2 of agricultural soils were contaminated with Cd in China (Chen, 1996), which has aroused major concerns because of the possibility of accumulation through food chain. Members of Brassicaceae family from genus *Brassica* are heavy metal accumulators (Kumar *et al*., 1995). Oilseed rape, *Brassica napus*, along with *Brassica juncea* and *Brassica campestris* are some of the widely grown crops in China. Studies have shown that some of the oilseed rape varieties had the capability to accumulate Cd and therefore had phytoremediation potential for Cd contaminated soil (Su and Wong, 2004; Ru *et al*., 2004). Otherwise, some variety had lower Cd concentration in shoot compared with Cd-accumulating oilseed rape varieties. Utilizing low-uptake oilseed rape variety for food production may be a strategy for human health. Plants have adopted different strategies to cope with extremely high toxic metal concentrations in their growth medium (Wenzel *et al*., 2003). Our knowledge on the Cd uptake mechanisms of Cd accumulator and non-accumulator oilseed rape is insufficient. The rhizosphere environment should play an important role in plant uptake metals. Many researchers are interested in the chemical changes in the rhizosphere of hyperaccumulator plants (McGrath *et al*., 1997; Puschenreiter *et al*., 2003). Knight *et al*. (1997) found that after growth of *Thlaspi caerulescens*, the decrease of soluble and exchangeable Cd in the soil solution accounted for only about half of the total plant uptake. This observation suggested that hyperaccumulator plants might be able to mobilize insoluble Cd in soil.

The objectives of the present study were to investigate the characteristics of Cd uptake kinetics by two oilseed rape varieties with different Cd accumulator, and to identify the distribution and forms of Cd in rhizosphere.

1 Materials and methods

1.1 Growth response and Cd accumulation

The experiment was conducted in a greenhouse with soil culture. Two oilseed rape varieties, Zhucang Huazi and Chuan you II-93 were grown in soil supplemented with Cd applied as $CdSO_4$ (40 mg/kg), and no Cd addition as control. All experimental treatments were set up in triplicates. The general properties of the soil were as follows: pH 7.85, CEC (cation exchange capacity) 20.1 cmol/kg, organic carbon 1.26%, total Cd 0.06 mg/kg. The soil was air-dried and passed through a 2-mm grid sieve, then manually mixed with $CdSO_4$ solution to ensure homogeneity and incubated for 15 d. Five hundred gram soil samples were

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placed into plastic pots and 4 plants of oilseed rape per pot were allowed to grow for 42 d. Oven-dried plant samples were ground and digested with HNO_3 using a microwave oven (Mars-5, CEM Co., USA), cadmium concentration was then determined by Atomic Absorption Spectroscopy (PE-2100, Perkin-Elmer Co., USA). All samples were run together with certified reference samples (GBW08510) for assurance control.

1.2 Concentration-dependent kinetics of Cd uptake

Seeds of oilseed rape Zhucang Huazi and Chuan you II-93 were germinated on a mixture of perlite and vermiculite moistened with deionized water for one week, then with nutrient solution. After 35 d seedlings were transferred to vessels filled with a nutrient solution containing (μmol/L): NH_4NO_3, 1000; KH_2PO_4, 250; K_2SO_4, 500; $MgSO_4 \cdot 7H_2O$, 32.5; NaCl, 500; $CaSO_4$, 1000; $MnSO_4 \cdot H_2O$, 1.0; $CuSO_4 \cdot 5H_2O$, 0.4; $ZnSO_4 \cdot 7H_2O$, 0.4; $MoNa_2O_4 \cdot 2H_2O$, 0.1; H_3BO_3, 8.0; $FeSO_4 \cdot 7H_2O$, 4.0; Na_2EDTA, 4.0 (Su and Wong, 2004). Solution pH was maintained at 6.5 using 0.1 mol/L HCl/NaOH. The plants were grown in a controlled condition of 14 h/10 h (day/night) cycle at 20–30°C. The hydroponic solutions used were continuously aerated and changed every 3 d. Plants were grown under these conditions for 45 d. The nutrient solution was then replaced with a pretreatment solution containing 500 μmol/L $CaCl_2$ for 24 h. Nine different concentrations of Cd (0.5–140 μmol/L) were used to study the influx kinetics of Cd. Each treatment was replicated three times. After 9 h uptake, the plants were quickly rinsed with pretreatment solution and then transferred to vessels containing ice-cold desorption solutions (5 mmol/L $CaCl_2$, 100 μmol/L $ZnSO_4$). After desorption, the plants were separated into roots and shoots and fresh weight were recorded. Cd concentration of the solution was also determined.

1.3 Speciation changes of Cd in rhizosphere

Rhizobox (24 cm × 18 cm × 2.5 cm) consisted of a central root compartment (RC) with 0.5 cm thick filled with a mixture of perlite and vermiculite for the growth of oilseed rape. Soil bags on both sides of the RC containing 900 g soil supplied with 40 mg/kg Cd as $CdSO_4$ or $CdCO_3$ powder. The general properties of the soil were the same as introduced in Section 1.1. The soil bags were made of nylon net with a mesh size of 35 μm, and were used to prevent direct contact of roots with soil particles, but allowing soil solution to flow into the root surface across the nylon net. The rhizoboxes were arranged in a greenhouse in a completely randomized design. Each rhizobox contained three plants and were grown for 135 d. At the same time, rhizoboxs without planted oilseed rapes were set up to represent the non-rhizospheric soil. The water content of the soil in the rhizobox was kept at 60% of field water hold capacity by replenishing water daily to a constant weight. All experimental treatments were performed in triplicates. At harvest, plants were carefully removed and rhizospheric soil samples were collected from the soil bag surface of RC side. The soils were air dried. A sequential extraction scheme developed by Tessier *et al*. (1979) was used to partition Cd in the soils into several fractions. The fractions were defined as: (1) exchangeable (extracted by 1.0 mol/L $CaCl_2$); (2) carbonate (extracted by 1.0 mol/L NaOAc, at pH 5; (3) Fe-Mn oxides (extracted by 0.04 mol/L NH_4OH/HCl); (4) organic (extracted by 0.02 mol/L HNO_3 + 30% H_2O_2). Concentrations of Cd were measured using graphite furnace atomic absorption spectrometry (TAS-990, Puxi Analytical Instrumental Co., China).

All data were analyzed using the SAS statistical package. Two-way ANOVA was carried out to compare the means of different treatments; where significant F values were obtained, differences between individual means were tested using the least significant test (LSD, $p < 0.05$).

2 Results and discussion

2.1 Growth response and Cd accumulation

Shoot dry weight and Cd concentration of shoot and root of the two oilseed rape varieties are shown in Table 1. Relative shoot dry weight (defined as the percentage of shoot dry weight of oilseed rape grown on Cd-supplemented soil relative to that grown on non-Cd supplemented soil) was an index, showing plant tolerance to Cd toxicity. As shown in Table 1, Cd accumulator oilseed rape Zhucang Huazi and non-accumulator oilseed rape Chuan you II-93 have no significant difference in dry weight and relative shoot dry weight. This result suggested that the two oilseed rape varieties has the same Cd tolerance ability at the soil Cd concentration 40 mg/kg. Shoot Cd concentration of Zhucang Huazi was significantly higher than that of Chuan you II-93, while, there was no significant difference in root Cd concentrations between them. The ratio of shoot/root Cd concentration of Zhucang Huazi was higher than that of Chuan you II-93. These results indicated that Cd accumulator oilseed rape Zhucang Huazi has a higher root to shoot translocation efficiency of Cd than that of non-accumulator oilseed rape Chuan you II-93.

Table 1 Shoot biomass (dw) and Cd concentration of the Cd accumulator and non-accumulator oilseed rape varieties grown in soil supplemented with Cd

Oilseed rape variety	Relative shoot dry weight (%)	Shoot dry weight (g/pot)	Shoot Cd concentration (mg/kg)	Root Cd concentration (mg/kg)	Ratio of shoot/root Cd concentration
Zhucang Huazi	91	2.30 a*	74.2 a	196.1 a	0.38
Chuan you II-93	98	2.59 a	37.1 b	153.9 a	0.24

* Values followed by the same letter within the same column do not differ significantly at 5% level.

2.2 Kinetics of Cd uptake

Figure 1 shows the concentration-dependent kinetics of Cd uptake in roots of Cd accumulator Zhucang Huazi and the non-accumulator Chuan you II-93. The Cd accumulator showed a marked saturable component at high Cd concentration, whereas in the Cd non-accumulator this component was very weak. At lower concentrations the curves were dominated by a linear component for both the varieties.

Table 2 summarizes the parameters obtained for the two varieties. The lower K_m value indicates a higher Cd^{2+} affinity for Cd accumulator Zhucang Huazi. The maximal influx (V_{max}) for Cd^{2+} was significantly different between the two oilseed rape varieties. The value of V_{max} for Zhucang Huazi was two-fold greater than that for Chuan you II-93. The result implied that Cd accumulator has a higher density of a Cd transporting system on the root cell membranes than the non-accumulator or that transport systems which are expressed are more active. In addition, the slope for the line component was higher for the Cd accumulator than that for the non-accumulator. *T. caerulescens* is considered to be a Cd haperaccumulator (Baker *et al*., 1994). Lombi *et al*. (2001) reported that concentration dependent influx of Cd revealed marked differences between *T. caerulescens* ecotypes. There is strong physiological evidence for a high-affinity, highly expressed Cd transporter in the root cell plasma membranes of the Ganges ecotype of *T. caerulescens*.

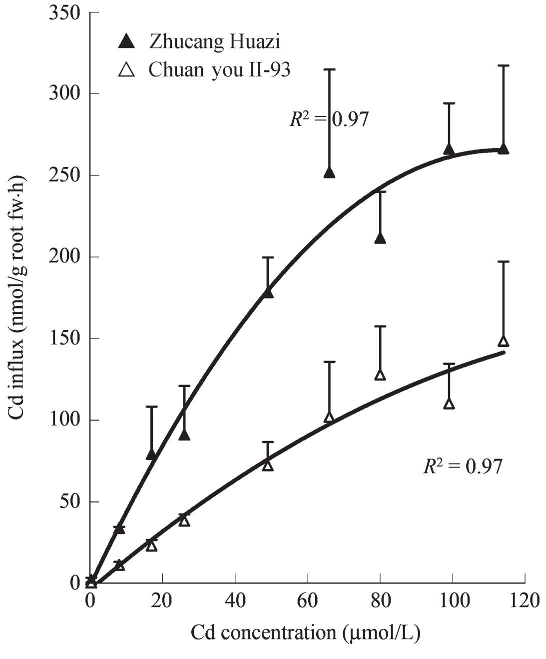

Fig. 1 Concentration-dependent kinetics of Cd uptake in roots of Cd-accumulator oilseed rape Zhucang Huazi and Cd non-accumulator oilseed rape Chuan you II-93. Data points and error bars represent mean values ± SE.

Table 2 Kinetic parameters for root Cd^{2+} influx of the two oilseed rape varieties

Oilseed rape variety	V_{max} (nmol/g root fw·h)	K_m (μmol/L)
Zhucang Huazi	119.6 (26.4)*	0.13 (0.05)
Chuan you II-93	58.0 (21.6)	0.77 (0.18)

* SE in parentheses.

2.3 Cd uptake and speciation changes in the rhizosphere

Table 3 shows the shoot dry weight and Cd concentration of the Cd accumulator and non-accumulator oilseed rape varieties grown on central root compartment (RC) of rhizobox, and soil on both sides of the RC applied with $CdSO_4$ and $CdCO_3$, respectively. Shoot dry weight of the two oilseed rape varieties have no significant difference when grown on simulated Cd contaminated soil with $CdSO_4$ or $CdCO_3$ as Cd source. However, Cd accumulator oilseed rape has a significantly higher shoot Cd concentration than that of non-accumulator oilseed rape for both the treatments. This result indicated that Cd accumulator Zhucang Huazi not only has a stronger Cd-uptake ability but also has a better ability to uptake insoluble Cd in the soil than that of non-accumulator Chuan you II-93. Table 3 also shows that shoot Cd concentration of the two oilseed rape varieties was significantly higher when grown in soil with $CdSO_4$ as Cd source than that grown in soil added with $CdCO_3$ as Cd source. This was largely due to the fact that $CdCO_3$ is an insoluble salt which has a lower availability for plant uptake.

Table 4 shows the distribution of Cd fractions in rhizosphere and non-rhizosphere of Cd-accumulator and non-accumulator oilseed rapes grown in Cd contaminated soil with $CdSO_4$ or $CdCO_3$ as Cd source. For both Cd-accumulator and non-accumulator oilseed rape, the exchangeable Cd concentration in the rhizosphere was significantly lower (decreased about 40%) than that in non-rhizospheric soil when $CdSO_4$ was used as Cd source. Exchangeable Cd was readily uptook by plant and the decrease in exchangeable Cd in the rhizosphere presumably resulted from the uptake of Cd by oilseed rapes. Although Cd accumulator oilseed rape has a stronger Cd uptake ability than non-accumulator oilseed rape, exchangeable Cd concentrations in the rhizosphere of the two kinds of oilseed rape had no significant difference. Table 4 also shows that no significant difference was found between rhizospheric and non-rhizospheic soil when $CdCO_3$ was applied as Cd source. $CdCO_3$ is insoluble salt which has a comparatively low availability for the plant, the exchangeable Cd concentration in the rhizosphere and non-rhizosphere were significantly lower in soil with $CdCO_3$ as Cd source than that with $CdSO_4$ as Cd source. This result

Table 3 Shoot biomass (dw) and Cd concentration of the Cd accumulator and non-accumulator oilseed rape varieties grown on rhizobox soil with $CdSO_4$ and $CdCO_3$ as Cd source

Cd source	Oilseed rape variety	Shoot dry weight (g/pot)	Shoot Cd concentration (mg/kg)
$CdSO_4$	Zhucang Huazi	31.5 ab	58.9 a
	Chuan you II-93	36.5 a	37.2 b
$CdCO_3$	Zhucang Huazi	26.2 b	8.0 c
	Chuan you II-93	28.4 ab	4.0 d

Values followed by the same letter within the same column do not differ significantly at 5% level.

Table 4 Change of Cd speciation in the rhizosphere of Cd accumulator and non-accumulator oilseed rape varieties grown on rhizobox with $CdSO_4$ or $CdCO_3$ as Cd source

Cd source	Soil	Exchangeable (mg/kg)	Carbonate (mg/kg)	Oxide (mg/kg)	Organic (mg/kg)
$CdSO_4$	Non-rhizosphere	12.5 a	13.1 c	7.61 a	1.39 a
	Zhucang Huazi rhizosphere	7.10 b	21.5 a	8.51 a	1.91 a
	Chuan you II-93 rhizosphere	7.17 b	17.5 b	8.30 a	1.45 a
$CdCO_3$	Non-rhizosphere	0.39 c	24.0 a	9.43 a	2.28 a
	Zhucang Huazi rhizosphere	0.23 c	19.9 ab	7.24 a	1.78 a
	Chuan you II-93 rhizosphere	0.28 c	24.1 a	9.18 a	1.71 a

Values followed by the same letter within the same column do not differ significantly at 5% level.

suggested that Cd accumulator oilseed rape may not be able to mobilize insoluble Cd in the soil. Zhao *et al*. (2001) also did not find root exudates of *T. caerulescens* involved in active mobilization of Zn and Cd in a contaminated calcareous soil.

Carbonate-bound Cd in the rhizosphere of Cd accumulator and non-accumulator oilseed rape was significantly higher than in the non-rhizospheric soil when $CdSO_4$ was used as Cd source, increasing by 79% and 34%, respectively. The mechanism was probably due to the release of CO_2 into the soil solution by the plant roots during their growth. Carbon dioxide combined with the free Cd ion surrounding the root to form the carbonate-bound Cd. Compared with Cd-accumulator and non-accumulator oilseed rape, carbonate-bound Cd in the rhizosphere of Zhucang Huazi was significantly higher than Chuan you II-93 when $CdSO_4$ was applied. This result was probably due to a higher root activity of Cd accumulator oilseed rape Zhucang Huazi than non-accumulator oilseed rape. No difference in carbonate-bound Cd was found between the rhizosphere and non-rhizosphere for Cd accumulator and non-accumulator oilseed rape when the simulated Cd contaminated soil using $CdCO_3$ as Cd source.

For Cd accumulator and non-accumulator oilseed rapes, no significant change in Fe-Mn oxide Cd and organically bound Cd were found between the rhizosphere and non-rhizosphere for both the Cd sources.

3 Conclusions

Concentration-dependent influx of Cd revealed marked differences between Cd accumulator and non-accumulator oilseed rape varieties. The value of V_{max} for the Cd accumulator oilseed rape Zhucang Huazi was two-fold greater than that for the oilseed rape Chuan you II-93. Exchangeable Cd concentration in the rhizosphere was significantly lower than in non-rhizosphere soil when the simulated Cd contaminated soil using $CdSO_4$ as Cd source for both the varieties. Carbonate-bound Cd in the rhizosphere of Cd accumulator oilseed rape was significantly higher than in the rhizosphere of non-accumulator oilseed rape and the non-rhizosphere soil. Cd accumulator oilseed rape has a higher Cd^{2+} affinity and a more active transport systems than non-accumulator oilseed rape varieties. Cd accumulator oilseed rape also has a stronger Cd-uptake ability and more ability to uptake insoluble Cd in the soil than that of non-accumulator oilseed rape.

Acknowledgments

The work was supported by the National Key Technology R&D Program of China (No. 2006BAD17B04), the Hi-Tech Research and Development Program (863) of China (No. 2007AA061001), and the National Basic Research Program (973) of China (No. 2002CB410804).

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