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Liu, H. J., Zhang, J. L., Christie, P., & Zhang, F. S. (2008). Influence of iron plaque on uptake and accumulation of Cd by rice (Oryza sativa L.) seedlings grown in soil. *Science of the Total Environment*, *394*(2-3), 361-368. https://doi.org/10.1016/j.scitotenv.2008.02.004

Published in:
Science of the Total Environment

available at www.sciencedirect.com

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Influence of iron plaque on uptake and accumulation of Cd by rice (*Oryza sativa* L.) seedlings grown in soil

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ARTICLE INFO

Article history:
Received 31 July 2007
Received in revised form
31 January 2008
Accepted 2 February 2008
Available online 5 March 2008

Keywords:
Cadmium
DCB (dithionite–citrate–bicarbonate)
Iron
Iron plaque
Oryza sativa

ABSTRACT

Iron plaque is ubiquitously formed on the root surfaces of rice. However, little is known about the role of iron plaque in Cd movement from soil to the plant aboveground parts. A pot experiment was conducted to investigate the influence of iron plaque in Cd uptake and accumulation by rice seedlings in soil. Rice seedlings were pre-cultivated in solution culture for 16 days. Two seedlings were transplanted in a nylon bag containing no substrate but surrounded by soil amended with Fe and Cd combined at rates of 0, 1, or 2 g Fe kg^{-1} and 0, 2.0, or 10 mg Cd kg^{-1} soil. Fe was added to induce different amounts of iron plaque, and Cd to simulate Cd-polluted soils. Plants were grown for a further 43 days and then harvested. The length of the longest leaf and SPAD values of the newly mature leaves were measured during plant growth. Fe and Cd concentrations were determined in dithionite–citrate–bicarbonate (DCB) soil extracts and in plant roots and shoots. Shoot and root dry weights were significantly affected by Fe supply level but not by added Cd. Root dry weight declined with increasing Fe supply but shoot dry weight decreased at 2 g Fe kg^{-1} and increased at 1 g Fe kg^{-1} (except at 2 mg Cd kg^{-1}). The length of the longest leaf and SPAD values of the newly mature leaves were significantly affected by plant growth stage and added Fe and Cd. Fe tended to diminish the negative effect of Cd on these two parameters. Cd concentrations in DCB extracts increased with increasing Cd and Fe supply. In contrast, external Fe supply markedly reduced shoot and root Cd concentrations and there was generally no significant difference between the two Fe supply levels. Shoot and root Cd concentrations increased with increasing Cd addition. Root Cd concentrations were negatively correlated with root Fe concentrations. The proportion of Cd in DCB extracts was significantly lower than in roots or shoots. The results indicate that enhanced Fe uptake by plants can diminish the negative effects of Cd to some extent and that iron plaque on root surfaces is of little significance in affecting uptake and accumulation of Cd by rice plants.

1. Introduction

Rice (*Oryza sativa*) is globally one of the most important food crops. Cadmium (Cd) is an element that is of great concern due to its potential toxicity to rice and, more importantly, its toxicity to humans through contamination of the food chain. Soil Cd pollution can occur naturally or from human activities such as waste disposal, mining and phosphate fertilizer application (Komarnicki, 2005). To reduce Cd uptake by rice plants is of great importance for minimizing grain Cd in rice production in Cd-polluted soils.

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doi:10.1016/j.scitotenv.2008.02.004

A common feature of aquatic plants such as rice is the formation of iron plaque on the root surfaces due to the release of O_2 and oxidants in the rhizosphere and the subsequent oxidation of ferrous to ferric iron with the precipitation of iron oxide or hydroxide on the root surface (Chen et al., 1980; Taylor and Crowder, 1983b; Taylor et al., 1984), and/or to biological oxidation by microorganisms (Emerson et al., 1999; Weiss et al., 2003). Iron plaque consists mainly of ferrihydrite (63%) in *Phalaris arundinacea* (Hansel et al., 2001) or goethite in *Juncus bulbosus* (Chabbi, 1999) together with minor levels of other forms of iron. Formation of iron plaque is generally considered to be one of the mechanisms that aquatic plants have evolved to acclimate to anaerobic conditions, particularly to very high concentrations of Fe^{2+}, Mn^{2+} and S^{2+} in the soil. In addition, due to the high capacity of functional groups on iron hydroxides to sequester metal(loid)s by adsorption and/or co-precipitation, the availability of the metal(loid)s in the rhizosphere can be affected by the iron plaque on the root surfaces, and this may lead to changes in the uptake and accumulation of elements by the plants. The presence of iron plaque can inhibit or enhance the uptake of phytotoxic metal(loid)s into plant tissues (Greipsson, 1994,1995; Ye et al., 2001; Batty et al., 2000, 2002; Chen et al., 2004; Liu et al., 2004a,b; Chen et al., 2006).

Like other wetland plant species, rice develops aerenchyma to transfer O_2 from the aerial parts to the roots, resulting in the oxidation of ferrous to ferric iron and the precipitation of iron oxides or hydroxides on root surfaces (Chen et al., 1980). The formation of iron plaque on rice root surfaces has been shown to alleviate Cu, Ni, Zn and Al toxicities (Greipsson and Crowder, 1992; Greipsson, 1994, 1995; Zhang et al., 1998; Chen et al., 2006) and to alter the uptake and translocation of P (Zhang et al., 1999) and As (Chen et al., 2004; Liu et al., 2004a,b, 2005) in solution culture experiments. However, little information is available on the relationship between iron plaque and Cd uptake and accumulation in rice grown in soil. The characteristics of iron plaque in soil differ substantially from those induced in solution culture in terms of the composition, distribution and properties of the iron plaque, microbial activity (Emerson et al., 1999; Weiss et al., 2003), and the extension of iron plaque into the rhizosphere (Taylor et al., 1984; Wild, 1988). For example, when *Typha latifolia* was grown in the field, Taylor and Crowder (1983a,b) found that concentrations of Cu and Ni in the shoots were lower in the presence of iron plaque than in its absence, and they argued that iron plaque decreased the uptake of various elements (Taylor and Crowder, 1983a). In contrast, in solution culture iron plaque did not inhibit elemental uptake by plants (Taylor and Crowder, 1983b). The objective of the present work was therefore to investigate the potential role of iron plaque in the growth and uptake and accumulation of Cd by rice seedlings growing in soil.

2. Materials and methods

2.1. Pre-cultivation of rice seedlings

Seeds of rice (*O. sativa* L. cv. II You 718) were surface sterilized in 30% v/v H_2O_2 for 30 min and washed thoroughly with deionized water. The seeds were dipped in saturated $CaSO_4$ solution for 6 h and then germinated in acid-washed quartz sand for ten days. Uniform seedlings were selected and transferred to a 1.2-l ceramic container and grown in a 1/2-strength nutrient solution for three days and thereafter in full strength solution for a further 16 days. The solution had the following composition: NH_4NO_3 500, $NaH_2PO_4{\cdot}2H_2O$ 60, K_2SO_4 230, $CaCl_2$ 210, $MgSO_4{\cdot}7H_2O$ 160, Fe-EDTA 10, $ZnSO_4{\cdot}7H_2O$ 0.5, $MnCl_2{\cdot}4H_2O$ 0.5, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.05, H_3BO_3 0.2, and $CuSO_4{\cdot}5H_2O$ 0.01 (μmol l^{-1}). The pH of the nutrient solution was adjusted to 5.0 with NaOH and HCl and the solution was changed every three days. The seedlings were then transplanted into soil with one of the designated treatments.

The soil was collected from a rice field in Sichuan Province, southwest China and is classified as an Aquorizem developed from alluvial deposits of the Minjiang River. Root debris and stones were removed and the soil was air-dried and sieved (<3 mm). The soil had the following properties: pH (10:1 distilled water:soil) 6.2, organic matter ($K_2Cr_2O_7$–H_2SO_4) 19.6 g kg^{-1}, total N (semi-quantitative titration) 1.84 g kg^{-1}, Olsen-P (0.5 M $NaHCO_3$) 17.9 mg kg^{-1}, available K (1.0 M NH_4OAc) 34 mg kg^{-1}, DTPA–Fe (2:1 extract solution:soil) 74.9 mg kg^{-1}, and DTPA–Cd 0.227 mg kg^{-1}. The soil was supplied with 0.2 g N kg^{-1} as $(NH_4)_2SO_4$, 0.15 g P_2O_5 kg^{-1} as KH_2PO_4 and 0.20 g K_2O kg^{-1} as K_2SO_4 as basal fertilizers. Subsequently the soil was amended with Fe and Cd solutions. Iron was supplied at a rate of 0, 1, or 2 g kg^{-1} (as $FeSO_4{\cdot}7H_2O$) and Cd at a rate of 0, 2.0, or 10 mg kg^{-1} (as $3CdSO_4{\cdot}8H_2O$). The Fe was supplied to induce iron plaque to varying extents and the Cd additions were made to simulate Cd contamination of polluted soil. Zhao et al. (2005) reported that Cd concentrations in Japanese rice paddy fields averaged 4.99 mg kg^{-1}. In some contaminated soils in China Cd concentrations were up to more than 100 mg kg^{-1}, and Cd concentrations in irrigated water were above 10 mg l^{-1} in Taiwan (Hsu and Kao, 2005). All chemical reagents were dissolved in water and then mixed thoroughly with the soil. The Fe and Cd were added in all possible combinations to give a total of nine treatments as follows: Fe0Cd0, Fe1Cd0, Fe2Cd0, Fe0Cd2, Fe1Cd2, Fe2Cd2, Fe0Cd10, Fe1Cd10, Fe2Cd10 and there were five replicate pots of each treatment.

2.2. Soil culture experiment

The plastic plant pots used were round and tapered with a mean diameter of 7.5 cm and a height of 15 cm. Each pot was filled with 600 g soil. Firstly, a small amount of soil (approximate 200 g) was placed in each pot and then a bag made of nylon net (8 cm wide × 15 cm long) of 30 μm pore size was placed upright in the centre of the pot. The nylon net was used to restrict root growth within the bag so that the roots could be easily separated from the soil at harvest. Finally, the remaining soil (approximate 400 g) was divided into two equal parts which were placed on either side of the bag. Seedlings were grown in mesh bags containing no substrate but surrounded by soil to avoid the difficulty of separating roots from soil and to minimize losses of root material at harvest. The soil was then moistened with deionized water and left to equilibrate for two weeks. Two rice seedlings were placed in each nylon net and the pots were then flooded with deionized water. The water level was maintained at about 1–2 cm above the soil surface during whole period of plant growth.

Table 1 – Dry weight of shoots and roots of rice seedlings grown in soil amended with different levels of Fe and Cd

Cd supplied (mg kg^{-1})	Fe supplied (g kg^{-1})	Shoot dry weight (g)	Root dry weight (g)
0	0	4.27±0.41	1.60±0.16
	1	4.74±0.42	1.47±0.13
	2	4.00±0.32	1.41±0.16
2	0	4.50±0.44	1.84±0.13
	1	4.40±0.06	1.51±0.24
	2	3.68±0.42	1.22±0.24
10	0	3.85±0.51	1.60±0.26
	1	5.15±0.36	1.70±0.11
	2	3.92±0.66	1.35±0.30
Significance[a] of			
Cd		NS	NS
Fe		***	***
Cd×Fe		*	NS

[a]By analysis of variance; ***P<0.001; *P<0.05; NS, not significant. Data are means±SD (n=5).

2.3. Plant growth and harvesting

The seedlings grew for 42 days from 22 June to 4 August 2005 in the greenhouse at the College of Resources and Environmental Sciences, China Agricultural University, Beijing. The average day/night regime was 15/9 h with a temperature regime of 35/15 °C. During plant growth the length of the longest leaf of each seedling was measured by ruler on 5, 12, 19, and 26 July and 3 August. The SPAD (Soil and Plant Analyzer Development) values of the newly mature leaves were also determined on 22, 25, 28, and 31 July and 3 August. At harvest the nylon nets were removed from the pots. Seedlings were separated into shoots and roots and the samples were rinsed with tap water and then with deionized water. Each entire root sample was firstly used for DCB extraction, and then the roots and shoots were oven dried at 70 °C for three days.

2.4. Chemical analysis of plant samples

Iron plaque on the surface of the fresh roots was extracted using a modified dithionite–citrate–bicarbonate (DCB) method (Taylor and Crowder, 1983c; Otte et al., 1989). The entire root systems of both rice seedlings from each pot were firstly incubated for 70 min at 25 °C in 80 ml 0.03 mol l^{-1} sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$) and 0.125 mol l^{-1} sodium bicarbonate ($NaHCO_3$) with the addition of 1.6 g sodium dithionite ($Na_2S_2O_4$). The extracts were then transferred into 100-ml glass flasks. Roots were rinsed three times with deionized water and the eluates were added to the DCB extracts. Finally the solutions were filtered into plastic containers for subsequent analysis.

Oven dried root and shoot samples were milled. Subsamples (0.25 g) were weighed into digestion tubes and moistened overnight with a mixed acid solution composed of 5 ml concentrated HNO_3 and 1 ml mixed concentrated acid (HNO_3:$HClO_4$=1:1). On the following day the samples were heated on a digestion block at 90 °C for 3 h, then at 140 °C for 5 h, and at 180 °C for a further 2 h until little solution remained in the tubes. After cooling, the digests were transferred to 25-ml flasks with deionized water and filtered into

Table 2 – Analysis of variance for Figs. 1 and 2

	Length of leaves	SPAD values
Significance[a] of		
Time	***	***
Cd	***	*
Fe	***	***
Time×Cd	**	NS
Time×Fe	***	***
Cd×Fe	***	*
Time×Cd×Fe	NS	NS

[a]By analysis of variance; ***P<0.001; **P<0.01; *P<0.05; NS, not significant.

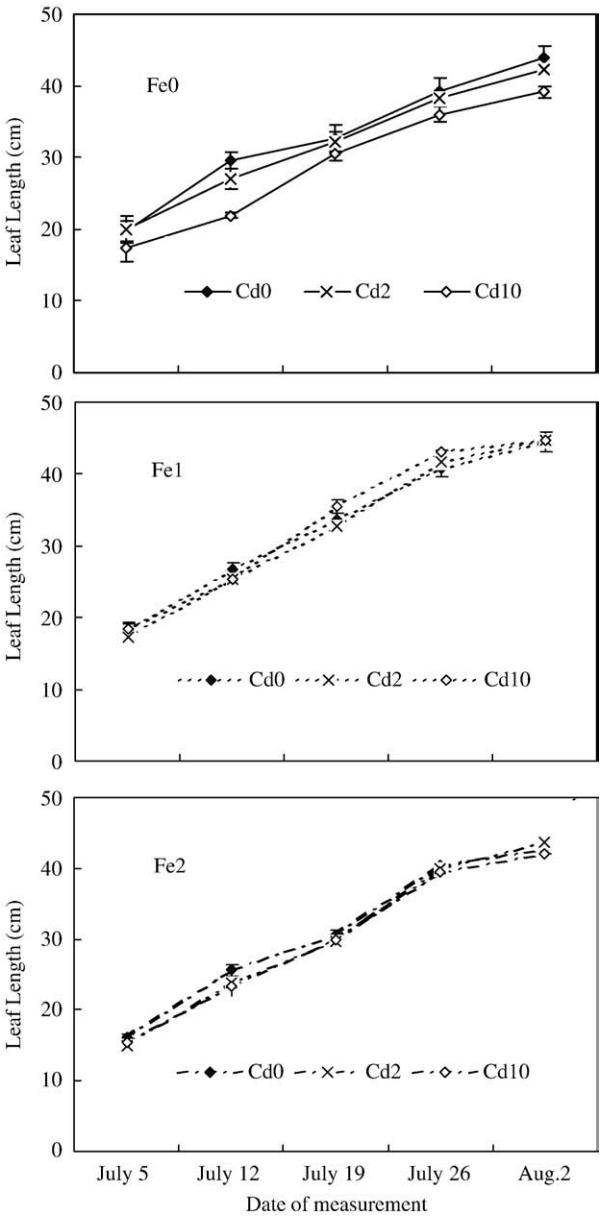

Fig. 1 – Length of the longest leaf of rice seedlings grown in soil amended with different levels of Fe and Cd.

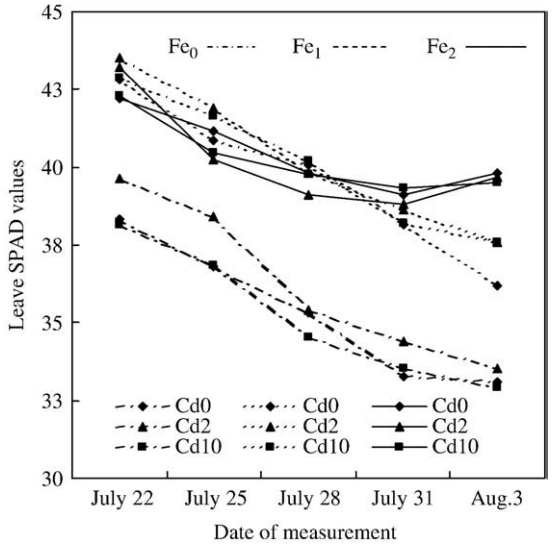

Fig. 2 – SPAD values of leaves of rice seedlings grown in soil amended with different levels of Fe and Cd.

plastic bottles. A reagent blank and a standard reference material (tomato, GSB 07-1264-2000, 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 DCB extracts and in the acid digests were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 3300 DV, Perkin Elmer, USA). The proportions of Cd in DCB extracts, roots and shoots were calculated as follows:

$$\text{DCB-Cd\%} = (\text{Cd content in DCB-extracts/plant total Cd content}) \times 100$$

$$\text{Root-Cd\%} = (\text{Root Cd content/plant total Cd content}) \times 100$$

$$\text{Shoot-Cd\%} = (\text{Shoot Cd content/plant total Cd content}) \times 100.$$

2.5. *Statistical analysis*

Data were tested for normality using the Kolomogorov–Smirnov test and results were analyzed by two-way or three-way analysis of variance (for length of longest leaf and SPAD values of leaves) using SAS for Windows (Version 8.2, SAS Institute Inc., Cary, NC, USA). Data presented are means ± SD (n=5), and the treatment means were compared using least significant difference (LSD) at the 5% level.

Table 3 – Analysis of variance for Figs. 3 and 4

	Fe in DCB	Fe in roots	Fe in shoots	Cd in DCB	Cd in roots	Cd in shoots
Significance[a] of						
Cd	NS	NS	NS	***	***	***
Fe	***	***	***	***	***	***
Cd×Fe	*	NS	NS	NS	***	***

[a]By analysis of variance; ***P<0.001; *P<0.05; NS, not significant.

3. Results

3.1. *Deposition of iron plaque on root surfaces*

At harvest, reddish iron plaque was visible on the surface of the rice roots. Iron plaque appeared as a light orange color at Fe0 and deep brown at Fe1 and Fe2, indicating that the roots were heavily coated with iron plaque. Iron plaque on the surface of lateral roots was deposited on the root segment more than 1 cm from the root tips. The entire older roots were heavily coated with iron plaque and these roots were short, thin, rigid, and easily broken down.

3.2. *Dry weights of shoots and roots*

The dry weights of shoots and roots were significantly affected by Fe additions but not by Cd additions. Cd and Fe interacted to affect shoot dry weight (P<0.05, Table 1). When no Cd was

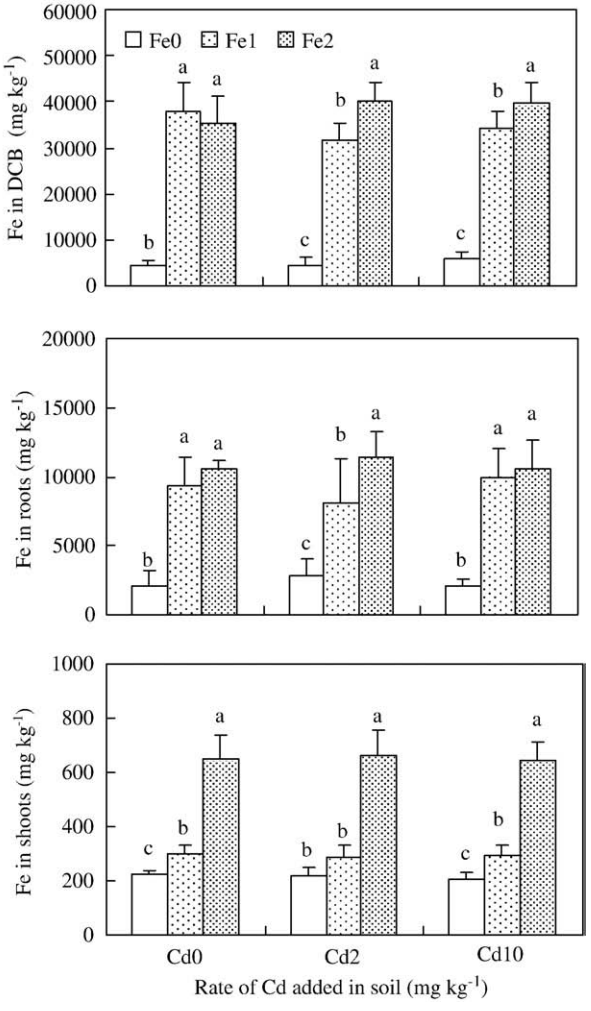

Fig. 3 – Mean Fe concentrations in soil DCB extracts and in roots and shoots of rice seedlings grown in soil amended with different levels of Fe and Cd. Data are means ± SD (n=5). Within each group of three values any two means sharing a lower case letter are significantly different by least significant difference at the 5% level.

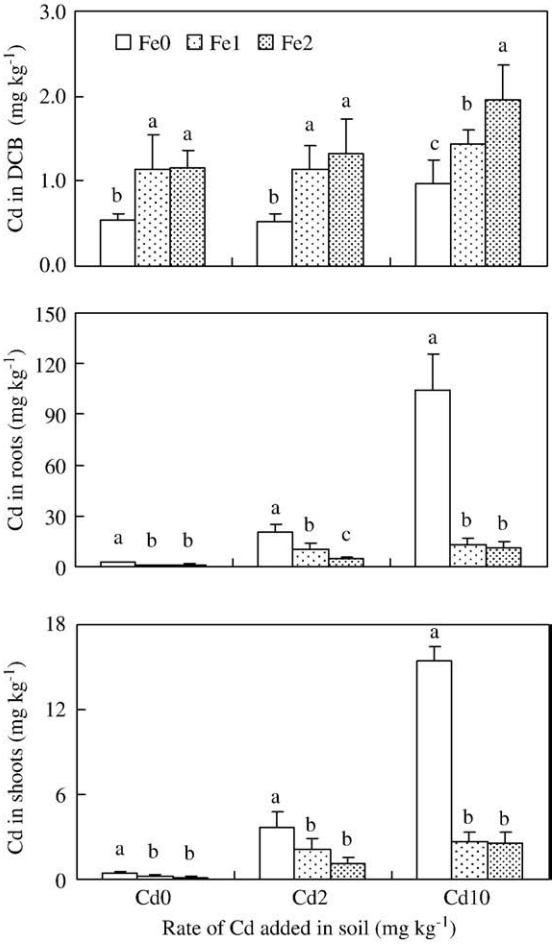

Fig. 4 – Mean Cd concentrations in soil DCB extracts and in roots and shoots of rice seedlings grown in soil amended with different levels of Fe and Cd. Data are means ± SD (n = 5). Within each group of three values any two means sharing a lower case letter are significantly different by least significant difference at the 5% level.

added to the soil, shoot dry weight at Fe0 was lower than at Fe1 and higher than at Fe2. Root dry weights at Fe1 and Fe2 appeared lower than at Fe0 but the difference was not significant. The dry weight of shoots and roots at Cd2 declined with increasing Fe supply. At Cd10 the dry weights of shoots and roots at Fe1were higher than at Fe0 and Fe2, and root dry weight at Fe2 was significantly lower than at Fe0.

3.3. *Length of the longest leaf and SPAD values of leaves*

The length of the longest leaf was significantly affected by plant growth period, Fe and Cd additions and by their two-way interactions (P<0.001, Table 2). At Cd0 and Cd2 the leaf lengths decreased at first (5 to 12 July) and subsequently increased with increasing Fe supply (Fig. 1). At Cd10 the leaf length increased at Fe1 over the whole growth period and at Fe2 it decreased initially and then increased. The Fe-induced growth promoting effect was greater at Fe1 than at Fe2 with all Cd additions. The leaf length at Fe0 declined with increasing Cd supply but at Fe1 and Fe2 it was not significantly affected by Cd supply level.

The SPAD values of mature leaves were significantly affected by plant growth period, Fe and Cd additions and their interactions (P<0.05, except time × Cd, Table 2). The SPAD values of leaves at Fe1 and Fe2 were significantly higher than at Fe0 irrespective of Cd supply level (Fig. 2). At Fe0 and Fe1 leaf SPAD values tended to decrease with the duration of the plant growth period, while at Fe2 the value no longer decreased from 25 July on. Addition of 2 mg kg^{-1} Cd increased leaf SPAD value at Fe0 but at Fe1 and Fe2 the addition of Cd usually had no significant effect on leaf SPAD value.

3.4. *Fe and Cd concentrations in DCB extracts and in roots and shoots*

Iron concentrations in DCB extracts and in rice seedling roots and shoots were significantly affected by Fe but not by Cd supply level (P<0.001, Table 3). The Fe concentrations in DCB extracts and roots were significantly higher when Fe was supplied than without added Fe (Fig. 3). In general, Fe concentrations in DCB extracts and roots at Fe2 were higher than at Fe1 and significant differences were observed for Fe concentrations in roots and DCB extracts at Cd2 and for DCB-Fe at Cd10. Shoot-Fe concentrations increased with increasing Fe supply level and the value at Fe2 was much higher than at Fe1 or Fe0.

The Cd concentrations in DCB extracts and in roots and shoots were significantly affected by Cd and Fe supply level (Table 3). The Cd concentrations in DCB extracts increased with increasing Fe supply level (except at Cd0, Fig. 4). The presence of Cd in DCB extracts when no Cd was added to the soil was most likely due to background Cd in the soil used. Cd concentrations in roots and shoots were significantly lower when Fe was supplied and in general there was no significant difference between the two Fe supply levels, except that root Cd concentrations at Fe2Cd2 were significantly lower than that at Fe1Cd2. The Fe-induced decreases in shoot and root Cd concentrations were greater at Cd10 than at Cd2. Cd concentrations in DCB extracts and in shoots and roots increased significantly with increasing Cd supply level and the trend was most pronounced at Fe0. Root Cd concentrations were shown

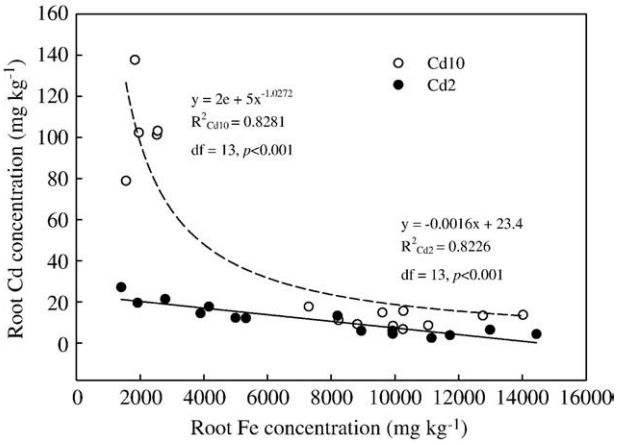

Fig. 5 – Correlation between concentrations of Fe and Cd in roots of rice seedlings grown in soil amended with different levels of Fe and Cd.

Table 4 – Fe percentages in DCB extracts and in roots and shoots of rice grown in soil amended with different levels of Fe and Cd

Cd supplied (mg kg^{-1})	Fe supplied (g kg^{-1})	DCB-Fe%	Root-Fe%	Shoot-Fe%
0	0	62.5±5.72	28.6±6.68	8.9±2.52
	1	78.1±6.41	19.8±6.21	2.0±0.23
	2	73.7±3.36	22.5±3.39	3.8±0.34
2	0	61.6±6.38	31.2±5.70	7.3±2.77
	1	78.5±5.25	19.4±5.58	2.1±0.53
	2	74.8±2.76	21.4±2.45	3.8±0.41
10	0	69.4±7.88	24.7±7.66	5.9±0.92
	1	76.1±2.50	21.9±2.47	2.0±0.40
	2	76.2±3.33	20.2±3.60	3.7±0.57
Significance[a] *of*				
Cd		NS	NS	NS
Fe		***	***	***
Cd×Fe		NS	NS	NS

Data are means±SD (n=5).
[a]By analysis of variance; ***P<0.001; NS, not significant.

to be negatively correlated with root Fe concentrations at both Cd2 and Cd10 addition levels (P<0.001, Fig. 5).

3.5. *Proportions of Fe and Cd in DCB extracts and in roots and shoots*

The proportions of Fe in DCB extracts and in roots and shoots were significantly affected by Fe supply level but not by Cd addition (Table 4). The proportion of Fe in the extracts was much higher than that in roots or shoots. DCB-Fe% was higher when Fe was supplied and the reverse was observed for the proportion of Fe in roots and shoots. The Fe-induced increases in DCB-Fe% and decreases in the proportion of Fe in roots and shoots were greater at Fe1 than at Fe2 except at Cd10 where the difference in DCB-Fe% and root Fe% between Fe1 and Fe2 was not significant.

The percentages of Cd distribution were significantly affected by Cd and Fe supply levels (except shoot Cd%) and by their interactions (Table 5). The proportion of Cd in roots was often greater than in DCB extracts and shoots (except at Fe1Cd0 and Fe2Cd0). Cd proportion in DCB extracts increased with increasing Fe supply level, whereas the proportion of root Cd decreased. Shoot Cd percentages were variable depending on external Cd addition. There was no significant difference in the proportion of Cd in roots and shoots between Fe1 and Fe2 except that root Cd% at Fe2Cd2 was significantly lower than at Fe1Cd2.

4. Discussion

Cadmium is not an essential element for plant growth and although its mammalian toxicity is of prime environmental concern it may also disturb various physiological processes in plants. Cadmium has been shown to inhibit root growth and leaf extension, decrease the content of chlorophyll and proteins (Lagriffoul et al., 1998), produce oxidative stress (Chien et al., 2001), influence photosynthesis (Mendelssohn et al., 2001), and affect the absorption of nutrient elements (Hsu and Kao, 2005). In the present experiment plant growth was significantly affected by Fe supply levels in the soil but not by Cd additions (Table 1), although the length of the longest leaf and leaf SPAD values were significantly affected by both factors and their interactions (Table 2). When no Fe was added to the soil Cd10 significantly decreased shoot dry weight (Table 1), and Cd2 and Cd10 inhibited extension of the longest leaf (Fig. 1). When Fe (1 or 2 g kg^{-1}) was added, there was no significant effect of Cd on dry weights of roots and shoots (except at Fe1Cd10), leaf length or SPAD values (Figs. 1 and 2). The results therefore indicate that Fe is the element that exerts the greater influence on rice growth and the negative effects of Cd additions on the physiological indices of rice seedlings are to some extent diminished by addition of Fe to the soil. Although aquatic plants have evolved a mechanism to oxidize Fe (II) to Fe (III) in the rhizosphere to form iron plaque to prevent excessive Fe uptake, high Fe concentrations in the substrate can potentially induce iron toxicity in these plants and consequently reduce plant growth (Batty and Younger, 2003). On the other hand, Fe has been shown to confer plant tolerance to excessive metals (DeKock, 1981) including Cu and Ni (Greipsson, 1994, 1995), and the beneficial effect of Fe has been attributed to strong competition of Fe with heavy metals for sensitive metabolic sites within the leaves (Taylor and Crowder 1983c; Kuo, 1986) or in the root tips (Chen et al., 2006).

More iron plaque was formed on root surfaces when additional Fe was added to the soil (Fig. 3). Iron plaque has been shown to have high affinity for metal(loid)s and thus affects the uptake of these elements by plants. Large amounts of iron plaque on root surfaces led to high deposition of Cd in Fe plaque as shown by the higher Cd in DCB extracts in plants treated with Fe (Fig. 4). Similar results were obtained by Liu et al. (2004b) who found that DCB-extracted As was correlated with the amount of iron plaque on root surfaces of rice. Zhang et al. (1998) also found that Zn concentrations in DCB extracts increased with increasing amount of iron plaque on root

Table 5 – Cd percentages in DCB extracts and in roots and shoots of rice grown in soil amended with different levels of Fe and Cd

Cd supplied (mg kg^{-1})	Fe supplied (g kg^{-1})	DCB-Cd %	Root Cd %	Shoot Cd %
0	0	13.3±2.76	57.9±2.51	28.9±2.11
	1	43.2±5.09	33.6±7.25	23.2±9.37
	2	46.6±1.01	36.0±3.82	17.4±3.75
2	0	1.8±0.51	67.7±5.15	30.5±5.45
	1	7.1±2.44	57.5±2.92	35.4±5.21
	2	15.6±5.64	47.5±3.07	36.9±4.51
10	0	0.7±0.29	72.8±3.31	26.5±3.04
	1	6.9±2.25	57.1±2.75	36.0±2.28
	2	9.3±1.82	54.5±2.21	36.2±2.69
Significance[a] *of*				
Cd		***	***	***
Fe		***	***	NS
Cd×Fe		***	***	***

Data are means±SD (n=5).
[a] By analysis of variance; ***P<0.001; NS, not significant.

surfaces. However, higher Cd concentrations in DCB extracts did not lead to increasing shoot or root Cd concentrations. Cadmium concentrations in shoots and roots of rice seedlings with higher amounts of iron plaque on the root surfaces were substantially lower than those of seedlings with low amounts of iron plaque (Fig. 4). One may presume that iron plaque impeded the uptake and translocation of Cd in the plants as shown in many other studies for other elements (Greipsson, 1994, 1995; Peverly et al., 1995; Christensen and Sand-Jensen, 1998; Christensen et al., 1998; Hansel et al., 2001, 2002; Liu et al., 2005; Chen et al., 2006). However, the substantial decreases in Cd concentrations in shoots and roots cannot be attributed solely to the sequestration of Cd in Fe plaque. As shown in Table 5, at Cd2 and Cd10 DCB-extracted Cd accounted for only a small proportion of Cd in the plants (0.7–15.6%) in comparison with 47.5–72.8% of Cd in roots and 26.5–36.9% in shoots. This is in marked contrast to the much higher proportion of As found in DCB extracts on rice root surfaces when rice was grown in solution culture (Liu et al., 2004a,b) and in soil (Bravin et al., 2008). In addition, the iron-induced decreases in root Cd concentrations were approximately 100% (Fe1Cd2) and 372% (Fe2Cd2) relative to Fe0Cd2, and 721% (Fe1Cd10) and 840% (Fe2Cd10) relative to Fe0Cd10. The corresponding values for shoot Cd concentrations were 76% (Fe1Cd2) and 236% (Fe2Cd2), and 485% (Fe1Cd10) and 507% (Fe2Cd10) (Fig. 4). Furthermore, root Cd concentrations were negatively correlated with root Fe concentrations (Fig. 5). It can be inferred that root tissues may be the main barrier preventing Cd from entering the roots, and enhanced Fe nutrition in the roots may partially depress Cd uptake by plant roots but the underlying mechanism needs further investigation. This is further supported by the observation that even though Fe concentrations in shoots and roots of plants grown at Fe2 were substantially higher than at Fe1, shoot Cd concentrations at Fe2 and Fe1 did not differ significantly irrespective of Cd additional level. Clearly, high Fe concentrations in shoots did not affect Cd uptake and lower Cd concentrations in shoots of plants treated with Fe in comparison to those without added Fe due to lower Cd concentrations in the roots, and thus the lower translocation of Cd from roots to shoots. Other studies using solution culture also showed that root tissue is important in preventing metal(loid)s from entering the plants, including As when arsenite was supplied in the substrate (Liu et al. 2005), Pb and Cd (Ye et al., 1998), Cu, Zn and Pb (Vesk et al., 1999), Fe, Mn, Cu and Zn (Chabbi, 2003), and Cd (Liu et al., 2007).

Recently Bravin et al. (2008) showed that redox potential varied temporally and spatially in the rhizosphere of rice seedlings, which may lead to a change in iron plaque in the rhizosphere. Liu et al. (2006) showed that the amount of Fe plaque differed among different sections of the roots (root tips>middle>base) and among rice genotypes tested. Our results and those of other workers indicate that the processes involved and the role of iron plaque on root surfaces of rice plants in sequestering heavy metals are more complicated than previously thought, particularly when the plants are grown in soil rather than synthetic media. Further research, particularly using *in situ* techniques, is required to clarify the relationship between iron plaque formation and Cd uptake and accumulation in rice plants.

In conclusion, our results indicate that Fe plaque may adsorb and sequester Cd onto the root surfaces. However, in comparison with root tissues, iron plaque on root surfaces is of little significance in preventing Cd from entering rice roots and subsequently restricting its translocation from roots to shoots. Further research is required to examine further the interactions between Cd and Fe and to study the movement of Cd in more detail within the soil–plant system. Our study has indicated that improving plant Fe nutrition may be of relevance in preventing Cd from entering rice plants. Application of Fe to Cd contaminated soils may have the potential to enhance Fe concentrations while reducing Cd concentrations in rice, and this has important implications for the production of rice with high Fe but low Cd contents for the benefit of human health.

Acknowledgments

This study was supported by the 'Program for Innovative Research Teams in Universities' of the Chinese Ministry of Education (Grant IRT0511) and the British Council (Project DelPHE 1.64). We thank Mr. Shihua Lu of the Institute of Soils and Fertilizers, Sichuan Academy of Agricultural Sciences, for his generous gift of the rice seeds. We also thank two anonymous reviewers whose helpful suggestions have greatly improved the manuscript.

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