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# CADMIUM UPTAKE BY AND TRANSLOCATION WITHIN RICE (*ORYZA SATIVA* L.) SEEDLINGS AS AFFECTED BY IRON PLAQUE AND $FE_2O_3$

YAN LAI[1*Φ], BO XU[1,2Φ], LI HE[3], MING LIN[4], LEI CAO[5], YANG WU[3], SHAOLIANG MOU[1] AND SHUILIN HE[6]

[1]*School of Life Sciences, Fujian Agriculture and Forestry University, 350002 Fuzhou, China*
[2]*Institute of Urban Environment, Chinese Academy of Sciences, 361021 Xiamen, China*
[3]*School of Life Sciences, Jinggangshan University, 343009 Jian, China*
[4]*Supply and Marketing Cooperatives of Fujian Province, 350003 Fuzhou, China*
[5]*Zhangzhou Agricultural Vocation School, 363000 Zhangzhou, China*
[6]*College of Crop sciences, Fujian Agriculture and Forestry University, 350002 Fuzhou, China*
[*]*Corresponding author's e-mail: laiyan1010@yahoo.com.cn; Ph: +86 591 83793159; Fax: +86 591 83727618*
[Φ]*These authors contributed equally to the work*

**Abstract**

A hydroponics culture experiment was carried out to investigate the effect of iron plaque and/or $Fe_2O_3$ on Cadmium (Cd) uptake by and translocation within rice seedlings. Uniform rice seedlings grown in nutrient solution for two weeks were selected and transferred to nutrient solution containing ferrous iron ($Fe^{2+}$) (30 mg/L) for 24 h to induce the formation of iron plaque on the root surface. Then rice seedlings were exposed to different level of Cd (1.0 mg/L and 0.1 mg/L), and simultaneously $Fe_2O_3$ was added into hydroponic system for three days. At harvest Cd content in dithionite-citrate-bicarbonate (DCB) extracts, roots and shoots were determined. The results of this study showed that iron plaque could sequester more Cd on root surfaces of rice seedlings, however, $Fe_2O_3$ reduced Cd adsorbed on root surfaces. Both of iron plaque and/or $Fe_2O_3$did not block Cd uptake by and translocation within rice seedlings. Although iron plaque could alleviate Cd toxicity to rice seedlings under low concentration of Cd (0.1 mg/L), the root tissue played more important role in reducing Cd translocation into shoot. And the long period experiment of hydroponic and soil culture was still needed to verify the potential effect of iron plaque and/or $Fe_2O_3$ on alleviating Cd toxicity to rice seedlings.

## Introduction

Rice is one of the most important staple food which is the staff of life for 3 billion people in the world, especially in East and South Asia, the Middle East, Latin America, and the West Indies (Stone, 2008). However, many biotic and abiotic stresses cause negative impacts on its production and quality. Heavy metal contamination in rice field is an important problem directly posing serious health risk for human beings (Zhang Z. W. *et al.*, 1998b, Bennett *et al.*, 2000, Rogan *et al.*, 2009, Cao *et al.*, 2010). Cadmium (Cd) is one of most toxic elements in nature and is soluble and non-degradable contamination which can be transformed from soil to plants (Satarug *et al.*, 2003). It has been reported that germination and growth of plants can be adversely affected by Cd (Farooqi *et al*., 2009, Shafi *et al.*, 2010), and rice has become the leading dietary source of Cd intake in Asia (Moon *et al.*, 1995, Zhang *et al.*, 1998b, Shimbo *et al.*, 2001). Long-term consumption of Cd contaminated rice will result in Cd-related diseases for human beings. For example, it can cause renal dysfunction when Cd reaches a level of about 200 μg/g in the human renal tubules (Nordberg, 1974) and gives rise to the occurrence of an epidemic of osteomalacia in Japan (Obata & Umebayashi, 1997). The reason inducing Itai-itai disease in Japan in the 1930s seems to be the widespread Cd contamination of rice (Ishihara *et al.*, 2001, Inaba *et al.*, 2005). Although Cd risk has been paying attention for several decades, it is still a difficult problem needing to be solved.

Iron plaque widely exists on root surface of plants in flooded condition and its formation is due to 2 crucial factors: one is the release of oxygen and oxidants into the rhizosphere; another is the ferrous iron which can be oxidized to ferric iron precipitated on root surfaces of plants as the iron oxide or hydroxide (Armstrong, 1967, Chen *et al.*, 1980). Iron plaque is considered to be able to block or reduce the heavy metals translocation into the plants. It is shown that the toxicity of aluminum (Al), arsenic (As), copper (Cu), zinc (Zn) and nickel (Ni) can be alleviated by iron plaque (Greipsson & Crowder, 1992, Greipsson, 1994, 1995, Liu *et al.*, 2005, Chen *et al.*, 2006). However, there is very limited information on Cd translocation and accumulation in rice seedlings with iron plaque. In addition, iron oxide also can adsorb contaminations in the environment and reduce the heavy metals uptake by plants (Wang *et al.*, 2009). Red soil is a typical southern soil in China and consists of several iron oxides and other components. Hematite ($Fe_2O_3$) is one of the iron oxides, which accounts for the most proportion in red soil (Yin & Guo, 2006). It has been reported that Cd content in red soil is higher than in others (black soil, yellow brown soil, laterite soil, etc.), and it also shows high Cd level in crop grown in red soil (Xia, 1992). Therefore, $Fe_2O_3$ as a main iron oxide in red soil is selected to study Cd destiny in rice plants. The objective of the present study was to investigate the effects of iron plaque and/or $Fe_2O_3$ on Cd uptake by and translocation within rice seedlings.

## Materials and Methods

**Preparation of rice seedlings:** Seeds of rice (Cultivar: TY180) were sterilized in $H_2O_2$ solution for 15 minutes and then washed thoroughly with deionized water. The seeds were geminated in acid-washed quartz sand. After 10 days uniform seedlings were selected and transplanted to plastic pots (1 L). Seedlings were grown in 1/3-strength Hoagland solution and the composition of nutrient solution was as following (mg/L): $Ca(NO_3)_2{\cdot}4H_2O$, 1181; $KNO_3$, 510; $MgSO_4{\cdot}7H_20$, 490; $KH_2PO_4$, 136.09; $H_3BO_3$, 2.86; $MnCl_2{\cdot}4H_2O$, 1.81; $CuSO_4{\cdot}5H_2O$, 0.08; $ZnSO_4{\cdot}7H_2O$, 0.22; $H_2MoO_4{\cdot}4H_2O$, 0.09; $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$, 0.019; $FeSO_4{\cdot}7H_2O$, 0.014. The nutrient solution was adjusted to pH 5.5 with 0.1M NaOH or HCl and changed every 3 days. The experiments were carried out in a controlled growth chamber with a 14 h light period (150 μmol $m^{-1}$ $s^{-1}$), and the temperature was kept at 28 °C during the day and 20 °C during the night.

**Experiment treatments:** After 2 weeks, seedlings were grown in deionized water for 12 h to minimize any interference from other elements with iron. And then they were transferred into 1 L solution with 30 mg/L of ferrous ion for 24 h ($Fe^{2+}$ as $FeSO_4{\cdot}7H_2O$). Solution pH was adjusted to 5.5 using 0.1 M KOH or HCl. Seedlings were subsequently grown in 1/3-strength nutrient solution including two levels of Cd (1.0 mg/L and 0.1 mg/L, $Cd^{2+}$ as $Cd(NO_3)_2{\cdot}4H_2O$) and/or $Fe_2O_3$ for 3d. The plants and $Fe_2O_3$ (1 g/L) were simultaneously transplanted into the culture pots with the following conditions: (1) no iron plaque, no $Fe_2O_3$, $Cd^{2+}$ (1.0 mg/L and 0.1 mg/L); (2) no iron plaque, with $Fe_2O_3$, $Cd^{2+}$ (1.0 mg/L and 0.1 mg/L); (3) iron plaque, no $Fe_2O_3$, $Cd^{2+}$ (1.0 mg/L and 0.1 mg/L); (4) iron plaque, $Fe_2O_3$, $Cd^{2+}$ (1.0 mg/L and 0.1 mg/L).

**DCB extraction of iron plaque from roots**: At harvest, seedlings were removed from each pot, separated into shoots and roots and rinsed with deionized water 3 times. Iron plaque from fresh root surfaces was extracted using dithionite-citrate-bicarbonate (DCB) according to the method of Taylor and Crowder, (1983) and Otte *et al.,* (1991). The whole root of each seedling was incubated for 60 min at 25°C in 30 mL 0.03 mol/L sodium citrate and 0.125 mol/L sodium bicarbonate with the addition of 0.6 g sodium dithionite. Then the extracts were transferred to 50 mL glass flasks and filtered into plastic bottle. After DCB extraction, roots and shoots were dried at 70°C for 3 days and weighed.

**Plant analysis:** Dried root and shoot samples were ground and chose some for digest. The samples of roots and shoots were acid digested in 5 mL of 30% $HNO_3$ at 90°C for 3 h, then at 140°C for 5 h, and 180°C for a further 2 h until a little solution remained without complete drying out. After the tubes had cooled, the digests were transferred to 25 mL flasks with deionized water and filtered into plastic bottles. A reagent blank and a standard reference material (tomato, GSBZ 51001-94, Chinese National Certified Reference Material) were included to verify the accuracy and precision of the digestion procedure and subsequent analysis. The concentrations of Fe and Cd in the digests were determined by atomic absorption spectrophotometry (AAS). Total uptake of the elements and the distributions of the elements in DCB extracts, roots and shoots were calculated according to Liu *et al.*, (2004).

**Statistical analysis:** Data on metal concentrations were analyzed by one-way ANOVA using SPSS 13 system for Windows. Data presented were means ± S.E. (n = 3) and were tested by least significant difference at the 5% level.

## Results

As shown in Fig. 1, the amounts of Fe in DCB extracts on rice root surfaces in the presence of iron plaque were significantly higher than in the absence of iron plaque. The iron content (approximate 55 g/kg Dry Weight (DW)) of root surfaces with iron plaque was approximately 10 times higher than that without plaque, indicating that exogenous ferrous Fe added into the hydroponic system was oxidized to ferric iron precipitated on rice root surface due to radical oxygen loss from rice root, meanwhile, which alleviated the toxicity of excess Fe to rice seedlings. It was found from Fig. 2a & 2d that the content of Cd in shoots increased with iron plaque and/or $Fe_2O_3$ than without iron plaque and/or $Fe_2O_3$. Fig. 2b & 2e respectively showed that Cd concentrations in roots generally revealed consistent trends (except root Cd (0.1 mg/L) with iron plaque without), suggesting that iron plaque and $Fe_2O_3$ did not alter Cd translocation into rice roots. As shown in Fig. 2c & 2f, when $Fe_2O_3$ was added into the hydroponic system, the concentrations of Cd in DCB extracts decreased under two levels of Cd both with and without iron plaque. However, Cd content in DCB extracts was significantly increased in the presence of iron plaque. The concentrations of Cd in DCB extracts also significantly increased under the combined treatments of iron plaque and $Fe_2O_3$. In addition, it was found that the roots of rice seedlings accumulated higher level of Cd than shoots and root surfaces expect for Cd in DCB extracts with iron plque under Cd treatment of 1.0 mg/L. This indicated that iron plaque could adsorb Cd on root surfaces, especially for excess Cd. Nevertheless, the root tissue became the main contributor to blocking Cd translocation in comparison with iron plaque.

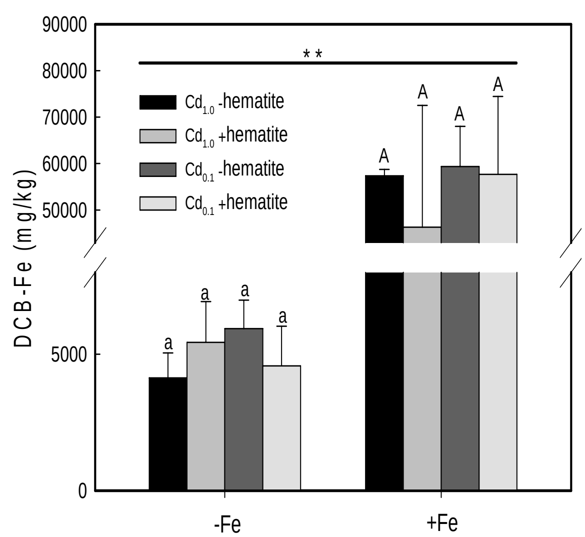


Fig. 1. The amount of iron in dithionite-citrate-bicarbonate (DCB)-extracts on root surfaces of rice seedlings with/without iron plaque. Data are means ± SE (n = 3). Lower case a and upper case A indicate significant differences between the two groups (with iron plaque (-Fe) and without iron plaque (+Fe)) according to least significant difference test. ** means extremely significant difference ($p$<0.001)

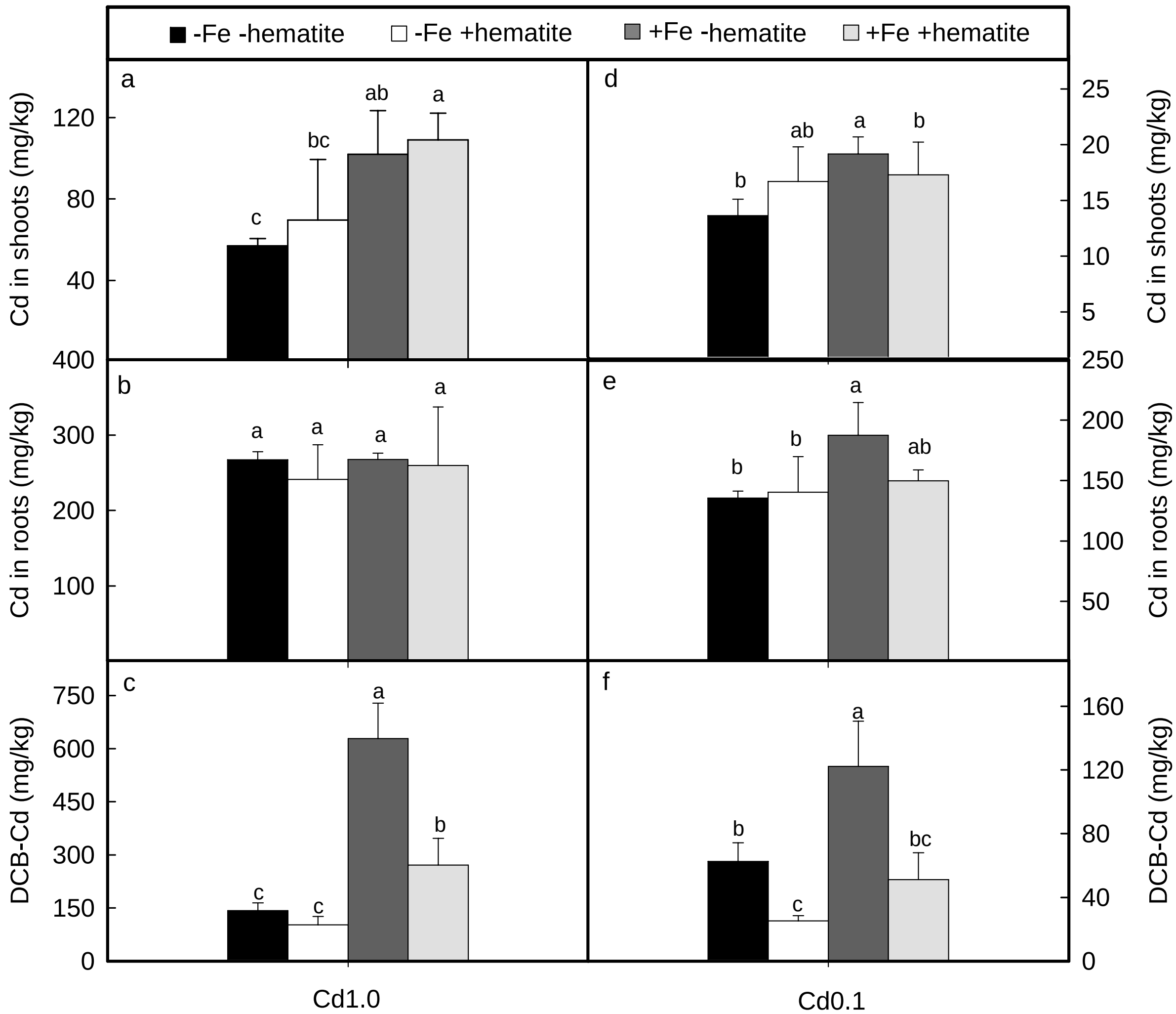


Fig. 2. Cadmium concentrations in dithionite–citrate–bicarbonate (DCB)-extracts on the root surface, in roots and shoots of rice seedlings grown in nutrient solution under different treatments. The different letters indicate the results of multiple comparisons according to least significant difference test.

Cd distribution in different components of rice seedlings was shown in Fig. 3. Under the condition of 1.0 mg/L Cd, the percentage of Cd in shoots was not reduced by iron plaque and/or $Fe_2O_3$, but the percentage of Cd in roots was reduced. The percentage of Cd in DCB extracts slightly decreased in the presence of $Fe_2O_3$, but increased with the iron plaque and the combined effects of iron plaque and $Fe_2O_3$. Under the condition of 0.1 mg/L $Cd^{2+}$, it was interesting to find that the percentage of Cd in shoots and roots showed decreasing trends in the presence of iron plaque, but increasing trends under the effects of $Fe_2O_3$ and combined treatments of iron plaque and $Fe_2O_3$. In contrast, the percentage of Cd in DCB extracts increased with iron plaque, and decreased with $Fe_2O_3$ and the combined treatments of iron plaque and $Fe_2O_3$.

## Discussion

In the present experiment, our results showed that the amount of Fe on the root surfaces of rice seedlings was 5.0 g/kg without iron plaque, and 55.2 g/kg with iron plaque (30 mg/L $Fe^{2+}$). Fe as an essential element for plants played an important role on plants growth (Talbot & Etherington, 1987, Wei *et al.*, 1994). However, deficiency of Fe element which could result in some negative symptoms like as chlorosis was a world-wide nutrient problem (Vose, 1982). Thus, iron plaque on the roots surface of plants could act as an elements reservoir for plants and mediate the uptake of nutrient and metals by plants (Zhang *et al.*, 1999, Liang *et al.*, 2006). But the interaction among elements and utilization of nutrients elements by plants are so very complicated that the effects of iron plaque on elemental uptake by plants are controversial. Some studies have reported on the effects of iron plaque that can adsorb As, Al, Cu, Zn and Ni and block translocation of these elements into plants (Greipsson & Crowder, 1992, Greipsson, 1994, 1995, Liu *et al.*, 2005, Chen *et al.*, 2006). On the other hand, there are some studies about iron plaque showing little effect on reducing uptake and translocation of Cu, Ni, Zn, lead (Pb) and Cd (Ye *et al.*, 1997, 1998). There are many factors resulting in the opposite results including different amount of iron plaque, different characteristic of element, different plant species, different culture period, different culture style and so on. For example, when the amounts of iron plaque on root surface of rice reached 24.9 g/kg, Zn concentrations in shoots were reduced (Zhang *et al.*, 1998a). Rice and *Typha latifolia* showed different effects on Zn translocation under the action of iron plaque (Ye *et al.*, 1998, Zhang *et al.*, 1998a). Different culture style (hydroponic culture and soil culture) also revealed different results of Cd accumulated by plants (Liu *et al.*, 2007, Liu *et al.*, 2008).

In our study, iron plaque and/or $Fe_2O_3$ did not block Cd translocation and uptake by rice seedlings. $Fe_2O_3$ reduced the accumulation of Cd on the root surface, but did not block Cd translocation into plants. Iron plaque enhanced the accumulation of Cd on the root surface, but still did not depress Cd translocation into plants. These results were similar to the studies from Liu *et al.*, (2007). Liu *et al.*, (2007) reported that although iron plaque could accumulate Cd on root surfaces, root tissue rather than iron plaque became a main barrier blocking Cd uptake and translocation within rice seedlings. In the system of soil culture, however, the concentrations of Cd in roots and shoots were significantly reduced in the presence of iron plaque (Liu *et al.*, 2008). The complex factors like metallic minerals in soil could possibly contributed a lot to reduction of Cd in plants (Wang *et al.*, 2009).

Furthermore, it was found from the Fig. 3 that the percentage of Cd in shoots and roots respectively showed increasing and decreasing trends with iron plaque under Cd treatment of high concentration (1.0 mg/L). However, the percentage of Cd in shoots and roots showed decreasing trends in the presence of iron plaque under Cd treatment of low concentration (0.1 mg/L). Liu *et al.*, (2008) also found that the percentage of Cd in shoots and roots was reduced with iron plaque under Cd treatment of low concentration, and shoots and roots respectively showed increasing and decreasing trends with iron plaque of rice seedlings exposed to excessive Cd. This indicated that iron plaque could alleviate Cd toxicity to rice seedlings under low concentration of Cd, however, the alleviative effect of iron plaque on Cd translocation was weakened under excess Cd. In addition, it was found although $Fe_2O_3$ supply shared some pressure from Cd stress for root surface, it did not reduce Cd translocation into rice plant.

In conclusion, our results show that although iron plaque and $Fe_2O_3$ can increase and reduce Cd adsorption on root surfaces, respectively, both of them can not block Cd uptake by and translocation within rice seedlings. The root tissue may play more important role in reducing Cd translocation into shoot with or without iron plaque and/or $Fe_2O_3$ under low level of Cd. Meanwhile, long period experiment of hydroponic and soil culture is still needed to verify the effect of iron plaque and $Fe_2O_3$ on Cd uptake by and translocation within rice seedlings.

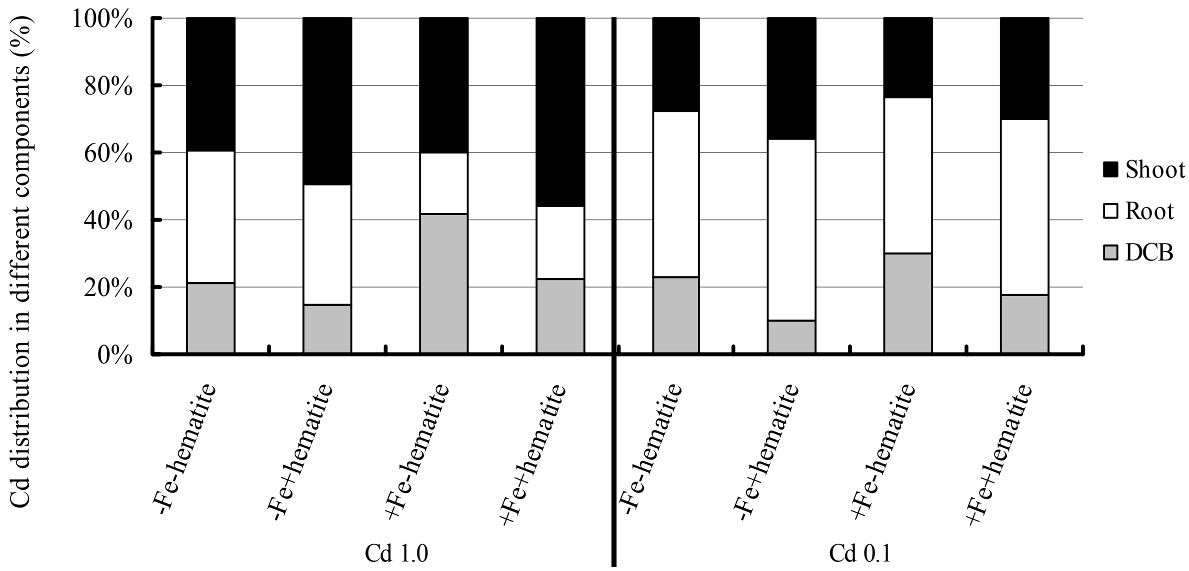


Fig. 3. Percentages of cadmium in different components of rice seedlings with $Fe_2O_3$ and/or iron plaque grown in nutrient solution with Cd treatments of 1.0 mg/L and 0.1 mg/L.

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