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Removal of Cd from Actual Soils Polluted with Cd and Zn and Cd–added Soils by *Brassica juncea* and *Zea mays*

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We have alternated the 3–week cultivation of corn (*Zea mays* L.) and that of Indian mustard (*Brassica juncea* (L.) Czern) in a pot with the sequential rotation, corn–Indian mustard–corn–Indian mustard, in order to remove Cd from two actual soils polluted with Cd and Zn, one (low Cd polluted (LP)) containing 6.5 mg Cd kg^{-1} and 715 mg Zn kg^{-1} and another (high Cd polluted (HP)) with 13.6 mg Cd kg^{-1} and 836 mg Zn kg^{-1}.

Fertilizer applications to soil in a rotational cropping can inhibit the decrease in the phytoextraction efficiency. As the total of the all cultivations, the Cd removal from the soils was 2.1% of the total soil Cd amount in the LP plot and 1.0% in the HP plot, whereas the Zn removal was lower than the Cd cases, 0.57% in the LP plot and 0.17% in the HP plot, respectively.

The Cd absorptions by both plants were significantly greater in the metal–added soils than in the actual polluted soils in all cultivations ($p < 0.05$). This would be caused by the high concentrations of exchangeable Cd in the added soils. These results indicated that the use of metal– added soils is likely to overestimate the efficiency of some plants in phytoextraction experiments involving actual polluted soils with its variation by the experimental conditions.

INTRODUCTION

In Japan, cadmium (Cd)–soil pollution has been one of the major environmental problems over the past several decades (Kitagishi and Yamane, 1981). It is quite likely that Cd is easily absorbed by rice in comparison to other heavy metals (e.g. Cu, Zn or Pb) (Asami, 2001). Therefore, some brown rice accumulates high levels of Cd in a certain area. The continuous intake of Cd–accumulated rice induces syndromes represented by the Itai–Itai disease. In fact, Cd concentrations in rice have been constantly monitored by the administrative–organ for several decades. If the brown rice contains more Cd than the standard value, 1.0 mg kg^{-1}, the consumption of the brown rice is prohibited by the Food Agency (Asami, 2001). In order to reduce the Cd–accumulated rice, it is necessary to remove Cd from relatively low (e.g. 1.0 mg kg^{-1}) polluted soils. The immobilization of Cd by increase of soil pH is a countermeasure to inhibit Cd absorption by plants (Kabata–Pendias and Pendias, 2001). However, this is not the final solution because the soil pH decreases due to heavy precipitation and acid rain (Sato, 1989) in Japan.

Soils have often polluted by Cd together with zinc (Zn) because Cd, which exists in Zn minerals, is released into the environment from the smelting process of Zn ores (Kabata–Pendias and Pendias, 2001). Remediation of Cd–polluted soils should be associated with co–existing Zn in soils.

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A lot of work, cost, and time are required using conventional techniques (land-fill–excavation) for the remediation of soils contaminated by heavy metals. It has been proposed that phytoextraction is likely to be a novel method that could restore the soils using conventional crop cultivation (Kumar *et al.*, 1995; Cunningham and Ow, 1996; Blaylock and Huang, 2000; McGrath *et al.*, 2000; Garbisu and Alkorta, 2001). A plant's ability for phytoextraction may be evaluated based on the biomass of a plant as well as with the heavy metal absorption capacity, in other words, heavy metal concentration (Boyjian and Garreira, 1997; Glass, 2000).

Indian mustard (*Brassica juncea* (L.) Czern) has been frequently studied for heavy metal phytoextractions due to its high accumulation capabilities (Blaylock *et al.*, 1997; Ebbs and Kochian, 1997, 1998; Quartacci *et al.*, 2001; Jiang *et al.*, 2003). However, the biomass production of Indian mustard is extremely low (about 0.5 g per plant in dry weight) (Quartacci *et al.*, 2001). Therefore, corn (*Zea mays* L.) is often used as an experimental species for phytoextraction because of its high biomass production and comparatively strong heavy metal resistance (Huang *et al.*, 1997; Wu *et al.*, 1999; Lombi *et al.*, 2001; Ali *et al.*, 2002; Wenger *et al.*, 2002). Many researchers have been using the single cropping, but there is no report about the phytoremediation using rotation cropping associated with several plant species.

It is difficult to get actual heavy metal polluted soil because of various problems in a polluted place. There are not few examples that use heavy–metal–added soil as substitution of polluted soil. Comparison of polluted soil and added soil may change the fractionation of heavy metal. Therefore, phytoremediation of heavy metals may not be evaluated correctly.

The purpose of the present study is to remove Cd from actual soils polluted with Cd and Zn and the corresponding metal–added soils by corn and Indian mustard, together with investigating the relationships between the heavy metal fractionation in the soils for plant absorption of the metals.

MATERIALS AND METHODS

Soil samples.

Two actual polluted paddy soils, collected in Omuta, Fukuoka, Japan, were used in this study. One (low Cd polluted (LP)) contained 6.5 mg Cd kg^{-1} soil and 715 mg Zn kg^{-1} soil and another (high Cd polluted (HP)) had 13.6 mg Cd kg^{-1} soil and 836 mg Zn kg^{-1} soil. A paddy soil (BLK) collected in Sedaka, Fukuoka, was used as the control soil. The soil properties are indicated in Table 1. The BLK soil was classified as Gleysol (sandy loam), and the two polluted soils as Gleysol (sandy clay). The pH and CEC of the HP soil were

Table 1. Soil properties.

	clay	silt	sand	pH(H2O)	EC	OM	CEC	extracted with HCl		$HClO_4$ digestion	
	%	%	%		dS m^{-1}	%	cmolc kg^{-1}	μgCd g^{-1}	μgZn g^{-1}	μgCd g^{-1}	μgZn g^{-1}
BLK	13	21	66	5.2	0.04	5.6	9.5	0.3	9.0	0.2	121.2
LP	19	16	65	5.4	0.10	7.9	12.7	6.4	199.2	6.5	714.8
HP	16	20	64	6.6	0.22	8.2	21.3	13.2	265.5	13.6	836.2

higher than those of the two other soils. Asami (2001) summarized that the Cd and Zn levels of non–polluted soils in Japan were 0.056 to 0.801 mg kg^{-1} and 16.0 to 105 mg kg^{-1}, respectively. The BLK soil had Cd and Zn concentrations within these levels, and regarded as a non–polluted soil.

Sequential chemical extraction.

It is essential to analyze the amount and the forms of the heavy metals in soils for evaluating the phytoextraction. Sequential chemical extraction methods are often used to determine the forms or fractions of heavy metals in the soils and sediments (McLaren and Crawford, 1973; Tessier *et al.*, 1974; Sadamoto *et al.*, 1994; Saeki *et al.* 1993a; 1993b). In the present study, the Cd and Zn fractions of the soils were analyzed by a method modified by Sadamoto *et al.* (1994). Three g of soil was shaken for 24 h with 30 mL of 0.05 mol L^{-1} potassium nitrate in a 50 mL tube. The supernatant taken from the tube after centrifuging and filtering through a 0.22 μm pore size filter was used for the analysis (ion–exchangeable form (EX fraction)). The residue from the above extraction was shaken for 24 h with 30 mL of 25 g L^{-1} acetic acid. The supernatant obtained by centrifuging and filtering was used for the analysis (form specifically adsorbed on clay minerals (AC fraction)). The residue was digested with 30 mL of 6% hydrogen peroxide, dried, and then extracted with 30 mL of 25 g L^{-1} acetic acid with shaking for 24 h. The supernatant obtained by centrifuging and filtrating was used for the analysis (form specifically adsorbed in organic matters (ORG fraction)). One gram of the residue was added to a 30 mL mixture of 0.1 mol L^{-1} oxalic acid and 0.175 mol L^{-1} ammonium oxalate and with 1 g ascorbic acid powder, then shaken for 1 h in a boiling water bath. The supernatant obtained by centrifuging and filtrating was used for the analysis (form occluded in oxides (OX fraction)). The final residue was digested by perchloric acid and the liquid phase obtained by filtrating was used for the analysis (residue (LA fraction)). The Cd and Zn in the solutions from each fraction were determined using atomic absorption spectrometry (AAS) (Shimadzu Co., Ltd., AA–670).

Cultivations in the polluted soils.

We alternated the 3–week cultivation of corn (cv. Pioneer 33G26) and that of Indian mustard (cv. Hakarashina) in 350 mL pots (diameter, 12 cm; depth, 10 cm) of the BLK pots, the HP pots and the LP pots, with the sequential rotation, corn – Indian mustard – corn – Indian mustard. One corn was grown in one pot, and Indian mustard was grown two plants in one pot because of small size of its plants. The cultivations were done inside a phytotron glass room at a temperature of 298 K and relative humidity of 70% in June 2002 (1st), August 2002 (2nd), October 2002 (3rd) and November 2002 (4th). Distilled water was added to the soils to maintain the field water capacity (0.60 m^3 m^{-3}) every day during the cultivation. Before the 3rd and 4th cultivations, these soils were fertilized. In order to determine the amount of fertilization of ammonium sulfate, corn was cultivated in the Cd–and Zn–added soil. Compared with the 0 g N kg^{-1} pot, the corn growth was inferior in the 0.1 g N kg^{-1} pot. And corn did not emerge in the 0.5 and 1.0 g N kg^{-1} pots. Therefore, the amount of fertilization was determined as 0.05 g N kg^{-1}. 0.05 g N kg^{-1} soil as $(NH_4)_2SO_4$ and 0.05 g K kg^{-1} soil as KH_2PO_4 were added to each soil in all the pots.

Shoots and roots were sampled 3 weeks after the sowing, dried at 343 K and then milled. In the laboratory, the root samples were carefully separated from the soils and washed with distilled water. The soils separated the roots were air dried and transferred to a 2–mm sieve. And the soils used for the next cultivation. The milled shoot– or root–samples were ashed with conc. HNO_3. The Cd and Zn concentration of the digested solutions was measured by AAS.

Cultivations in the Cd–added soils.

Two Cd–added soils (low Cd–added soil (LA) and high Cd–added soil (HA)) made by adding to a non–polluted soil (BLK) with the corresponding amounts of $CdCl_2$ to the concentrations in the LP and the HP soils, respectively. The Cd added soils were mixed with water to 50% of the field water capacity and incubated for 1 week at room temperature (about 293 K), then used for the cultivation. Lime was added to the HA soil in order to adjust the soil pH to that of the HP soil. The same rotation cropping was done in these plots of the lime–added soil to compare with the results from the plots of the polluted soils.

Extractions of Cd and Zn from the soils by the fertilizers

Five g of metal–added soil (6.5 mg Cd kg^{-1}, 715 mg Zn kg^{-1}) was shaken at 303 K for 1 h with 25 mL of 0.02, 0.2, or 2 g N L^{-1} $(NH_4)_2SO_4$ (or g K L^{-1} KH_2PO_4) solution in a 50 mL tube. The Cd and Zn in the supernatant from these solutions were analyzed using AAS (Shimadzu Co., Ltd., AA–670).

Statistical analysis.

Each experiment was performed in triplicate. The variance for each of the results was analyzed and the differences between the averaged values were tested using MS–Excell and Tukey's method (Shinjo, 1996).

RESULTS AND DISCUSSION

Fractionation of Cd and Zn in soil by sequential chemical extraction

The fractionations of Cd and Zn in soil were analyzed before cultivation in order to

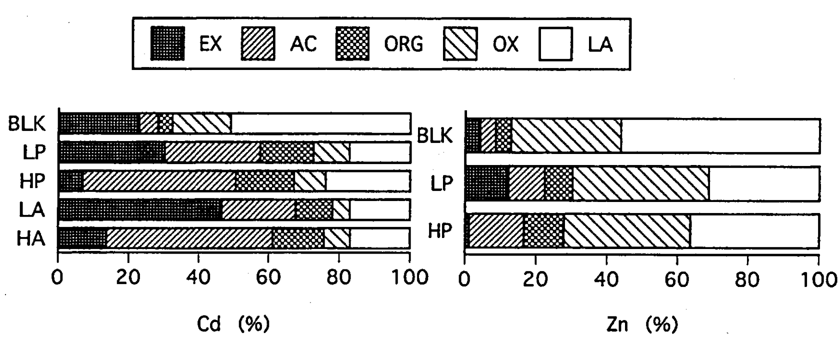

Fig. 1. Fractionation of soil Cd and Zn before cultivation.

evaluate the plant–available metal fractions (Fig. 1). The exchangeable Cd and Zn concentrations in the LP soil were significantly higher than those in the HP soil ($p<0.05$ in both metals). The solubility of soil Cd decreases with the soil pH increasing (Soon, 1981). The Cd proportions of the AC and ORG fractions in the HP soil were higher than those in the LP soil. The high soil pH is considered as one of the causes.

Plant productions

Fig. 2 shows the shoot and root weight changes of the corn and Indian mustard throughout the rotation. The shoots and the roots of both plants decreased from the 1st and the 2nd crops to the 3rd and the 4th crops, respectively. This may be due to the decrease in duration of sunshine from summer to autumn in the greenhouse, although the cultivation temperature was constant. The nutrition in the soils may have decreased by continuing cultivation. The shoot productions of the 1st corn crop in both the HP and LP plots were greater than that in the BLK plot. The 3rd crop in which the fertilizers were added to the soils had no significant difference in the shoot production between the polluted soil plot and the BLK plot. The shoot of the 2nd Indian mustard crop in the LP plot was small in comparison to the other plots. Indian mustard would suffer from excessive Zn in the soil because the soil exchangeable Zn fraction in the LP soil was higher than in the other soils, as shown in Fig. 1.

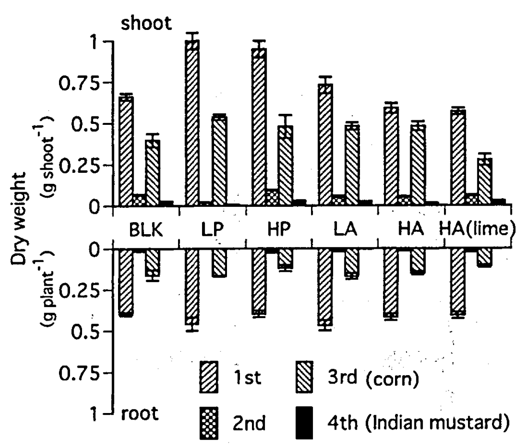

Fig. 2. Shoot and root dry weight changes of corn and Indian mustard.

Cd and Zn removals by crop rotation

Figs. 3 and 4 showed the concentrations of Cd and Zn in shoots of each crop for 21–day cultivation. The plant Cd concentrations increased from the 1st to the 3rd crop of corn and from the 2nd to the 4th crop of Indian mustard in both the LP and HP plots (Fig. 3). For shoot Cd concentration, there were no significant difference between the BLK plot and the polluted soil plots except for 4th LP plot. The shoot Cd concentrations in the

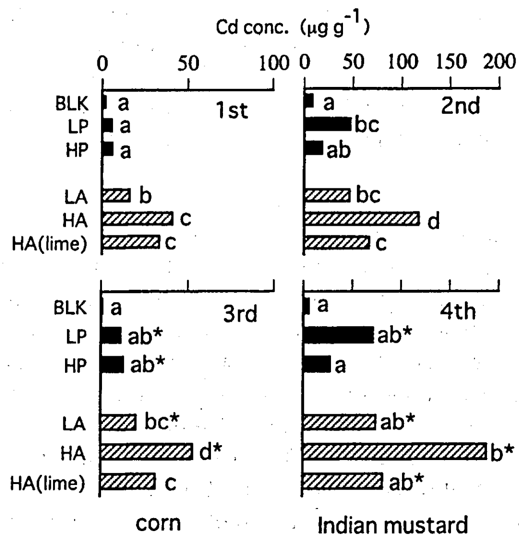

Fig. 3. Cd concentration in shoot of corn and Indian mustard at 21 days after sowing. Different letters show 5% significant difference.

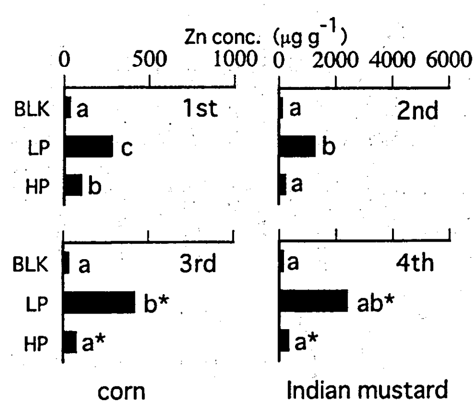

Fig. 4. Zn concentration in shoot of corn and Indian mustard at 21 days after sowing. Different letters show 5% significant difference.

Cd–added plots were higher than that in the BLK plot except for 4th cultivation. The plant Zn concentrations also rose from the 1st to the 3rd crop and from the 2nd to the 4th crop in the LP plot (Fig. 4). These increases were due to the fertilizer application to the soils before the 3rd and 4th cultivations. Fig. 5 shows that the extractions of Cd and Zn from the soil by 2 g L^{-1} of $(NH_4)_2SO_4$ and KH_2PO_4 solutions were apparently greater than by

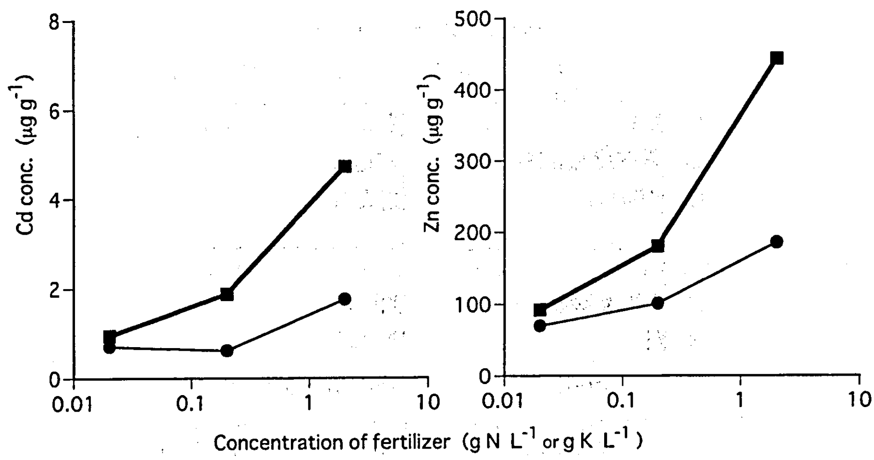

Fig. 5. Extractions of Cd and Zn from the soil by fertilizers. ■, $(NH_4)_2SO_4$; ●, KH_2PO_4.

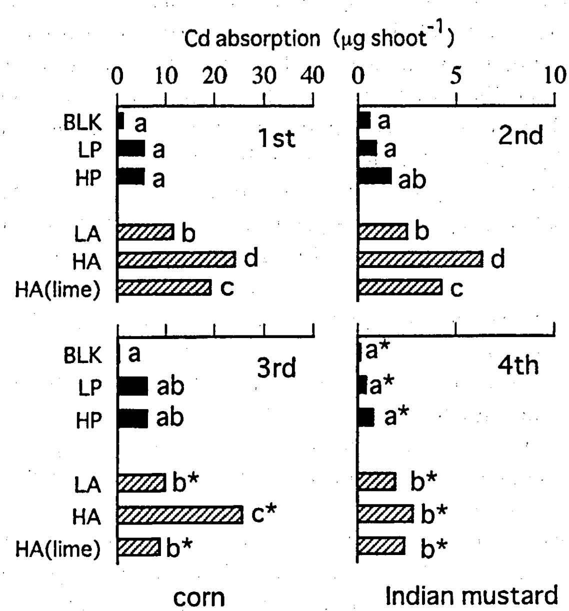

Fig. 6. Cd absorption in shoot of corn and Indian mustard at 21 days after sowing. Different letters show 5% significant difference.

water (0.81 μg Cd g^{-1}, 107 μg Zn g^{-1}). These salt applications would induce the increases in the plant available Cd and Zn fractions in the soils. The authors assumed that sequential cropping decreases heavy metal accumulations in plants by cropping and lowers the

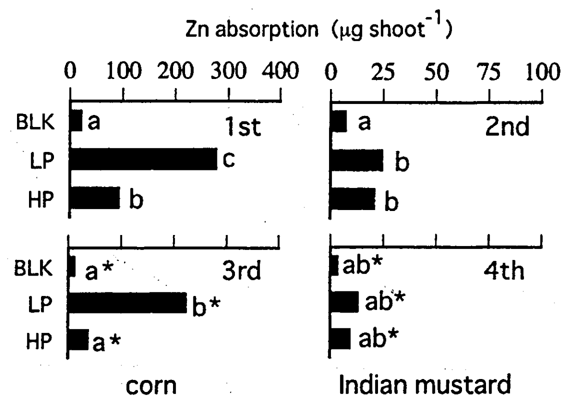

Fig. 7. Zn absorption in shoot of corn and Indian mustard at 21 days after sowing. Different letters show 5% significant difference.

efficiency of the phytoextraction by cropping because plants absorb more plant–available metals easily. The present results indicated that fertilizer application to soil during rotational cropping could inhibit the decrease in the phytoextraction efficiency.

Figs. 6 and 7 showed the absorptions of Cd and Zn in shoots of each crop for 21– day cultivation. The Cd absorptions by Indian mustard were smaller than those by corn because of the low shoot production in spite of the high concentration of the metal in the plant (Fig. 6). However, it may be possible to get a similar Cd removal per unit area in Indian mustard to that in corn by dense planting. The Zn concentrations in the root of Indian mustard were very high in the 2nd LP plot ($4040 \mu g\ g^{-1}$), the 4th LP plot ($3770 \mu g\ g^{-1}$), and the 4th HP plot ($1730 \mu g\ g^{-1}$). It is quite likely that the amount of absorbed Cd by Indian mustard decreased in these plots due to the low shoot production caused by the toxicity of the soluble Zn in the soil.

Fig. 4 shows that the shoot Zn concentrations was low in the HP plot although the HP soil had higher Zn amounts than the LP soil in the total Zn. Consequently, the Zn absorptions by corn were significantly higher in the LP soil than those in the HP soil ($p < 0.05$) (Fig. 7), probably because of high pH of the HP soil. Most of the Zn fraction in the soil may be non plant–available because of the high soil pH.

Table 2 shows the Cd removals by the plants calculated from the total plant absorptions of the shoots and roots and from the total Cd amounts in a pot of the soils. As the total of the all cultivations, the Cd removals from the soils were 2.1% of total soil Cd amount in the LP plot and 1.0% in the HP plot, whereas the Zn removals were lower than the Cd cases. It is inferred from this result that Cd can be efficiently removed rather than Zn in spite of the lower content in the soils. In order to remove the heavy metals by plants from the polluted soils, it is important to select the optimum plant that conforms to temperature during the cultivation (Inoue *et al.*, 2003). In this study, the rotation cannot be compared with a single cropping. In view of the temperature during the cultivation, the Cd absorption by plants may increase by the rotation compared with a single cropping.

Table 2. Cd and Zn removal rate by the plants.

	Accumulation in shoot μg	Accumulation in root μg	Total metal* amounts in soil μg	Removal rate by shoot %	Total removal** rate %
Cd					
BLK	2.9	2.6	50	5.8	10.9
LP	14.0	19.8	1625	0.9	2.1
HP	16.3	18.1	3400	0.5	1.0
LA	30.3	78.1	1625	1.9	6.7
HA	67.9	116.0	3400	2.0	5.4
HA (lime)	41.3	94.6	3400	1.2	4.0
Zn					
BLK	53.7	54.7	30300	0.18	0.36
LP	575.8	448.9	178700	0.32	0.57
HP	189.6	159.6	209050	0.09	0.17

Total metal amounts in soil* = (concentration in soil) × (soil weight/pot)
Removal rate** = (accumulation in shoot or whole plant)/(total metal amounts in soil) × 100

Comparison between the actually polluted soils and the Cd–added soils

In order to investigate the differences in the heavy metal forms in the soils and in the metal absorptions by plants between the artificially metal–added soils and the actually–polluted soils, the same rotation cropping combined with corn and Indian mustard were done in the BLK soils with an added reagent, $CdCl_2$, to the same levels as the polluted soils. Fig. 1 shows the higher Cd percentage of the exchangeable fraction in the added soils than that in the polluted soils. The corn's Cd concentrations of the 1st crop in the low added (LA) soil were significantly higher than those in the LP plot ($p < 0.05$) (Fig. 3). This would be caused by the high concentrations of exchangeable Cd in the added soils. Both of these plants had more Cd in the HA plots than in the HP plots (Fig. 6). One factor would be the high pH of the HP soils (Table 1), then an application of lime to the HA soil was made in order to adjust soil pH to the HP soil. But, Fig. 3 indicates that the lime application did not lower the Cd concentrations in the plants to the level of the HP plot. Even if soil pH was changed by lime, it is possible that the forms of Cd in added–soils do not become like these in actual polluted–soils.

Table 2 shows that the Cd removals by the plants were 6.7% of the total soil Cd in the LA soil, and 5.4% in the HA soil in a total sum of the all cropping. The Cd removal in the metal–added soils was apparently higher than those in the actually polluted soils. When the plants are grown in the heavy–metal–added soil, there are many examples that the plants absorb the heavy metals so much. However, these plants may uptake much plant–available metals in these added soils. It is a problem to use these results for evaluation of phytoremediation directly. The results of this study indicated that the use of metal–added soils in the experiments for phytoextraction is likely to overestimate the efficiency of any plants in actual polluted soils with its variation by the experimental conditions.

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