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Original Research

How Red Mud-Induced Enhancement of Iron Plaque Formation Reduces Cadmium Accumulation in Rice with Different Radial Oxygen Loss

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Abstract

In situ stabilization of cadmium (Cd) in soil by the addition of Fe-rich amendments (e.g., red mud or RM) has been suggested as an effective and low-cost method. A combined soil-sand pot experiment was conducted to investigate the influence of the addition of RM on iron plaque formation and Cd accumulation in rice plants. Two experiments were conducted:

1. A hydroponic trial with 20 cultivars for screening the rice cultivars with different radial oxygen loss (ROL).
2. A rhizobag trial using the three selected rice cultivars (Zheyou12, Qianyou 1, Chunjiangnuo 2) with different ROL at 2 and 5 mg Cd kg^{-1} exposures amended by three rates of RM application (0, 0.5%, 1%).

The results indicated that the three rice cultivars with different ROLs on average showed better growth performance, less Cd uptake, and more iron (Fe) plaque on root surface and in the rhizosphere under RM treatments. In addition, the rice cultivar with higher ROL tended to have higher Fe plaque and Cd adsorption on the roots and in their rhizosphere with increasing RM additions. These results suggested that rice plants (especially high ROL-ability cultivars) amended by Fe-rich amendments tend to possess a high ability to increase Fe plaque on root surface and in the rhizosphere, as well as decrease Cd uptake and translocate from root to grain.

Keywords: cadmium pollution, paddy soil, remediation, red mud, rice

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Introduction

Increasing cadmium (Cd) accumulation in agricultural soils has become a growing concern globally because of increased fertilizer- and biosolids-borne Cd in soils [1-3]. Rice grown on Cd-contaminated paddy soil in China can accumulate high levels of Cd in shoots and grains [4-6]. In many mildly Cd-contaminated paddy soils, the pressure to produce is so high that farmers cannot afford to fallow paddy soils for remediation. It is therefore urgent to remediate mildly Cd-contaminated paddy soils economically and properly, which could prevent Cd uptake by rice and its transfer to the food chain [1].

A number of wetland plants, including rice, are known to form iron (Fe) plaque on their roots by oxidizing Fe^{2+} to Fe^{3+}, resulting from the radial oxygen loss (ROL) of roots and associated microorganisms [7]. The main constituents of iron plaque are ferric hydroxides, goethite, and lepidocrocite [8]. Recently, it has been discovered that ROL of root has an important effect on As and Cd accumulation by rice [6, 9, 10]. The ROL of plant root and oxidizing capacity are considered the most important biotic factors controlling Fe plaque formation [11]. In addition, ROL of paddy rice oxygenates its rhizosphere [12]. Due to the high adsorption capacity of functional groups on iron hydroxides, Fe plaque on the root surface and in the rhizosphere of wetland plants (including rice) could influence plant uptake of heavy metals to some extent, and may act as a barrier or a buffer to the metals [13-18].

Today, *in situ* remediation techniques of mildly Cd-contaminated soils are considered possible effective approaches to address the issues of excessive plant Cd concentrations. During the last decade, the possibility of Cd immobilization in soils through the addition of different amendments or sorbent has been extensively investigated in order to reduce the risk of groundwater contamination, plant uptake, and exposure to living organisms [19-22]. Among these amendments or sorbents, red mud (RM), a by-product of aluminium (Al) manufacturing, was found to be effective in increasing Cd sorption and decreasing soluble Cd concentrations in Cd-contaminated and acidic soils under pot trials [5, 20, 23-26] and field studies [27-29], and led to a reduction in Cd uptake by plants. Red mud is known to be rich in oxy-hydroxides of Fe, including hematite and goethite [22]. Most recently, some studies have indicated that RM additions can effectively decrease Cd uptake in rice under pot and field trials [30, 31]. However, there are few reports on the interactions between ROL, Cd, RM, and Fe plaque formation in Cd accumulation by rice. Therefore, we hypothesized that RM addition could enhance Fe plaque on root surface and in the rhizosphere, which may further enhance Cd adsorption and reduce Cd accumulation in rice. The major aims of the present study were to investigate variations and correlations in the degree of Fe plaque formation and uptake and distribution of Cd in shoot and root tissues, and in Fe plaque on root surfaces and in the rhizospheres in the presence of RM by rice with different rates of ROL, in order to evaluate RM additions and Fe plaque on Cd accumulation and distribution within the rice plant.

Materials and Methods

Hydroponic Experiment under Deoxygenated Nutrition Conditions

Rice Selected and Cultivation

Seeds of 20 rice (*O. sativa* L.) cultivars were obtained from the Rice Research Institute in Guangdong Province, China. The rice seeds were sterilized in 10% H_2O_2 (v/v) solution for 30 min, followed by thoroughly washing with deionized water and then placed in moist filter paper for five days for germination at 26ºC. After germination, seedlings were subsequently exposed to 0.5 strength Hoagland nutrient solutions [32] for 25 days and then four uniform seedlings for each cultivar were selected and transplanted to PVC pots (7 cm diameter and 15 cm high, one plant per pot) filled with deoxygenated nutrient solution containing 0.1% (w/v) agar, which can mimic the changes in gas composition found in waterlogged soil [9]. The pots were placed in a greenhouse and arranged in a completely randomized design, and the solutions were renewed once every seven days. After 28 days the plants were measured for ROL.

Measuring ROL

ROL rates of root were determined according to the Ti^{3+}-citrate method [9, 33]. All steps of the operation were carried out in a sealed box filled with N_2 gas. After 6 h of reaction, the absorbance of the partly oxidized Ti^{3+}-citrate solution was measured on a Perkin-Elmer 3 UV/VIS spectrophotometer at 527 nm. The released O_2 was monitored by Ti^{3+}-citrate oxidation and calculated from a standard curve.

ROL for one plant can be expressed in the following equations:

$$\text{ROL amount} = c\ (y\text{-}z) \qquad (1)$$

$$\text{Rate of ROL} = c\ (y\text{-}z)\ /\ g \qquad (2)$$

…where ROL amount is the amount of radial oxygen loss per plant (mmol O_2 $plant^{-1}$ h^{-1}), rate of ROL is the rate of radial oxygen loss (mmol O_2 kg^{-1} root d.w. h^{-1}), c is initial volume of Ti^{3+}-citrate added to each tube (L), y is concentration of Ti^{3+}-citrate solution of control (without plants) (μmol Ti^{3+} L^{-1}), z is concentration of Ti^{3+}-citrate solution after 6 h with plants (μmol Ti^{3+} L^{-1}), and g is root dry weight in kg.

Pot Trials

Experimental Design

The soil used in the pot trial was collected from an alluvial loamy paddy field (0-20 cm) located at the Soil Fertility and Fertilizer Efficiency Long-Term Monitoring Base of Qiyang, Hunan Province, China. It was thoroughly mixed, air-dried, and ground to pass a < 2 mm sieve. The physical and chemical properties of the soil were analyzed as follows: pH: 5.31, organic matter 22.6 g kg^{-1}, total N 1.65 g kg^{-1}, total Cd 0.11 mg kg^{-1}, available Cd 0.014 mg kg^{-1}, available Fe 128.7 mg kg^{-1}, and available Mn 25.4 mg kg^{-1}.

An RM (pH = 11.1) sample was collected from the Shandong Aluminum Limited Company (Zibo, Shandong Province, China), which was the residue of the Bayer process for extracting alumina from bauxite. The mineralogical composition of RM sample (XRD analysis) is a mixture of SiO_2 (20%), Fe_2O_3 (28%), Al_2O_3 (21%), CaO (6.2%), MgO (1.3%), TiO_2 (3.3%), K_2O (0.26%), and Na_2O (11%). The specific surface area, determined by the BET/N_2-adsorption method (Sorptomatic CarloErba), was 12.2 m^2g^{-1} for RM. The RM sample was dried overnight at 105ºC, finely ground, and sieved to <1 mm.

There were three treatments of RM (0, 0.5%, and 1%) (w/w) combined with the 2 and 5 mg Cd kg^{-1} exposure. The Cd-contaminated soils were prepared by spiking the soils with Cd (2 and 5 mg kg^{-1} as $CdCl_2$), then mixing thoroughly and equilibrating for two months. During equilibration, soil moisture was maintained at 70% of maximum water holding capacity by weighing. After two months the soil samples were air-dried and passed through a 2-mm sieve, and then the air-dried soil and RM were used in each pot for the experiments.

Based on our early pilot studies, three rice cultivars with diverse rates of ROL were used in this experiment, and they are Zheyou12 (ZY12, low ROL ability), Qianyou 1 (QY1, moderate ROL ability), and Chunjiangnuo 2 (CJN2, high ROL ability). The treatments were employed with three levels of Cd (as $CdCl_2$) [without Cd (Cd0), 2 mg Cd kg^{-1} (Cd2), and 5 mg Cd kg^{-1} (Cd5)] combined with three levels of RM [RM0, RM0.5% (w/w), RM1%(w/w)]. Each treatment was replicated four times. Rice seeds were sterilized in 10% H_2O_2 (v/v) solution for 30 min, followed by thoroughly washing with deionized water. In each pot, two uniform germinated seeds were transplanted into each rhizobag and grown for 120 days. Soil moisture content was brought up to 100% of water holding capacity before seedling emergence, and then kept submerged with deionized water for the whole growth period. Eighty-four pots were arranged randomly in the greenhouse with a relative humidity of 85% and light/dark cycle of 14 h day/10 h night.

A rhizobag system with a soil-sand combination was used to collect rhizosphere and non-rhizosphere soils separately and to study the effect of RM-induced transformation of iron plaque on Cd uptake by rice. The rhizobags were made of nylon net with a mesh size of 40 μm. Bag size was 12 cm in diameter and 10 cm in height. In total, 1.5 kg dried soil was placed in each pot. Soil in rhizobags received (per kg) a basal application of 100 mg P as KH_2PO_4, 125 mg K as KH_2PO_4, and 110 mg N as urea. The fertilizers were added to the soil as solution and mixed thoroughly before potting. Cadmium and RM were added to the soil as solution and mixed thoroughly with the soil. Each rhizobag, filled with 300 g washed quartz sand, was placed in the centre of each pot (15 cm diameter, 17 cm height), and the gap between the rhizobag and the pot was filled with 1.5 kg soil for top-layer surface level inside and outside the rhizosphere [17]. Soils were balanced at 100% of the water-holding capacity by the weighing method for two weeks.

Plant height was measured before harvesting. Rice plants were harvested by carefully moving the rhizobags out of the pots. Roots were separated from the quartz sand, and plants were divided into two parts as roots and shoots for determining biomass, concentrations of Cd in root and shoot tissues, and concentrations of Cd, Fe, and Mn on root surface. The quartz sand inside the rhizobags was referred to as the rhizosphere material. The soils in the pots were homogenized thoroughly, and further referred to as the non-rhizosphere soils [17].

Extraction of Iron Plaque

Cadmium, Fe, and Mn in Fe plaque on root surfaces and in the rhizosphere were extracted by dithionite-citrate-bicarbonate (DCB) method [14]. The quartz sand collected from the rhizosphere was extracted by DCB-extraction [17]. Rice roots or quartz sand were respectively immersed in 45 mL DCB solution, and then shaken at 280 rpm for 3 h at 25ºC. They were filtered with quantitative filter papers to 100 mL volumetric flasks and rinsed four times, then diluted to volume with deionized water. The concentrations of Cd, Fe, and Mn remaining in root tissues were determined after washing. The washed roots were then dried to constant weight at 60ºC and digested.

Chemical Analysis

Soil pH was measured in the soil: water ratio of 1:2.5 (v/v). Soil organic C was analyzed by potassium dichromate (K_2CrO_7) and total N by the Kjeldahl method [34]. To determine total Cd content the soil samples were digested in 4 mL of "aqua-regia" (HNO_3/HCl = 1/3, v/v) [35] and determined by inductively coupled plasma optical emission spectrometry (ICP-MS, Elan 5000, Perkin Elmer, USA). Available Cd, Fe, and Mn in soils were extracted by diethylenetriamine pentaacetic acid (DTPA: 0.005 M DTPA, 0.1 M triethanolamine and 0.01 M $CaCl_2$ at pH 7.3) in a soil:solution ratio of 1:2 (v/v) [36] and determined by ICP-MS for Cd and inductively coupled plasma optical emission spectromety (ICP-OES, Optima 2100, Perkin Elmer, USA) for Fe and Mn.

Oven-dried shoot and root samples were ground using a Retsch-grinder (Type: 2 mm, made in Germany), and digested with HNO_3 [37]. Samples of plant (0.2 g) were weighed into 100 mL block digestion tubes, and concentrated nitric acid (10 mL) was added and allowed to stand overnight. They were then heated for 3 hr at 60ºC, followed by 6 hr at 110ºC. After cooling, the digests were passed through a pre-washed filter (Whatman No. 540), the digestion tubes were rinsed four times, passing through the filter and the filtrates made up to 50 mL volume using ultra-pure water. Blank and bush leaf material (BGW-07603) (China Standard Materials Research Center, Beijing, PR China) were used for quality control.

In the plant digestion solution, DCB extracts and Cd concentrations were determined using ICP-MS, and Fe and Mn concentrations were determined using ICP-OES. The recovery rates of Cd, Fe, and Mn were 90 ±10%.

Data Analysis

Total Cd in iron plaque of the root surface ($T_{DCB–Cd}$) or rice tissue (root, straw, and grain) ($T_{Rice\ tissue\text{-}Cd}$) were calculated by multiplying Cd concentration in iron plaque or rice tissue by rice tissue weight. The total Cd amount (T_{Cd}) was calculated as the sum of $T_{DCB–Cd}$ and $\sum T_{Rice\ tissue\text{-}Cd}$. Therefore, Cd distribution in iron plaque or rice tissue was calculated by dividing $T_{DCB–Cd}$ or $T_{Rice\ tissue\text{-}Cd}$ by TCd.

All results were presented as arithmetic means with standard errors and analyzed using the SPSS 11.0 statistical package. Data on plant performance were tested for their normality and variance prior to a one-way analysis of variance (ANOVA). No data transformation was required. If the differences between RM treatments for each rice cultivar were significant at the 5% level, the least significant difference (LSD) was calculated as a *post hoc.* Correlation coefficient analyses were conducted using program of Origin 7.0.

Results

ROL Rate

ROL rates for the 20 rice cultivars were measured after being grown in 0.1% agar nutrient solutions for 28 days (Fig. 1). The results showed that the rates of ROL of rice varied due to genetic differences among the cultivars. ROL values ranged from 14.89 (Youyou 998) to 30.57 (Chunjiangnuo 2) mmo1 O_2 kg^{-1} root d.w. h^{-1} (average 22.46 mmo1 O_2 kg^{-1} root d.w. h^{-1}).

Effects of Red Mud on Rice Growth

In general, the height, root, straw and grain tissue of dry weight of three rice cultivars (ZY12, QY1, CJN2) in the RM0.5 and RM1 treatments were significantly higher than those in the control (Table 1). For example, straw increment (in terms of % of the control) of the three rice cultivars varied from 32.6% (CJN2) to 48.5% (ZY12) in the RM0.5% treatment, and from 34.9% (ZY12) to 50.0% (CJN2) in the RM1% treatment at the 2 mg Cd kg^{-1} exposure level, indicating that the biomass increment was more significant as RM addition increased.

Cadmium Concentrations in Rice Tissues

In the Cd-polluted soils without RM treatments, among the three cultivars (ZY12, QY1, CJN2), CJN2 showed the lowest Cd concentrations in root, straw, and grain, and ZY12 the highest ($P < 0.05$, Table 2). When RM was added to soils, the concentrations of Cd in root, straw, and grain of the three rice cultivars decreased significantly ($P < 0.05$) in the RM treatments. For example, the degree of reduction in root, straw, and grain for the three rice cultivars ranged from 20.9% to 31.1%, 23.8% to 42.6%,

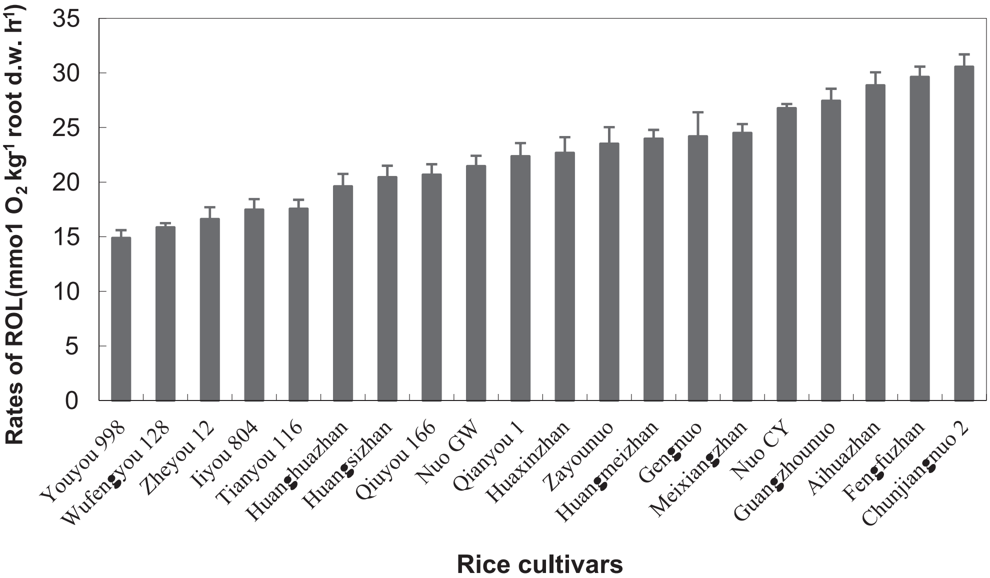

Fig. 1. Rate of ROL (mmo1 O_2 kg^{-1} root d.w. h^{-1}) of the 20 rice cultivars exposed to 0.1% agar with 1/2 strength Hoagland solution for 28 days (mean ±SE, n = 4).

Table 1. Biomass (g plant^{-1}) of root, straw, and grain, and height (cm) of the three rice cultivars (ZY12, QY1, CJN2) in different RM treatments under the 2 and 5 mg Cd kg^{-1} exposures (mean ±SE, n = 4).

Treatment			Height	Root wt.	Straw wt.	Grain wt.
Cd level (mg kg)	RM level (W/W, %)	Cultivars				
0	0	ZY12	52.6±2.33a	0.89±0.06b	2.86±0.22a	1.21±0.04c
		QY1	50.1±0.83a	0.99±0.07b	3.11±0.04a	1.33±0.02b
		CJN2	54.7±0.57a	1.21±0.03a	3.19±0.05a	1.54±0.02a
2	0	ZY12	53.9±3.29de	0.83±0.05e	2.35±0.11g	0.85±0.07h
		QY1	52.2±0.71e	1.14±0.03cd	3.19±0.05f	1.24±0.02g
		CJN2	55.1±0.75cde	1.19±0.02c	3.24±0.03f	1.59±0.03e
	0.5	ZY12	58.9±1.38bc	1.18±0.02c	3.49±0.09e	1.97±0.05d
		QY1	60.3±0.40bc	1.21±0.03c	4.23±0.03d	2.39±0.05c
		CJN2	67.8±0.61a	1.42±0.03b	4.67±0.04b	2.78±0.04b
	1	ZY12	57.4±2.48cd	1.09±0.03d	3.17±0.05f	2.14±0.04d
		QY1	62.7±0.55b	1.34±0.05b	4.41±0.04c	2.48±0.11c
		CJN2	68.5±0.85a	1.58±0.03a	4.86±0.03a	2.97±0.04a
5	0	ZY12	47.6±0.47e	0.74±0.01e	2.14±0.04h	0.51±0.03j
		QY1	49.7±0.47d	0.81±0.03e	2.94±0.07g	0.94±0.07h
		CJN2	51.6±0.47cd	1.06±0.02d	3.08±0.03f	1.34±0.04g
	0.5	ZY12	51.1±0.72cd	1.24±0.03c	3.21±0.05f	1.61±0.03f
		QY1	57.4±0.53c	1.23±0.03c	3.99±0.06d	2.14±0.03d
		CJN2	64.3±0.75b	1.39±0.06b	4.21±0.05b	2.54±0.04b
	1	ZY12	52.7±1.17c	1.18±0.04c	3.35±0.03e	1.84±0.03e
		QY1	63.8±0.63b	1.27±0.05c	4.17±0.04c	2.37±0.04c
		CJN2	68.4±0.40a	1.51±0.03a	4.41±0.04a	2.72±0.05a

Note: Different letters after means within the three rice cultivars (ZY12, QY1, CJN2) under the same Cd treatment indicate significant differences in height or biomass between the treatments (CK, RM0.5%, RM1%) at $P < 0.05$ according to one-way ANOVA followed by LSD tests.

and 45.5% to 61.1% in the RM0.5% treatment, and the matching values in root, straw, and grain were from 40.1% to 49.8%, 36.9% to 61.1%, and 66.7% to 77.8% in the RM1% treatment at the 2 mg Cd kg^{-1} exposure, respectively (Table 2).

Concentrations of Cd, Fe, and Mn on Root Surfaces (Plaque on Root) and on Sand Surfaces (Plaque in Rhizosphere)

The concentrations of Cd, Fe, and Mn on root surfaces and on sand surfaces of the three rice plants tested significantly increased with increasing RM treatments (Table 3). The degrees of increment of Cd, Fe, and Mn concentrations on root surfaces were significantly different ($P < 0.01$) between the rice cultivars tested, ranging from 30.1% to 40.9%, 56.9% to 107%, and 13.7% to 50.9% in the RM-amended soils. The equivalent ranges on sand surface in the RM treatments were 31.4% to 123%, 67.1% to 174%, and 19.1% to 63.2%, respectively. Iron concentrations were also higher than for Cd or Mn on both root and sand surfaces.

Cadmium Distribution in Rice Tissues and Root Plaque

In the Cd-polluted soils without RM treatments, most Cd of the three rice cultivars (ZY12, QY1, CJN2) tested was mainly distributed in the root tissues, followed by straw, Fe plaque, and grain (Table 4). Under the RM treatments most Cd was distributed in the Fe plaque, followed by straw, root tissues, and grain. Among the three rice cultivars, CJN2 showed the highest Cd distribution in Fe plaque, and ZY12 the lowest under the RM treatments.

pH and DTPA-Cd Concentrations of Non-Rhizosphere Soil

The pH value in non-rhizosphere of the three rice plants tested significantly ($P < 0.05$) increased with the

Table 2. Concentrations of Cd (mg kg^{-1}) in grain, straw, and roots of the three rice cultivars (ZY12, QY1, CJN2) in different RM treatments under the 2 and 5 mg Cd kg^{-1} exposures (mean ±SE, n = 4).

Treatment			Root Cd	Straw Cd.	Grain Cd
Cd level (mg kg)	RM level (W/W,%)	Cultivars			
0	0	ZY12	0.21±0.02a	0.13±0.02a	BDL
		QY1	0.14±0.02b	0.11±0.01ab	BDL
		CJN2	0.13±0.01b	0.08±0.01b	BDL
2	0	ZY12	0.92±0.03a	0.84±0.041a	0.33±0.01a
		QY1	0.81±0.02b	0.67±0.05b	0.27±0.02b
		CJN2	0.61±0.03de	0.54±0.02c	0.18±0.01c
	0.5	ZY12	0.72±0.03c	0.64±0.03b	0.18±0.01c
		QY1	0.64±0.02d	0.47±0.02c	0.12±0.01d
		CJN2	0.42±0.02f	0.31±0.01d	0.07±0.01gh
	1	ZY12	0.55±0.04e	0.43±0.04c	0.11±0.01df
		QY1	0.41±0.01fg	0.32±0.02d	0.08±0.004fg
		CJN2	0.34±0.01g	0.21±0.02e	0.04±0.004h
5	0	ZY12	5.31±0.03a	2.64±0.04a	0.69±0.04a
		QY1	4.97±0.05b	2.51±0.03b	0.64±0.02a
		CJN2	4.92±0.05b	2.44±0.03b	0.46±0.02b
	0.5	ZY12	4.23±0.03c	2.11±0.01c	0.41±0.02b
		QY1	3.91±0.02d	1.95±0.03d	0.32±0.01c
		CJN2	3.61±0.04e	1.74±0.02e	0.26±0.01d
	1	ZY12	3.87±0.06d	1.74±0.02e	0.34±0.01c
		QY1	3.51±0.02e	1.67±0.04e	0.19±0.01e
		CJN2	3.01±0.03f	1.26±0.02f	0.14±0.01e

Note: Different letters after means within the three rice cultivars (ZY12, QY1, CJN2) under the same Cd treatment indicate significant differences in Cd concentrations of grain, straw, or roots between the RM treatments (CK, RM0.5%, RM1%) at $P < 0.05$ according to one-way ANOVA followed by LSD tests.

increasing RM treatments, in a range of 5.71-5.79 pH units in the RM0.5% treatment and 5.83-5.95 pH units in the RM1% treatment (Table 5). DTPA-Cd concentrations of non-rhizosphere of the rice plants decreased with increasing RM treatments. The decrease ranged from 0.021 mg kg^{-1} to 0.207 mg kg^{-1} in the RM treatments under both Cd exposures (Table 5).

Correlations between Concentrations of Cd in Straw, Grain, and the Concentrations of Cd, Fe, and Mn on Root and Sand Surfaces

Significant correlations were apparent between concentrations of Cd in straw and grain, and the concentrations of Cd, Fe, and Mn on root and sand surfaces in the RM treatments (Table 6). Positive correlations were found between the Cd concentrations in root and the concentrations of Fe and Mn on root surfaces and on sand surfaces in the RM treatments. Negative correlations were suggested between the Cd concentrations in grain and the concentrations of Fe and Mn on root surfaces and on sand surfaces in the RM treatments. Positive correlations were also found between Cd and Fe and Mn concentrations on root surfaces and on sand surfaces in the RM treatments.

Discussion

Plant Growth and Fe Formation under Different RM Additions by Rice with Different ROLs

In this study, 5 mg Cd kg^{-1} exposure significantly ($P < 0.05$) decreased root, shoot, and grain weight of the three rice cultivars (ZY12, QY1, CJN2) compared without RM treatments. When the three rice cultivars were cultivated in Cd-treated soils, RM addition exhibited an advantageous effect on rice growth (Table 1). This finding was in good agreement with the results obtained from rice and other plants [29-33] reported that RM treatments resulted in shoot biomass in cucumber being about two-fold greater than RM addition. The results indicated that RM could alleviate Cd stress on rice.

RM additions may alleviate the influence of Cd on rice growth in two ways. Firstly, RM additions provided some

Table 3. The concentrations of Fe, Mn, and Cd in plaque on root surface (Plaque$_{root}$) and plaque in rhizosphere (Plaque$_{rhizo}$) of the three rice cultivars (ZY12, QY1, CJN2) in different RM treatments under the 2 and 5 mg Cd kg^{-1} exposures (mean ±SE, n = 4).

Treatment			Root surface			Rhizosphere		
Cd level (mg kg)	RM level (w/w, %)	Cultivars	Fe (g kg^{-1})	Mn (mg kg^{-1})	Cd (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cd (µg kg^{-1})
0	0	ZY12	26.32±1.83b	94.32±3.24c	0.39±0.03c	14.29±0.77c	1.36±0.15b	1.23±0.10a
		QY1	29.37±1.05b	102.63±1.46b	0.56±0.02b	21.3±0.91b	1.42±0.03b	1.32±0.04a
		CJN2	34.35±1.36a	112.34±2.05a	0.74±0.02a	27.4±0.98a	1.74±0.07a	1.31±0.03a
2	0	ZY12	35.64±1.99g	110.21±4.92f	1.13±0.04f	26.90±3.18g	1.54±0.14f	6.78±0.19e
		QY1	39.41±1.60g	117.61±1.54ef	1.14±0.03f	38.90±0.73f	1.57±0.02f	8.23±0.05de
		CJN2	48.64±1.26f	121.48±3.82e	1.34±0.02e	48.63±1.07e	1.88±0.05e	12.89±0.45c
	0.5	ZY12	58.67±3.84e	125.48±4.52de	1.47±0.04e	55.48±2.96e	1.95±0.11de	8.91±0.19d
		QY1	66.97±1.42d	133.74±2.15cd	1.88±0.07d	67.10±1.44d	1.99±0.06cde	13.28±0.32c
		CJN2	76.31±1.50c	141.31±4.94bc	2.24±0.04c	81.24±1.79c	2.24±0.03bc	21.69±0.53b
	1	ZY12	79.34±5.59c	144.82±5.02b	1.92±0.05d	82.37±4.48c	2.19±0.20bcd	14.30±1.05c
		QY1	89.34±1.50b	151.23±1.94b	2.41±0.06b	99.21±1.48b	2.31±0.05b	20.39±0.89b
		CJN2	99.21±1.98a	168.74±2.35a	2.97±0.04a	124.9±1.78a	2.99±0.07a	39.39±1.40a
5	0	ZY12	43.36±0.44h	114.39±2.01f	3.04±0.05h	36.91±1.45j	1.74±0.05j	13.91±0.29f
		QY1	49.61±1.20g	124.39±1.66e	3.36±0.04g	46.90±1.62h	1.88±0.05f	17.67±1.14e
		CJN2	54.32±1.08f	129.67±2.18e	3.67±0.05f	53.21±1.01g	2.09±0.03e	26.84±1.23c
	0.5	ZY12	71.32±0.81e	139.67±1.97d	3.84±0.04e	71.54±1.31f	2.09±0.04e	16.32±0.39ef
		QY1	80.23±1.65d	143.58±1.37d	4.21±0.05d	88.64±1.04e	2.28±0.03d	26.97±0.60c
		CJN2	88.91±1.42c	156.45±5.09c	4.81±0.06b	123.64±2.28b	2.67±0.05b	41.57±0.84b
	1	ZY12	89.54±0.87c	164.35±2.25c	4.11±0.03d	99.37±2.09d	2.31±0.03d	21.67±0.45d
		QY1	94.51±1.58b	178.63±3.51b	4.56±0.04c	116.34±1.25c	2.51±0.05c	37.58±1.17c
		CJN2	112.21±1.19a	195.64±6.29a	5.17±0.03a	145.68±2.62a	3.41±0.03a	59.81±1.51a

Note: Different letters after means within the three rice cultivars (ZY12, QY1, CJN2) under the same Cd treatment indicate significant differences in Fe, Mn, and Cd concentrations of root surfaces or rhizosphere between the RM treatments (CK, RM0.5%, RM1%) at $P < 0.05$ according to one-way ANOVA followed by LSD tests.

Table 4. Distribution (%) of Cd in iron plaque on root surface, grain, straw, and roots of the three rice cultivars (ZY12, QY1, CJN2) in different RM treatments under the 2 and 5 mg Cd kg^{-1} exposures (mean ±SE, n = 4).

Treatment			Grain	Straw	Root	Root plaque
Cd level (mg kg)	RM level (w/w, %)	Cultivars				
0	0	ZY12	BDL	40.79±3.39a	20.98±2.55a	38.24±1.59c
		QY1	BDL	33.14±2.17a	13.40±1.59b	53.46±0.72b
		CJN2	BDL	21.22±1.37b	11.68±0.82b	67.10±1.89a
2	0	ZY12	5.51±0.45ab	39.11±2.94a	36.99±2.03a	18.40±0.99g
		QY1	5.53±0.42ab	35.24±2.02ab	37.74±1.28a	21.49±0.77f
		CJN2	5.28±0.40ab	32.26±2.85b	33.08±1.59b	29.38±0.27e
	0.5	ZY12	5.56±0.57a	34.83±1.49ab	32.63±1.04b	26.98±0.76e
		QY1	4.44±0.28b	30.75±0.96b	29.64±0.54bc	35.18±1.31d
		CJN2	3.09±0.39c	23.03±1.25c	23.39±1.52de	50.49±0.86b
	1	ZY12	4.32±0.31b	30.63±1.83b	26.82±0.98cd	38.24±1.48c
		QY1	3.21±0.22c	22.77±0.65c	21.91±0.55ef	52.11±0.66b
		CJN2	1.66±0.15d	14.23±1.16d	18.52±0.32f	65.59±1.07a
5	0	ZY12	2.90±0.28cd	46.37±0.24b	32.27±0.37a	18.46±0.20f
		QY1	4.12±0.46a	50.09±1.32a	27.33±1.04de	18.46±0.54f
		CJN2	3.57±0.12abc	43.61±0.28c	30.25±0.31bc	22.57±0.32e
	0.5	ZY12	3.78±0.15ab	38.86±0.94de	30.32±0.31b	27.29±0.46d
		QY1	3.72±0.20ab	42.17±0.62c	26.06±0.46ef	28.05±0.45d
		CJN2	3.35±0.14bc	37.28±0.89de	25.46±0.46f	33.91±0.40b
	1	ZY12	3.95±0.20ab	36.76±0.88e	28.75±0.51cd	30.54±0.54c
		QY1	2.56±0.17de	39.45±0.85d	25.22±0.38f	32.77±0.66b
		CJN2	2.08±0.27e	30.41±0.81f	24.84±0.27f	42.67±0.52a

Note: Different letters after means within the three rice cultivars (ZY12, QY1, CJN2) under the same Cd treatment indicate significant differences in Cd distribution of grain, straw, root, or plaque between the RM treatments (CK, RM0.5%, RM1%) at $P < 0.05$ according to one-way ANOVA followed by LSD tests.

nutrients for plant growth and thus increased biomass of plants. In this study, RM0.5 and RM1 treatments caused obvious increase in root, shoot, and grain biomasses in the rice cultivars with Cd stress (Table 1). Moreover, an increase in biomass induced by RM additions, especially of leaves, will increase photosynthesis and consequently lead to increases in the ROL from the entire roots of rice grown in the Cd-polluted soils. Secondly, the application of RM decreased Cd uptake into rice by decreasing Cd availability in soil and increment of Fe plaque (Table 6). In the present study, rice roots appeared reddish with RM applications (RM0.5 and RM1) at 2 and 5 mg kg^{-1} Cd exposure, which indicated that RM addition resulted in increments of Fe^{2+} concentrations under Cd exposure, which increased Fe plaque on their roots by oxidizing Fe^{2+} to Fe^{3+} (Mendelssohn et al., 1995). The Fe plaque on roots of rice when supplied with RM followed the order of CJN2 > QY1 > ZY12, suggesting that increasing RM additions could induce more Fe plaque formation on root surfaces of rice with higher rates of ROL. Furthermore, the rice cultivar (CJN2) with the highest rates of ROL had the growth performance and Fe plaque in the rhizosphere under the same RM treatment by the two Cd exposures, which indicated that ROL and RM application had a synergistic effect on rice plant growth and Fe plaque formation under Cd stress.

Cadmium Accumulation and Uptake in Plants under RM Additions by Rice with Different ROLs

Previous studies have shown that the addition of RM could effectively reduce Cd uptake and accumulation in plants [20, 23-25, 27-29, 33] The present study also shows that a remarkable decrease in Cd concentrations can be observed in the straw and grain of the three rice cultivars (ZY12, QY1, CJN2) treated with increasing rates of RM application compared with unamended soil at 2 and 5 mg kg^{-1} Cd exposure (Table 2). This result strongly suggests that the application of RM could significantly decrease Cd uptake by rice.

Table 5. Concentrations of DTPA-extractable Cd (mg kg^{-1}) and pH in the non-rhizosphere of the three rice cultivars (ZY12, QY1, CJN2) in different RM treatments under the 2 and 5 mg Cd kg^{-1} exposures (mean ±SE, n = 4).

Treatment			pH	DTPA-extractable Cd
Cd level (mg kg)	RM level (w/w, %)	Cultivars		
0	0	ZY12	5.54±0.02a	0.029±0.001a
		QY1	5.57±0.02a	0.024±0.001a
		CJN2	5.49±0.01b	0.027±0.002a
2	0	ZY12	5.62±0.01d	0.196±0.002ab
		QY1	5.64±0.01d	0.201±0.006a
		CJN2	5.52±0.01e	0.188±0.005b
	0.5	ZY12	5.71±0.01c	0.161±0.006c
		QY1	5.74±0.01c	0.167±0.006c
		CJN2	5.71±0.01c	0.167±0.003c
	1	ZY12	5.95±0.01a	0.102±0.004d
		QY1	5.88±0.02b	0.112±0.002d
		CJN2	5.92±0.01ab	0.106±0.004d
5	0	ZY12	5.67±0.04d	0.426±0.005a
		QY1	5.67±0.06d	0.433±0.004a
		CJN2	5.58±0.02e	0.401±0.006b
	0.5	ZY12	5.74±0.02c	0.314±0.004c
		QY1	5.77±0.03bc	0.324±0.004c
		CJN2	5.79±0.02abc	0.317±0.006c
	1	ZY12	5.87±0.02a	0.219±0.006e
		QY1	5.83±0.02abc	0.237±0.005d
		CJN2	5.86±0.02ab	0.221±0.004e

Note: Different letters after means within the three rice cultivars (ZY12, QY1, CJN2) under the same Cd treatment indicate significant differences in pH and DTPA-Cd concentrations of non-rhizosphere between the RM treatments (CK, RM0.5%, RM1%) at $P < 0.05$ according to one-way ANOVA followed by LSD tests.

Table 6. Correlation among concentrations of Cd in root, straw, grain, and DCB-extractable Fe, Mn, and Cd on root surface and in the rhizosphere of rice (n = 84).

	Cd_{root}	Cd_{straw}	Cd_{grain}	$Cd_{root\ surface}$	$Fe_{root\ surface}$	$Mn_{root\ surface}$	$Cd_{rhizosphere}$	$Fe_{rhizosphere}$	$Mn_{rhizosphere}$
Cd_{root}	1	0.988**	0.84**	0.773**	0.221*	0.268*	0.418**	0.226*	0.233*
Cd_{straw}		1	0.895**	0.717**	0.158	0.198	0.348**	0.158	0.160
Cd_{grain}			1	0.426**	-0.105	-0.073	0.068	-0.119	-0.101
$Cd_{root\ surface}$				1	0.760**	0.774**	0.854**	0.780**	0.744**
$Fe_{root\ surface}$					1	0.937**	0.841**	0.968**	0.883**
$Mn_{root\ surface}$						1	0.868**	0.945**	0.896**
$Cd_{rhizosphere}$							1	0.897**	0.906**
$Fe_{rhizosphere}$								1	0.927**
$Mn_{rhizosphere}$									1

Note: ** $P < 0.01$, * $P < 0.05$.

Results from the present study indicate that Cd concentrations and uptake of the rice cultivar with higher rates of ROL (CJN2) were significantly lower than those with lower ROL (ZY12) (Table 6). In the present study, the application of RM (0.5%, w/w,) and RM (1%, w/w,) to the soil had no obvious increase on soil pH (Table 5), which indicated that the slight increase (0.09-0.40) in pH value was not the prime cause. Furthermore, results from the present study show that the decrease of DTPA-Cd concentrations of soils treated with RMs ranged 0.021 mg kg^{-1} to 0.207 mg kg^{-1} under both Cd exposures, which suggested that the decrease in Cd availability was also not the prime cause. Therefore, the prime cause was the increment of formation of iron plaque on the root surface and in the rhizosphere induced by the application of red mud (Table 3). Root surfaces and rhizosphere material (sand) of the rice appeared more reddish with the application of red mud. A significant positive correlation between red mud additions and DCB-extractable Fe/Mn on iron plaque of roots and in the rhizosphere of the studied rice was demonstrated. These results suggested that RM addition could induce the formation of iron-manganese oxides plaque on the root surface and on the rhizosphere sand. RM-induced enhancement of plaque formation is probably due to an increase in concentrations of sorbed Fe in the rhizosphere with RM addition. In addition, in the present study negative correlations were suggested between the Cd concentrations in grain and the concentrations of Fe and Mn on root surfaces and on sand surfaces in the RM treatments (Table 6). And our study showed that the concentrations of Cd on root surfaces were significantly correlated to Fe ($P < 0.01$) and Mn ($P < 0.01$) concentrations on root surfaces when the rice were grown in soils amended with RM. Similarly, a positive correlation was also observed between Cd in rhizosphere and Fe ($P < 0.01$) or Mn ($P < 0.01$) concentrations in the rhizosphere in Table 6. These results suggested that the enhancement in the formation of Fe and Mn plaques on rice can increase the Cd adsorption on root surfaces and in the rhizosphere. Compared to terrestrial plants, wetland plants such as rice could release more oxygen to the rhizosphere and tend to form more iron plaque because of oxidizing Fe(II) to Fe(III) on their root surfaces and in rhizosphere and prevent toxic oxidative zone [13].

It is believed that metal concentrations on root surfaces are related to sediment geochemistry (concentrations of labile metal) [7], and that the Fe and Mn oxides on root surfaces are considered a consequence of oxidizing activity of roots due to radial oxygen loss (ROL) of root [38]. Due to the fact that concentrations of Cd on root surfaces and in the rhizosphere were positively correlated to Fe and Mn as indicated above, the enhancement in the formation of Fe plaque by the red mud addition would increase the concentrations of Cd, Fe, and Mn on root surfaces and in the rhizosphere. Therefore, the rice cultivar with higher Fe plaque (e.g. CNJ2) increased Cd adsorption and decreased Cd uptake into roots and translocation to shoots in rice. [5] found that the rice cultivar, having the highest ROL among 25 rice plants studied, forms the highest extent of Fe plaque on root surfaces and immobilizes the highest Cd concentration in Fe plaque. This phenomenon had also been observed for other metals such As and Pb [6, 9, 18]. Furthermore, some results indicated that Fe plaque in the rhizosphere may have a more important role than that on root surfaces [33, 39]. In addition, [40] showed that redox potential (Eh) varied temporally and spatially in the rhizosphere soil of rice, which could lead to change the Fe plaque formation, concurrent with Fe and Mn oxidation in the rhizosphere. Therefore, more in-depth studies on the roles of Fe plaque both in the rhizosphere and on root surfaces of rice plants are still clearly needed.

In summary, our results indicated that the overall effect of RM on Cd uptake into rice plants have been mainly caused by the enhanced formation of Fe plaque by the RM addition. In addition, changes of pH and (Eh) in rhizosphere soils caused by root activities (e.g. ROL) of rice plants may be temporal and spatial, thus more in-depth studies on the effect of RM on changes of Cd fractions are clearly needed both in rhizosphere soils and bulk soils in the future.

Conclusions

These results obtained from the current study demonstrated that the increasing RM additions could significantly decrease Cd uptake by rice with different ROLs. In addition, the rice cultivar with higher ROL tended to have higher Fe plaque and Cd adsorption on the roots and in their rhizosphere with increasing RM additions. The principal mechanism could be due to RM-induced enhancement of the formation of iron plaque on root surface and in the rhizosphere. It is envisaged that RM-induced enhancement of the formation of iron plaque on the root surface and in the rhizosphere of rice – especially high ROL-ability cultivars – may be significant for the development of practical approaches to reducing Cd accumulation in rice.

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