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Running head: Effect of Fe(0) on Cd uptake in rice plants

Title: Effect of zero-valent iron application on cadmium uptake in rice plants grown in cadmium-contaminated soils

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ABSTRACT

Cadmium (Cd) contamination in soils is a serious problem for crop production in the world. Zero-valent iron (Fe(0)) is a reactive material with reducing power capable of stabilizing toxic elements in a solution. In the present study, we examined the effect of zero-valent iron (Fe(0)) application on Cd accumulation in rice plants growing in Cd-contaminated paddy soils. The Fe(0) application significantly decreased Cd accumulation in the leaves and seeds of rice plants grown in Cd-contaminated soils. The form of Cd in soil was determined by sequential extraction. The Fe(0) application increased the free-oxides-occluded (less available) Cd content, and decreased the exchangeable and Fe-Mn-oxides-bound (more available) Cd content, in Cd-contaminated soils.

Keywords: Cadmium-contaminated soils; *Oryza sativa* L.; Reducing power; Sequential extraction method; Zero-valent iron

INTRODUCTION

Cadmium (Cd) contamination in soils is a significant worldwide environmental problem. The crops cultivated in such contaminated soils often contain significant levels of Cd that can impair human health. In Japan, many rice fields have been affected by Cd derived mainly from human activities such as old mines. It has been estimated that about half of the Cd intake of Japanese people comes from rice (Toyoda et al., 1998). Therefore, the Cd content in rice needs to be reduced to levels as low as possible. There are several methods for remediating Cd-contaminated soils, including soil washing and phytoremediation (Mulligan et al., 2001); however, the problems are the cost and the delayed effect, respectively. Another effective means of remediation, immobilization of Cd in soils, has also been proposed.

Zero-valent iron (Fe(0)) is an inexpensive reactive material with reducing power for the stabilization of toxic elements such as arsenic (Lien and Wilkin, 2005), selenium (Mondal et al., 2004), and several heavy metals (Rangsivek and Jekel, 2005), and for the decomposition of pesticides (Ghauch, 2001) and chlorinated hydrocarbons (Janda et al., 2004) in a solution. For example, a substantial portion of Cu^{2+} is reduced and transformed to insoluble forms of Cu(0) and Cu_2O in stormwater runoff (Rangsivek and Jekel, 2005). Moreover, Fe(0) can be applied to stabilize several elements in contaminated soils. Kumpiene et al. (2006) reported that Fe(0)

application decreased As and Cr concentrations in shoots of plants (a mixture of grasses and herbs) grown in a chromated copper arsenate-contaminated soil.

Because it is known that the reduction of Cd^{2+} in soil solution can induce Cd-insolubilization in soils (Kashem and Singh, 2001), the application of Fe(0) may be effective for inexpensive and rapid remediation of Cd-contaminated soils. In this study, therefore, we examined the effect of Fe(0) application on Cd accumulation in rice plants grown in Cd-contaminated soils. Additionally, the effect of Fe(0) application on the form of Cd in soils was investigated.

MATERIALS AND METHODS

Experiment 1: Removal of Cd in water using Fe(0)

Before the cultivation experiment, the batch experiment was conducted to assess the ability of Fe(0) to eliminate Cd in water. Four levels of Fe(0) (0, 0.5, 2, and 5 g) were added to the solution containing 100 mL of 100 mg L^{-1} Cd solution (cadmium chloride, pH4.7). Fe(0) used in the present study was a segregate form of nanoscale iron particles, and was obtained from JFE Steel Corporation (Figure 1). The solution with Fe(0) was shaken for 1 h, and filtered through a membrane filter (pore size = 0.45 μm). The concentration of Cd remaining in the solution was

determined by inductively coupled plasma emission spectrophotometry (ICPS-7000, Shimadzu, Kyoto, Japan). The solution pH was determined by a pH meter.

Experiment 2: Effect of Fe(0) application on Cd accumulation in rice grown in Cd-contaminated soils

Cd-contaminated soils were prepared by adding appropriate amounts of CdCl₂ to the fertilized field soils at Hokkaido University (brown lowland soil). The soils were incubated in a greenhouse for 6 months with occasional watering. At the end of this treatment, the contaminated soils contained 10 mg kg⁻¹ Cd (0.1 M HCl extractable). Four levels of Fe(0) (0, 0.5, 1, and 5 g) were individually mixed with 100 g of the Cd-contaminated soil. The mixed soils were put in a 100-mL test tube and water was added to mimic paddy conditions. The soils were incubated at 23 °C for 10 days under continuously submerged conditions.

Seeds of rice (*Oryza sativa* L. cv. Kirara 397) were surface sterilized with sodium hypochlorite for 10 min, washed with deionized water, and sown on a moderately moist peat moss in a greenhouse at Hokkaido University. Uniform seedlings (shoot height = 5 cm) were transplanted into the test tubes containing the incubated soils and cultivated in a growth chamber (16 h day / 8 h night, 23 °C) for 90 days. The soils were watered every day to maintain

the submerged condition. Plant shoots and soils were sampled after cultivation. Shoots were separated into stems, leaves, chaffs, and seeds. These samples were dried in a forced-air oven at 80 °C for 72 h, weighed, and ground with a vibrating sample mill (TI-100, CMT, Saitama, Japan). The soils with two Fe(0) treatments (0 and 5 g) were air-dried at room-temperature for 2 weeks and then passed through a 2 mm sieve to prepare them for chemical analysis.

After being ground, 100 mg of the dry leaf and seed samples were digested by H₂SO₄-H₂O₂, and Cd and Zn concentrations were determined by inductively coupled plasma emission spectrophotometry. The sequential extraction method reported by Sadamoto et al. (1994) was used to fractionate Cd in the soils (5 fractions). Soil samples (0.5 g) were shaken with 5 mL of 0.05 M Ca(NO₃)₂ at 30 °C for 24 hours, and then centrifuged at 3000 rpm for 10 min. The supernatant was defined as the exchangeable fraction. The residue was shaken with 5 mL of 2.5% CH₃COOH at 30 °C for 24 hours, and then centrifuged at 3500 rpm for 15 min. The supernatant was defined as Fe-Mn oxides-bound fraction. The residue was digested with H₂O₂ at 100 °C until the H₂O₂ evaporated. Then, the residues were shaken with 5 mL of 2.5 % CH₃COOH at 30 °C for 24 hours, and centrifuged at 3500 rpm for 20 min. The supernatant was defined as the organic-matter-bound fraction. The residue was extracted with 15 mL of acid ammonium oxalate (0.1 M oxalic acid, 0.236 M ascorbic acid, and 0.175 M ammonium oxalate) at 100 °C for 1 hour, and centrifuged at 15000 rpm for 10 min. The supernatant was defined as

the free-oxides-occluded fraction. The residue was vacuum-dried and ground in a mortar. One hundred mg of ground samples were digested with HF-HCl-HNO₃. This fraction was defined as the residual fraction.

Statistics

The results were analyzed with Tukey's multiple comparison test or the Student's *t*-test ($P < 0.05$).

RESULTS AND DISCUSSION

As described in the Introduction, the daily Cd intake of Japanese people is mostly derived from rice. Rice plants accumulate relatively high concentrations of Cd (Arao and Ae, 2003). Ishikawa et al. (2006) reported that rice exhibited a superior ability for mobilizing and absorbing Cd from soils contaminated with low levels of Cd, compared with indian mustard and maize. Here, we apply Fe(0) to soils in an attempt to reduce Cd accumulation in rice plants.

Before cultivating rice, we conducted a simple batch experiment (Experiment 1). Application of Fe(0) significantly decreased the pH of the solution containing Cd (Figure 2). When Fe(0)

was applied to solutions not containing Cd, the pH after 1-hour shaking was nearly neutral (6.64 ± 0.22 , $n=3$), indicating that the Fe(0)-induced pH decline in the Cd-contained solution was not due to spontaneous oxidation of Fe(0). Fe(0) application decreased soluble Cd concentration in the solution in a dose-dependent manner (Figure 2). About 80 % of Cd in the solution (100 mg L^{-1} , 100 mL) could be removed by the application of 5 g of Fe(0). Since this decrease in Cd concentration accompanied a pH decline in the solution (Figure 2), Cd insolubilization was not due to the neutralization of the solution but presumably to the reduction of Cd^{2+} . The pH decline might be caused by oxidation of Fe^{2+} produced in the process of Cd^{2+} reduction [$\text{Fe}(0) + \text{Cd}^{2+} \rightarrow \text{Fe}^{2+} + \text{Cd}(0)$]. Thus, Fe(0) application insolubilizes Cd in solution.

In the next experiment, we determined whether Fe(0) application could reduce Cd accumulation in shoots of rice plants grown in Cd-contaminated soils. Although it was not statistically significant, the Fe(0) application tended to stimulate the growth of rice (Figure 3). Since the Cd level in this experiment (10 mg kg^{-1} soil) did not inhibit the growth of rice (Figure 3), this stimulatory effect of Fe(0) may be due to the utilization of nutrients in soils. The Cd concentration in seeds and leaves of rice grown in Cd-contaminated soils were significantly reduced by the 1.0 and 0.5 mg Fe(0) applications, respectively (Figure 4). Cd concentration in the chaff was measured as trace levels in all the treatments (data not shown).

Then, how did the Fe(0) application reduce Cd uptake in rice? In order to know how the

forms of Cd changed in soils with the Fe(0) application, Cd in soil was fractionated by the sequential-extraction method (Sadamoto et al., 1994). This method yielded 5 fractions: exchangeable, Fe-Mn oxides-bound, organic matter-bound, free-oxides occluded, and residual fractions. The potential availability of Cd forms in soils for plant roots is: exchangeable > Fe-Mn oxides bound > organic matter bound > free-oxides occluded > residual. In the Cd-contaminated soils without the Fe(0) application, the Cd content in each fraction was in the following order: residual >> Fe-Mn oxides bound > exchangeable \approx free-oxides occluded > organic matter bound (Figure 5). As a result of the Fe(0) application, Cd in the exchangeable and Fe-Mn oxides bound fractions, the more bio-available forms, were significantly reduced, whereas the level of Cd in the free-oxides occluded fraction, a less bio-available form, was doubled (Figure 5). There was no significant change in the Cd content of the organic matter bound or residual fractions resulting from the Fe(0) application. In general, the inactivation (precipitation) of Cd in soils can be brought about a change in soil pH (Chuan et al., 1996); however, the soil pH after treatment was ca. 5.7, irrespective of the treatments (data not shown).

These results indicate that the decline of Cd accumulation in rice was attributed to the increase of free-oxides occluded (less available) Cd content in soils resulting from the Fe(0) application. This decline could be caused by the reducing power of Fe(0), directly or indirectly.

CONCLUSIONS

In conclusion, the present study demonstrated that Fe(0) application significantly reduced phyto-available Cd in Cd-contaminated soils, resulting in remarkable reduction of Cd accumulation in rice seeds and leaves without toxic effects on plant growth. Further practical study under field conditions is needed to apply the Fe(0) treatment to crops produced in Cd-contaminated soils.

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FIGURE LEGENDS

Figure 1. Fe(0) used in this study.

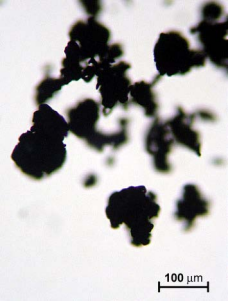
Figure 2. Effect of Fe(0) application on soluble Cd concentration (bar graph) and pH (line graph) in the solution. Data are means of three replicates \pm SE. In the line graph, SE is smaller than the symbol. Different letters indicate statistically significant differences at $P < 0.05$.

Figure 3. Dry weight of rice plants grown in soils with different treatments. Values are the means of three replicates. The range indicates \pm SE for each organ. Different letters indicate significant differences at $P < 0.05$ in total dry weight.

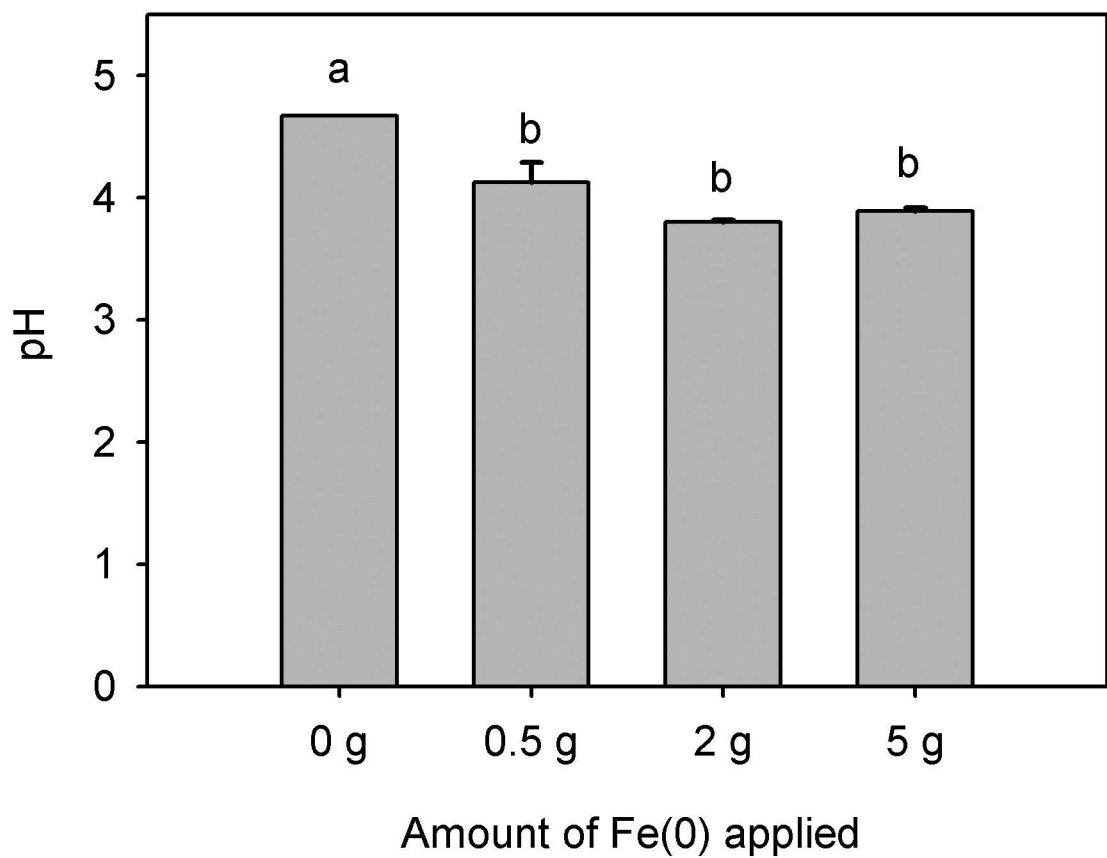
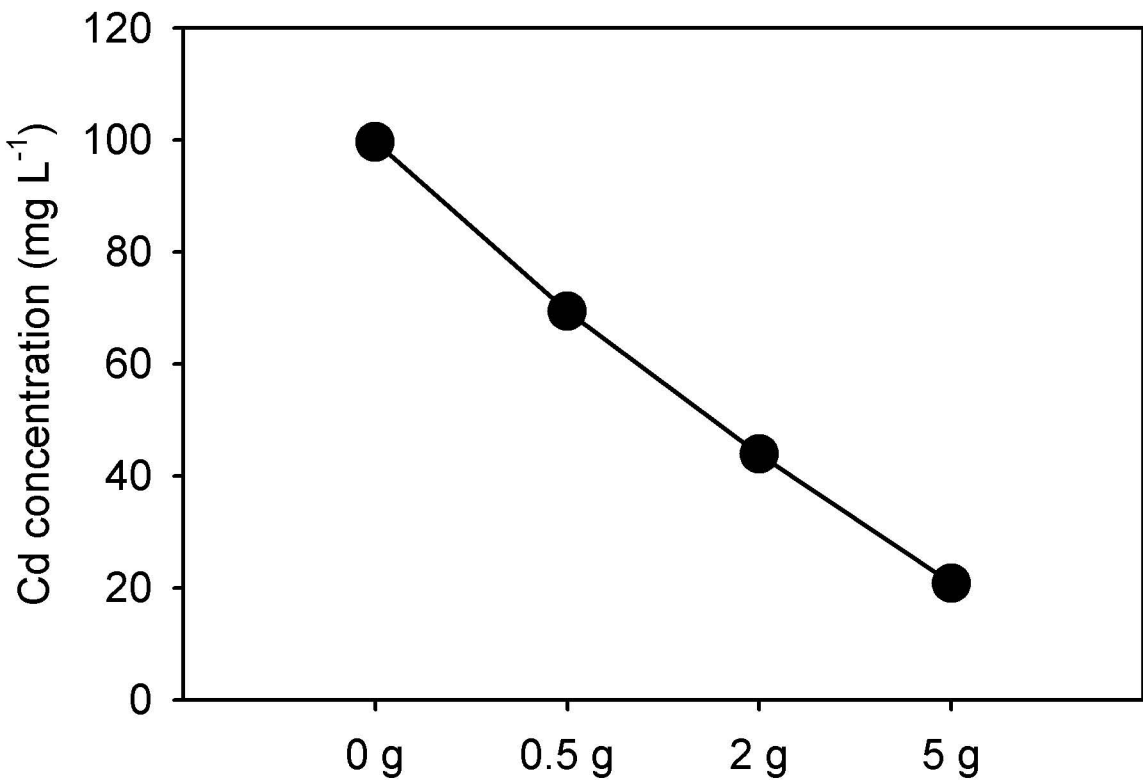
Figure 4. Cd concentration of seeds and leaves of rice plants grown in soils with different treatments. Values are the means of three replicates \pm SE. Different letters indicate significant differences at $P < 0.05$.

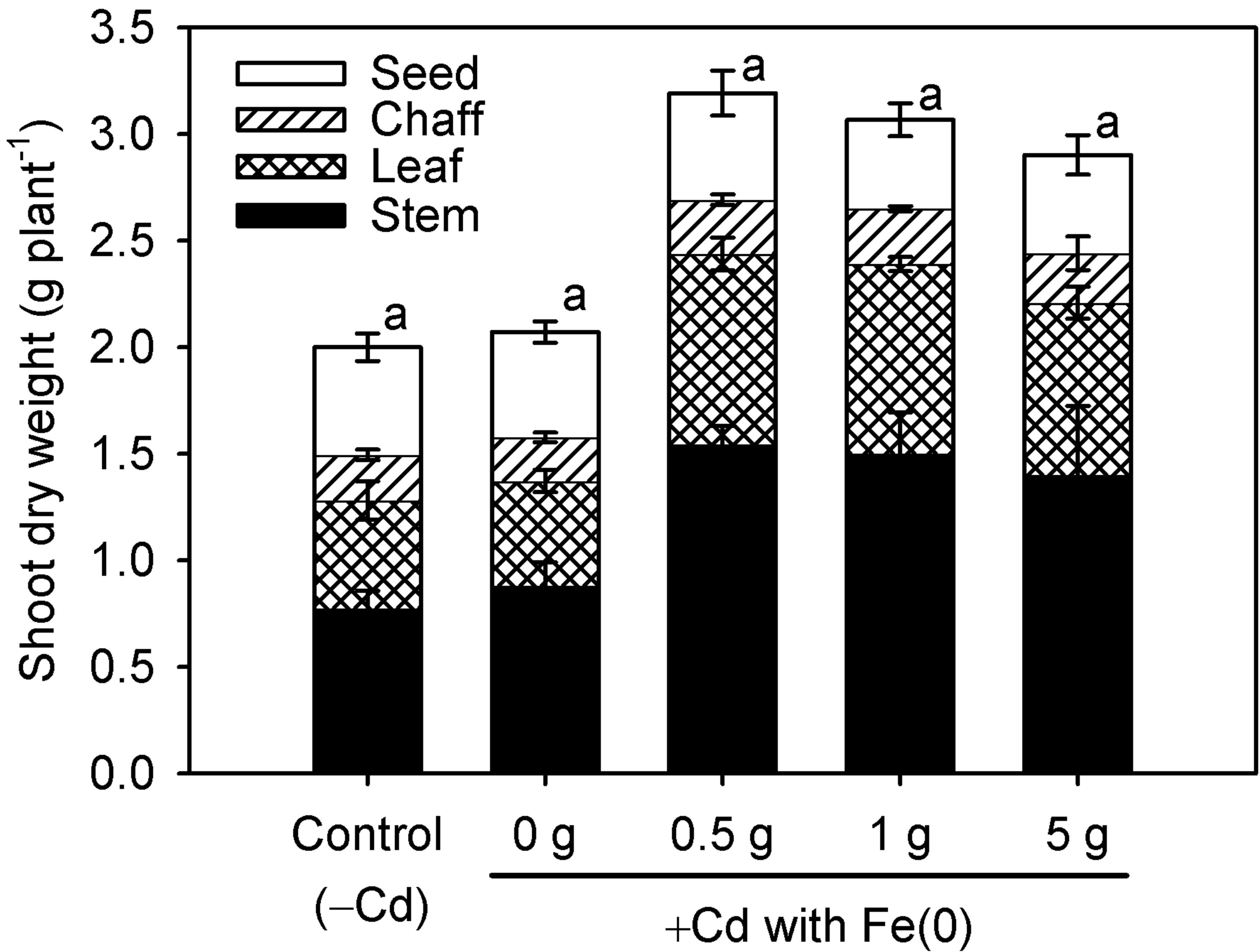
Figure 5. Effect of Fe(0) application on the Cd-form in Cd-contaminated soils. The range for each fraction indicates \pm SE. *, significantly different from the soils without Fe(0) application

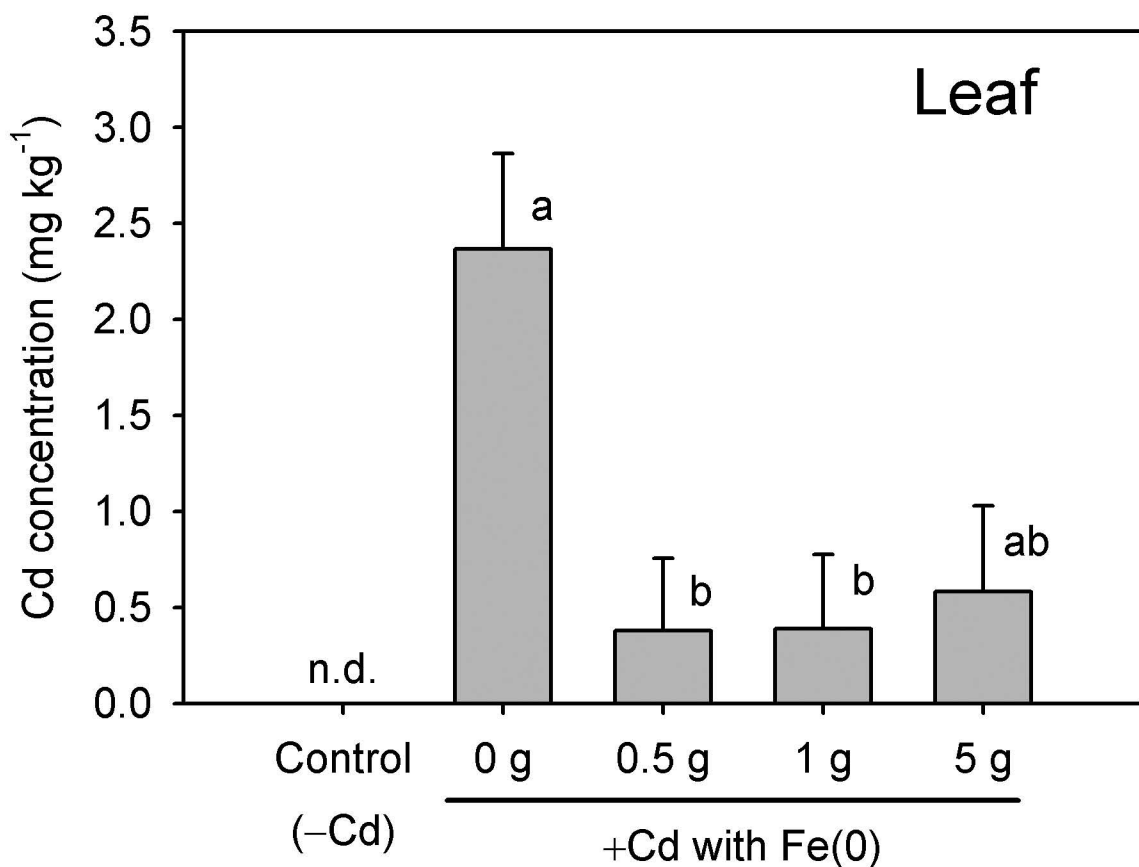
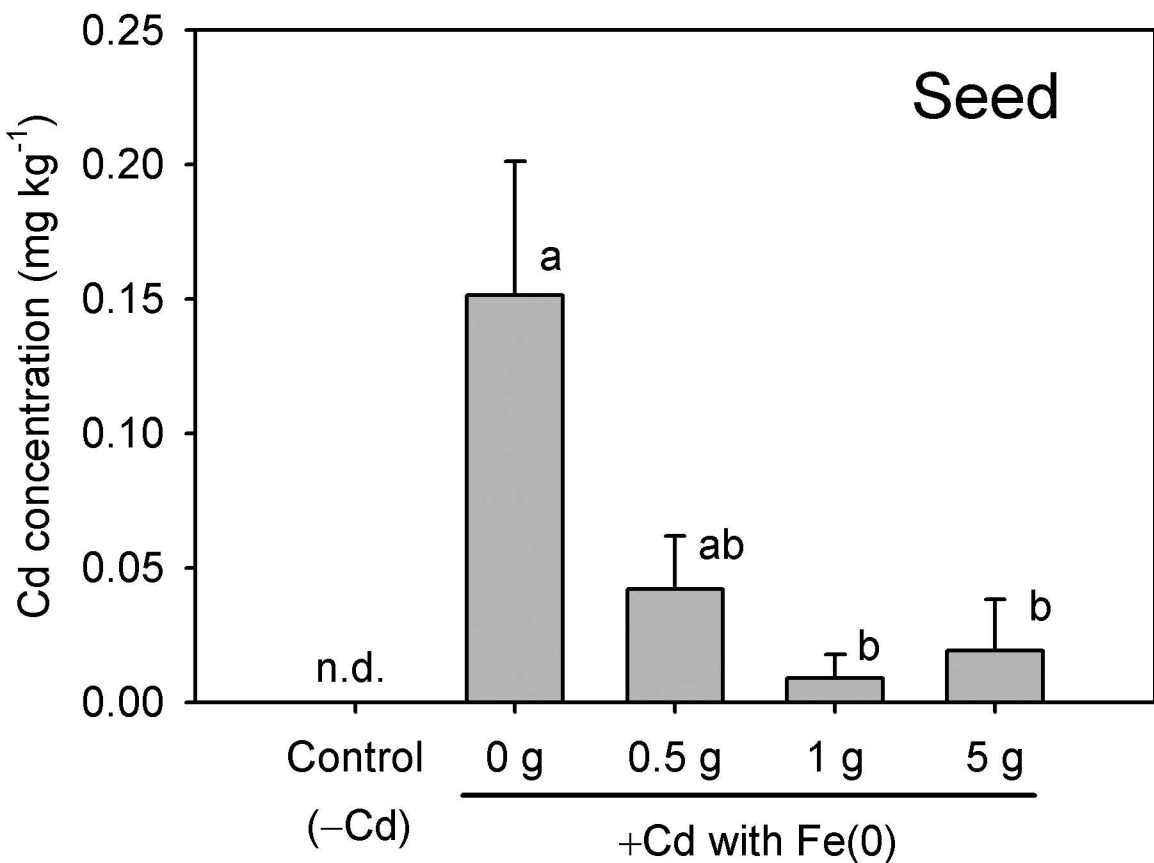
($P < 0.05$).

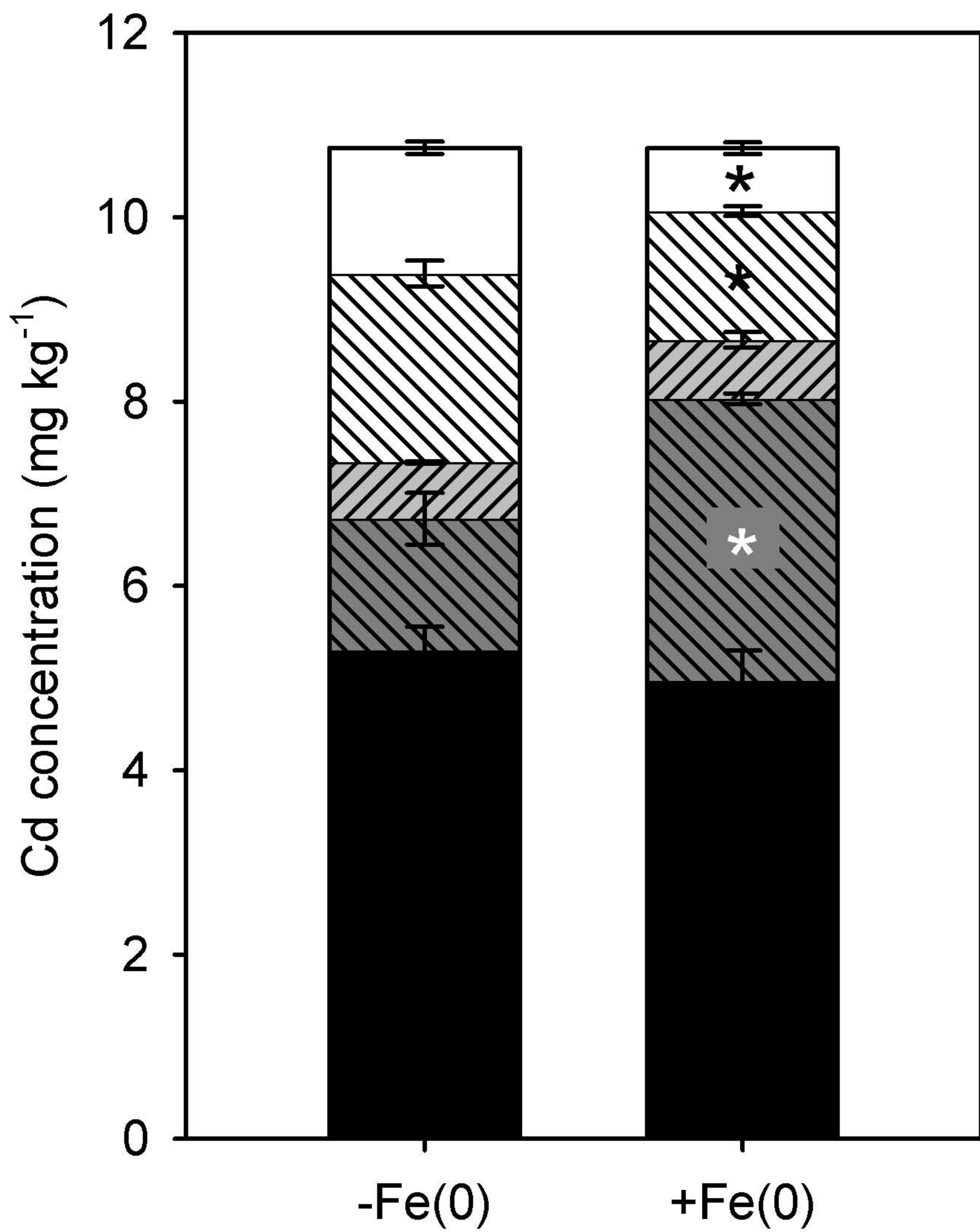


100 μm









- Exchangeable
- Fe-Mn oxides bound
- Organic bound
- Free oxides occluded
- Residual