



Iron fractions responsible for the variation of Cd bioavailability in paddy soil under variable pe+pH conditions

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HIGHLIGHTS

- Low pe + pH conditions facilitated the transformation of crystalline iron oxides into amorphous forms in soil.
- The increase of amorphous Fe promoted the immobilization of Cd in rice rhizosphere soil.
- The formation of iron plaque at low pe + pH levels was beneficial for Cd attachment on root surfaces.
- Different iron fractions were responsible for the variation of soil Cd bioavailability under variable pe + pH conditions.

ARTICLE INFO

Article history:

Received 2 December 2019
Received in revised form
13 February 2020
Accepted 25 February 2020
Available online 26 February 2020

Handling Editor: Lena Q. Ma

Keywords:

Cd availability
Amorphous iron oxides
pe+pH
Iron plaque
Rice

ABSTRACT

Iron (Fe) in soil is closely related to cadmium (Cd) uptake by rice plants, and soil pe + pH significantly influences Fe redox behavior. This study aimed to explore the influential mechanisms of varying pe + pH conditions on the transformation of iron oxides in the rhizosphere and the subsequent effect on Cd accumulation in rice plants. A two-month pot experiment was conducted to investigate the effect of soil pe + pH on the fractions of iron oxides and formation of iron plaque (IP), as well as the effect of these changes on Cd uptake by rice plants (*Oryza sativa* L.). Different irrigation strategies, 70% water holding capacity (DY), continuous flooding (FL), and alternate flooding/drying weekly (AWD), were used to achieve various soil pe + pH levels. The results showed that low pe + pH conditions (under the FL and AWD treatments) were more beneficial to the transformation of crystalline iron oxides into amorphous forms in rhizosphere soil and the precipitation of IP on rice roots. The increase of amorphous iron oxides resulted in the reduction of Cd availability in rhizosphere soil by immobilizing more Cd on Fe oxides. Moreover, Cd adsorbed on rice root surfaces reacted with IP, inhibiting Cd soil-to-root transport. The two mechanisms combinatively functioned at decreasing Cd concentration in rice shoots by 14.1–33.1% at low pe + pH conditions compared to that of the high pe + pH (DY treatment). These results indicate that lowering soil pe + pH effectively reduced Cd accumulation in rice plants, probably through the immobilization of amorphous Fe oxides on Cd and sequestration of iron-plaque on Cd.

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1. Introduction

Rice is an important food source for more than 1.3 billion Chinese people (Chen et al., 2017). In recent decades, paddy soils in

parts of south China have been subjected to Cd contamination, originating from anthropogenic activities such as mining, sewage irrigation, metal processing and so on (Yin et al., 2017). The efficient accumulation of Cd in rice grains has become an urgent problem for food safety in China because the dietary intake of rice is regarded as a major source of elevated Cd levels in the human body (Qiao et al., 2018).

Iron in soil decreases Cd uptake by rice plants (He et al., 2017; Wei et al., 2018). Two main mechanisms may explain this phenomenon. One explanation involves the adsorption and inner-sphere complexation of Cd on iron oxides (Muehe et al., 2013).

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The high specific surface area and abundant functional groups (such as hydroxyl groups) on the surface of iron oxides provide many sorption sites to bind Cd (Tessier et al., 1996). The iron oxides can reduce the bioavailability of Cd in soil by converting Cd from available Cd to Fe/Mn oxide-bonded Cd (Li et al., 2019). The biochemical and physical processes associated with microbial Fe redox, are accompanied by the production of secondary iron minerals (e.g. magnetite and goethite), which also play an important role in Cd immobilization (Zhu et al., 2014; Li et al., 2016). Besides, the iron plaque (IP) is deposited on a root surface, mainly in the form of ferrihydrite and goethite (Amaral et al., 2017; Hu et al., 2019). Cd adsorption/co-precipitation on IP is a physical barrier for Cd uptake by rice roots (Lux et al., 2011). Another mechanism should be that Cd can enter a rice plant through the Fe-uptake pathway, thus Fe competes with Cd for transporters (such as *OsNRAMP5*, *OsIRT1*) and reduces potential Cd uptake and translocation in rice (Takahashi et al., 2011).

Iron exists mainly in the form of iron (hydr)oxides in soil; the common types include goethite and hydrated iron oxide. However, environmental factors (such as pH and Eh) can cause phase transformation as well as changes in crystallinity and morphology of iron oxides in soil (Yu et al., 2016; Li et al., 2019). Previous studies have indicated that the rise of pH decreases the solubility of Fe(III) and stability of Fe(II); a high Eh is beneficial for Fe(II) oxidization, while a low Eh promotes Fe(III) reduction (Weber et al., 2006; Fan et al., 2014). In rice paddy ecosystems, the irrigation method of alternate drying-wetting, as well as the phenomenon of radial oxygen loss (ROL) from rice roots result in fluctuant pH and Eh in soil, which is closely related to Cd availability in soil (Liang et al., 2016; Yu et al., 2016). For example, both of the precipitation of Cd(OH)₂ at neutral or alkaline pH or CdS in low Eh can decrease soil Cd availability (Sun et al., 2007). And the soil surface is negative charged at pH higher than pH_{pzc} (point of zero charge), which enhances the electrostatic adsorption with Cd (Yang et al., 2020). As iron oxides are redox sensitive, the iron redox cycle (caused by the changes of pH and Eh) leads to repeated leaching-precipitation of iron, which is defined as ferrollysis (Brinkman, 1970). Ferrollysis significantly decreases free iron oxides while increases amorphous iron oxides; and the increase of amorphous iron oxides provides more active sorption sites for heavy metal binding (Jiang et al., 2017). Meanwhile, the changes of Eh and pH in rice rhizosphere soil caused by ROL play vital roles in the regulation of IP formation. Because the oxidation state is a prerequisite for the formation of IP, and recent studies reported that IP formed at pH levels above 6.5 and disappeared at pH levels below 4.5 (Zhang et al., 2019a).

The transformation of iron oxides is influenced by both pH and Eh. According to Brennan and Lindsay (1998), the electrons released by microbial decomposition of organic matter were accepted by Fe(III), which increased the solubility of Fe²⁺ ($\text{Fe}[\text{OH}]_3[\text{amorp}] + 3\text{H}^+ + \text{e}^- \leftrightarrow \text{Fe}^{2+} + 3\text{H}_2\text{O}$). Additionally, the activity of Fe²⁺ was expressed as $3\log \text{Fe}^{2+} = 40.65 - 2(\text{pe} + \text{pH}) - 6 \text{pH}$ ($\text{pe} = \text{Eh}/59.2$). Thus $\text{pe} + \text{pH}$ may be considered as an important redox parameter to assess the form of iron oxides present in, for example, a paddy soil environment. However, there is little research on the combined effect of Eh and pH ($\text{pe} + \text{pH}$) on changes in iron oxides and Cd bioavailability in paddy soils. Herein, we hypothesize that the form of iron oxides present in soils is attributed to changes in $\text{pe} + \text{pH}$, which in turn affects Cd bio-availability in soil and Cd accumulation in rice plants. The aims of this study are: 1) to explore the influence of changes in iron oxides on Cd availability in soil under various $\text{pe} + \text{pH}$ conditions; 2) to investigate the responses of morphology and quantity of IP on rice roots to unstable $\text{pe} + \text{pH}$ conditions; and 3) to reveal the relationships among iron oxide fractions, IP formation and Cd accumulation in rice.

2. Materials and methods

2.1. Soil samples

Typical paddy soils that were naturally Cd-polluted were sampled (0–20-cm depth) from Xiangtan (27° 49' N, 112° 56' E, XT) and Liuyang (28° 16' N, 113° 64' E, LY) in Hunan province, China. The Cd background values of contaminated soils were over three-fold higher than the concentration limits of 0.4 mg kg⁻¹ (5.5 < pH ≤ 6.5) and 0.6 mg kg⁻¹ (6.5 < pH ≤ 7.5) based on the national soil environmental quality standards (China, GB15618-2018). The soils were air dried and sieved to particle sizes of <2 mm and <0.15 mm for a pot experiment and analysis of soil properties. Soil texture, organic matter (OM), and cation exchange capacity (CEC), as well as the contents of Fe and Cd were determined by pipette method, potassium dichromate external-heating method, barium chloride method and ICP-MS method, respectively (Taubner et al., 2009; Qiao et al., 2018). The soil initial pH was measured by a pH meter (Denver Instrument UB-7, ultraBASIC, Denver Instrument, U.S.A.) after mixing samples with deionized water (soil: water = 1:2.5 w/w) and shaking for 1 h (Qiao et al., 2018). Relevant soil properties are shown in Table 1.

2.2. Pot experiments

For the pot experiment, each plastic pot (height 16 mm, diameter 13 mm) was filled with 3 kg of air-dried soil. NPK basal fertilizer containing 0.25 g urea/kg, 0.15 g KH₂PO₄/kg, and 0.04 g KCl/kg soil was first dissolved in deionized water, then mixed evenly with each pot. The soils were incubated at 70% maximum field capacity for eight weeks. Rice seeds (XS09) were sterilized in 10% H₂O₂ for 20 min, then thoroughly rinsed five times with deionized water, and germinated on a Petri dish with the bottom covered with wet filter paper. After germination, six young rice seedlings of uniform size were transferred into the pots and cultivated for another five days. And then the following treatments were established: (1) DY: 70% water holding capacity (WHC); (2) FL: continuous flooding (3–4 cm of standing surface water); and (3) AFD: weekly alternating of flooding and drying (soils were dry for one week without any visible layer of water on the soil surface and then flooded at a 2–3-cm depth for the next week and alternated every week). There were three replicates for each treatment for the detection of root IP and to measure accumulation of Cd in rice plants. The seedlings were cultivated in a greenhouse for two months in the Chinese Academy of Agricultural Sciences in Beijing, China under natural conditions with day/night temperatures of 25/18 ± 2 °C.

2.3. Sampling and analysis

At the end of the pot experiment, 2–3 cm layer of water was held over the soil surface in pots under FL and AFD treatments. Eh and pH in 5-cm depth soil was measured in situ with a pH/ORP meter (FJA-6, Nanjing Chuan-Di Instrument and Equipment Co. LTD, China) for three times in each pot. The samples of plant and soil were harvested manually. Rhizosphere soil samples were obtained by shaking roots of rice plants and collecting soil (or slurry) adhering to roots. The rhizosphere soil (or slurry) was immediately freeze-dried at -70 °C with a freeze dryer (ALPHA 1–2 LD plus, CHRIST, Germany) to maintain the redox state, followed by crushing and sieving at a mesh size of 0.15 mm before analysis. The free (Fed) and amorphous (Feo) Fe oxides in soil were respectively extracted with citrate-bicarbonate-dithionite (CBD) and 0.2 M ammonium oxalate (pH = 3.0) (Tan et al., 2019). The exchangeable

Table 1
Basic chemical properties of the test soils.

Soil sample	Texture	pH	OM %	CEC cmol kg ⁻¹	Fe g kg ⁻¹	Background Cd mg kg ⁻¹
XT	red soil	6.2	1.86	18.5	54.4	1.28
LY	red soil	6.7	0.94	15.8	56.2	1.81

phase and Fe/Mn-oxide-bound phase of Cd were extracted with DTPA-TEA-CaCl₂ and NH₂OH·HCl (25% HAc), respectively, according to the procedure proposed by Li et al. (2016). The extracts were filtered through a 0.22- μ m filter, and the concentration of Fe/Cd in filtrate was detected by ICP-MS (7700X, Agilent, USA).

After the retrieval from soil, rice plants were carefully rinsed with deionized water. The above-ground and below-ground parts of rice plants were separated. A modified DCB (dithionite-citrate-bicarbonate) method was used to extract the IP on the fresh rice root surfaces (Zhang et al., 2019b). An extracting solution was prepared by adding 3.0 g sodium dithionite (Na₂S₂O₄) to a mixture of sodium citrate (0.27 M) and sodium bicarbonate (0.11 M) and adjusting to pH 6.5. Whole fresh roots were immersed in 45 ml extracting solution and incubated for 7 h. The extracts were filtered and then we determined Fe/Cd concentrations in IPs with ICP-MS (7700X, Agilent, USA). Roots were washed with deionized water and oven-dried (60 °C) together with shoots to a constant dry weight. The dry biomass of above-ground and below-ground biomass were recorded. Then the samples of roots and shoots were finely ground and digested with HNO₃-H₂O₂ through microwave digestion. Cd concentrations of digestion solutions were measured with ICP-MS (7700X, Agilent, USA).

2.4. Calculation and statistical analysis

The pe + pH value was calculated using the equation of $pe + pH = pH + Eh (mV)/59.2$. The content of crystalline Fe oxides (Fec) was calculated from the difference between contents of Fed and Feo. One-way analysis of variance at the 5% probability level was applied to analyze all the data by SPSS 16.0 (SPSS, München, Germany). The means \pm SD (n = 3) of results were plotted. Pearson's correlation in SPSS v16.0 was used to evaluate the correlation of parameters.

3. Results and discussion

3.1. pe + pH and Fe fractions

The various water management conditions had significant effects on Eh and pH in paddy soils and resulted in different pe + pH values (Fig. 1). Among these, the pe + pH values under non-flooding treatments were higher than that under flooding treatments for both test soils. In flooding conditions, the oxidation of organic

matter and reduction of soil components in the waterlogged soils resulted in significantly lower soil Eh values; and the consumption of H⁺ due to the reduction of redox-sensitive elements in the soil contributed to increased pH in acidic soils (Sun et al., 2007). In contrast, gas exchange occurring between the atmosphere in drainage conditions and soil likely provided sufficient oxygen in the rhizosphere environment to rise soil Eh (Li et al., 2018). A slight increase in pH (as the initial pH was above 6.0, as shown in Table 1) and a sharp decrease in Eh under the flooded conditions resulted in significantly decreased pe + pH value in this study.

The change of pe + pH greatly influenced the iron oxide crystallinity in soil. As shown in Fig. 2, iron oxides existed primarily in crystalline form at a pe + pH of approximately 11, while the content of crystalline Fe oxides dropped by 28% (XT) and 19.3% (LY) as their pe + pH values declined to 4.29 and 4.72, respectively; and the content of amorphous iron oxides increased by 75%–153% with the decrease of pe + pH. Under flooding treatments, the crystalline ferric oxide can be promptly transformed into the amorphous phase (Calabrese and Porporato, 2019). Fe(III) in amorphous oxides

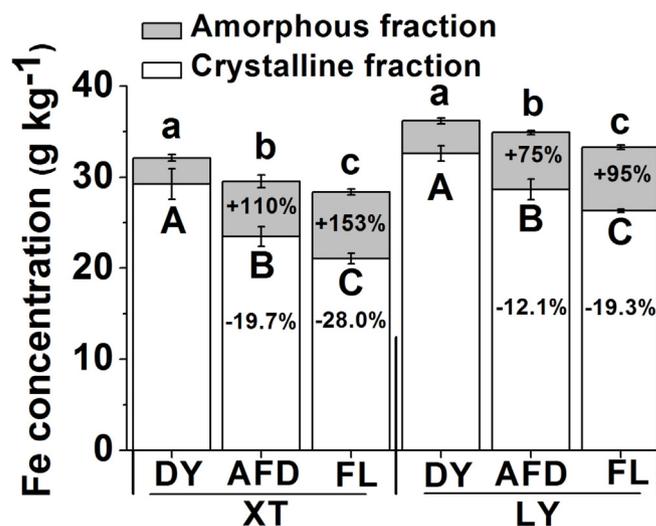


Fig. 2. Changes of Fe oxide fractions in rhizosphere soils under various pe + pH conditions. Standard errors are indicated by vertical bars. Different lowercase letters above columns indicate significant differences ($P < 0.05$) in fractions amorphous iron oxides among treatments. Different capital letters within different treatments denote significant differences ($P < 0.05$) in fractions of crystalline iron oxides.

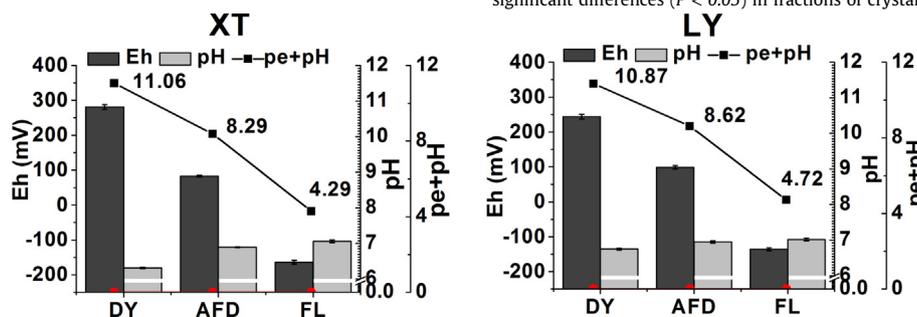


Fig. 1. Variation of pe + pH in soil under different water management conditions after a two-month cultivation period.

was largely reduced to Fe^{2+} due to the sharp decrease of Eh in flooded soils where soil pH was close to 7.0. Because the Fe(III) in soil was reduced and consequently dissolved when Eh dropped to 100 mV at pH = 7 (Dold et al., 2011). And then the hydrolysis of Fe^{2+} and Fe^{3+} in soil solutions initially produced unstable and poorly crystalline iron oxides (ferrihydrite) (Cudennec and Lecerf, 2006). However, metastable ferrihydrite can readily transform into more stable crystalline iron oxides, such as goethite and hematite in aerobic conditions or lepidocrocite under anaerobic conditions or with the catalysis of reducing ions (Liu et al., 2008). There are two potential ways for the transformation of ferrihydrite into stable crystalline iron oxides. The first is the dissolution/re-precipitation of ferrihydrite in which the $\text{Fe}(\text{OH})_2$ and $\text{Fe}(\text{OH})^{2+}$ are produced by ferrihydrite dissolution, reassembly and redeposit to form crystalline iron oxides. The other is the direct solid-state conversion of ferrihydrite into hematite through dehydration and rearrangement processes (Liu et al., 2005). Both pathways can be illustrated by electron transfer among different forms of iron, like the electron transfer between adsorbed ferrous iron and interfacial ferric iron (Liu et al., 2005). Reportedly, the presence of Fe(II) can accelerate the solid-phase conversion of ferrihydrite (Liu et al., 2005). In the present study, the drainage treatment gave rise to soil Fe(II) oxidation accompanied by electron release, as well as the incomplete oxidation of Fe(II), which likely promoted the ferrihydrite converting into more crystalline forms by both dissolution/re-precipitation and solid-state transformation. Accordingly, the amorphous iron oxides were partially converted to crystalline forms so that the crystallinity of iron oxides increased in soil. While under anaerobic and reduced conditions (low pe + pH), some Fe(III) in soils reduced to Fe(II), which inhibited the formation of ferrihydrite and its subsequent transformation to crystalline forms, thus facilitating the formation of amorphous iron oxides. Moreover, it has been reported that microorganisms also affect the transformation of iron oxides. For instance, the low pe + pH conditions may promote the activity of dissimilatory iron reducing bacteria to produce more Fe(II), which is readily re-oxidized to amorphous iron oxides (Li et al., 2018). This hypothesis still needs to be verified in future studies.

The change in crystallinity of iron oxide under the treatment of repeated submergence and drainage could also be attributed to ferrololysis. In a "flooding-drainage" cycle of soil, the concentration of Fe^{2+} significantly increased in the flooding process, while it declined after soil re-drying, which meant massive leaching and conversion of iron oxides in iron redox cycling (ferrololysis). This process led to the increased content of amorphous iron oxides but the decreased free iron oxides. Also, an amorphous intermediate has been reported to be produced by microbial action prior to the reduction of crystalline Fe(III) oxides in paddy soil; and this amorphous intermediate can react with soil organic matter by adsorption or co-precipitation against converting into crystalline iron oxides (Mikutta et al., 2014; Jiang et al., 2017). Therefore, under AWD treatment, the inhibited ferrihydrite transformation, ferrololysis, and the intermediate formation might all be associated with the increase of poorly crystallized iron oxides. Additionally, there was a slight non-significant reduction in the total amount of crystalline and amorphous iron oxides with the decrease of pe + pH levels (Fig. 2). Because the reduction of Fe(III) in amorphous oxides under reduction conditions can result in the release of dissolved Fe^{2+} into overlying water (Li et al., 2019).

3.2. Changes of Cd fractions in soil

The level of Fe/Mn oxide fraction of Cd (Fe/Mn–Cd) in soil increased significantly with the reduction of pe + pH (Fig. 3). The highest concentration of iron oxide-bound Cd was observed at

pe + pH level of 4.29 (XT) and 4.72 (LY), where the concentrations were respectively 31.8% and 24.7% higher than that at pe + pH of approximately 11. The substantially high concentration of Fe/Mn–Cd at low pe + pH corresponded to the higher content of amorphous iron oxides in flooding-treated soils (Fig. 2). The amorphous iron oxides have large surface areas and abundant functional groups on their surface, with more reactive sites to adsorb metal ions compared to crystalline forms (Jiang et al., 2017). Also, the secondary iron minerals produced from Fe^{2+} , which was released by microbial iron respiration in the anaerobic conditions, could also supply abundant hydroxyl groups ($\equiv\text{Fe}-\text{OH}$) to bind Cd^{2+} (Li et al., 2016). With the decrease of pe + pH, the enhanced reduction state of soil caused the formation of secondary iron minerals with more Cd immobilization on them, which led to the increase of Fe/Mn–Cd content. It should be noted that although a significant increase in amorphous iron oxides (75%–153%) was observed, the content of crystalline iron oxides accounted for the majority of total iron oxides in soil (Fig. 2). Therefore, the increase of Fe/Mn bound Cd in soil under flooded conditions may be also associated with the increase in adsorption capacity of iron oxides. According to previous research, the adsorption capacity of iron oxides to Cd was significantly enhanced at soil pH higher than 6.45 (Zhu et al., 2018). The increased pH in flooded soils could increase the negative charge on the surface of iron oxides, which enhanced the adsorption capacity of iron oxides to Cd.

In the present study, the concentration of DTPA-extractable Cd was used to represent Cd availability in soils because DTPA-extractable Cd has been widely used as an indicator of the pool of available metals or plant-available fraction of metals in soils. As shown in Fig. 3, the concentration of DTPA-extractable Cd in soil was significantly reduced as pe + pH decreased, and an opposite trend in response to the changes of Fe/Mn–Cd was observed (Fig. 3). Cd bound to Fe–Mn oxides in soil has been reported to difficultly desorb back into the soil solution and was considered as an unavailable fraction (Backes et al., 1995). Thus, the immobilization of Cd by Fe/Mn oxides greatly reduces its mobility and availability in soil. As expected, the amount of available Cd in tested soils at low pe + pH (4.29–8.62) levels was lower by 21%–33.9% (XT) and 15.3%–26.1% (LY) than in the respective soils at pe + pH close to 11. Besides the marked increase of Fe/Mn–Cd, the precipitation of Cd sulfides when the pe + pH dropped below 4.85 also contributed to the decrease of soil Cd availability (Brennan and Lindsay, 1996). In addition, the waterlogged conditions with a high concentration of Fe^{2+} in the rhizosphere and close to neutral soil pH, coupled with root oxygen release likely facilitate the formation of bacteriogenic iron oxides produced by the metabolism of Fe-oxidizing bacteria (Yu et al., 2016). These bacteriogenic iron oxides with high affinity for Cd^{2+} may have also contributed to reducing Cd mobility in soil (Yu et al., 2016). In addition, the bioavailability of Cd in soil is also influenced by other factors, such as organic matter (SOM), different ions. For example, SOM can effectively form complexes with Cd to reduce its mobility (Zeng et al., 2011). As the decomposition of organic matter is slower in submerged soils, compared to aerobic conditions, the existing of more SOM can be beneficial for the accumulation of Cd (Sahrawat, 2004).

3.3. Iron plaque formation and Cd adsorption

The content of DCB-extractable Fe represented the thickness of IP. In this study, the Fe levels in DCB extracts at pe + pH of 4.29–8.62 were 1.29–1.49-fold higher than those in the aerobic treatments with pe + pH close to 11 (Fig. 4). The root oxidation and soil Fe^{2+} concentration greatly influenced the deposition of the IP coating on rice roots (Fu et al., 2018). In the present study, the concentration of Fe^{2+} in rhizosphere soil increased significantly

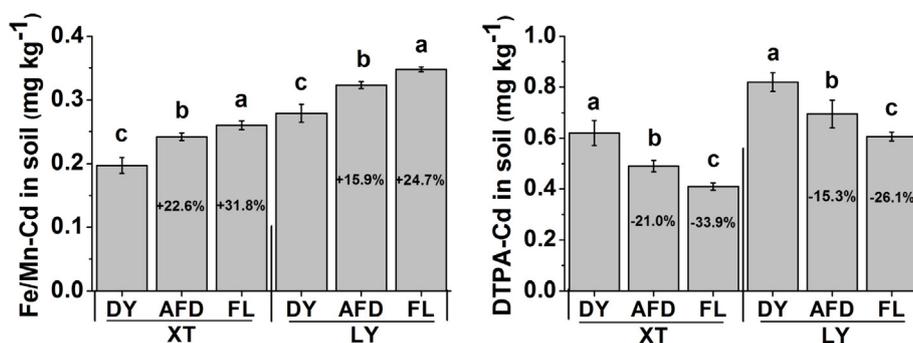


Fig. 3. Concentration of Fe/Mn oxide-bound Cd (Fe/Mn-Cd) and DTPA-extractable Cd (DTPA-Cd) in rhizosphere soils in response to different soil pe + pH conditions.

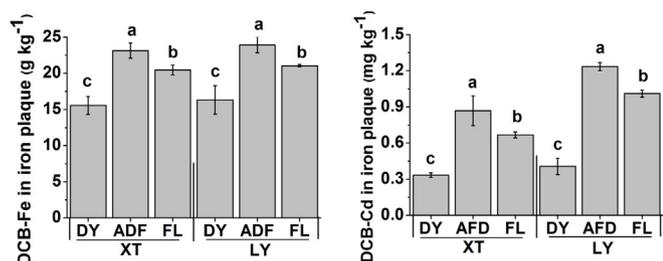


Fig. 4. Formation of iron plaque and the concentration of Fe (DCB-Fe) and Cd (DCB-Cd) in the iron plaque on rice root surfaces measured at the end of rice cultivation.

with the decrease of pe + pH (data not shown). At low pe + pH conditions, the oxygen release from the rice roots into the rhizosphere soil (radial oxygen loss), coupled with the biological reduction of Fe³⁺ into Fe²⁺, could be beneficial for IP formation (Neubauer et al., 2007; Li et al., 2016). In addition, soil species such as NO₃⁻, SO₄²⁻, Fe(III) and Mn(III/VI) that are present in oxide phases receive electrons in soils with low pe + pH, resulting in the formation of NO₂⁻, S²⁻, Fe²⁺, Mn²⁺, and organic acids with low molecular weight during the reduction reactions (De Livera et al., 2011). The IP was mainly composed of ferrihydrite and as previously mentioned, low pe + pH condition may limit the transformation of ferrihydrite that was readily converted into well-crystallized iron oxides under aerobic conditions. Therefore, more IP was developed under low pe + pH conditions when compared with pe + pH above 10. Interestingly, the amount of IP on rice root surface at pe + pH near 8 (AFD treatment) were significantly higher than those at pe + pH below 5 (FL treatments). We suspect that the flood-dry-cycle treatment provided more favorable conditions for IP formation than continuous flooding. The flooding condition was favorable for Fe²⁺ release through the reduction of Fe(III), while the subsequent drought conditions were conducive to IP deposition.

The amount of DCB-extractable Cd in plaque deposits ranged from 0.334 to 1.235 mg kg⁻¹ (Fig. 3), suggesting that the IP sequestered a portion of Cd from soil solution. The IP adsorbed or reacted with Cd in the rhizosphere due to its high specific surface area and abundant -OH functional groups on its surface (Zhang et al., 2019a). However, the capacity of plaque deposits to bind Cd was significantly affected by soil pe + pH. For example, the concentration of DCB-extractable Cd in the IP at pe + pH below 4.29–8.62 was 2.30–3.04-fold higher than that of the concentration in the IP at pe + pH close to 11. The difference may be primarily attributed to more IP deposition on rice roots under low pe + pH conditions, which provided more active sites for Cd adsorption. Also, the iron oxides coating on rice roots with more negative charges could adsorb more Cd, when soil pH raised above 6.45 in flooding conditions (refer to low pe + pH).

Additionally, the DCB-Cd concentration in IP ranked the treatments in the same order as IP thickness did: AFD > FL > DY. The Cd species in the IP were dominated by the fractions in the amorphous and poorly-crystallized hydrous iron oxides (ferrihydrite and goethite) (Liu et al., 2006). Under drainage and re-drying treatment, the fluctuant pH and Eh resulted in more low-crystalline iron oxides and was responsible for more Cd immobilization on iron oxides by adsorption and/or co-precipitation. Contrarily, the decreased IP in continuous flooding conditions may have resulted in Cd release (Yu et al., 2016). Thus, more Cd was sequestered on IP under the AFD than FL treatments.

However, there are inconsistent results regarding the role of IP in Cd sequestration. This study found that Cd sorption on IP further reduced Cd uptake by plants, which was in agreement with that reported by Xu et al. (2018). But a recent research found that flooding treatment decreased the formation of IP and its affinity for metals (Zhang et al., 2019b). The differences of these results may be probably caused by different experimental periods. Besides, even though IP has great effect on Cd immobilization in rhizosphere soil, the concentration of other elements (such as Fe, Mn, Zn, Se) in soil or plants can also influence Cd uptake by plants positively or negatively (Seshadri et al., 2016).

3.4. Cd distribution in rice tissues

Cd uptake by rice plants from soil was reduced with the decrease of soil pe + pH values (Fig. 5). Compared with non-flooding treatments (pe + pH 10.87 and 10.89), the flooding treatment (pe + pH 4.29 and 4.72 for both soils) significantly decreased Cd concentrations in rice roots by 33.1% (XT) and 25.8% (LY), and the alternate drying-wetting treatment (pe + pH 8.29 and 8.62) reduced by 19% (XT) and 14.7% (LY). A likely reason was the decrease of Cd availability in soil by the increase in Cd immobilization on iron oxides with decreasing pe + pH (Fig. 2). Another reason might be due to more IP deposition on rice roots under reducing conditions; IP with high affinity for Cd reduced Cd phytoavailability in the rhizosphere and thereby reduced the uptake of Cd by rice roots. Notably, part of the IP on rice roots may decompose in submerged soil to form new secondary Fe(III)/Fe(II) minerals that can retain Cd, and thus further lower the availability of Cd in the rhizosphere soil (Yu et al., 2016). Moreover, another viewpoint in a recent study by Zhang et al. (2019b) indicated that the decrease of IP on rice roots in flooded conditions reduced the affinity of root surfaces for Cd²⁺ and inhibited the transport of Cd to rice roots.

The above-ground tissues of rice plants contained much lower concentrations of Cd than roots (Fig. 5), due to the restricted transport of Cd from root to xylem (including the sequestration of Cd-chelates in the vacuole, and the formation of physical barriers to extracellular transport of Cd) (Lux et al., 2011). Significant

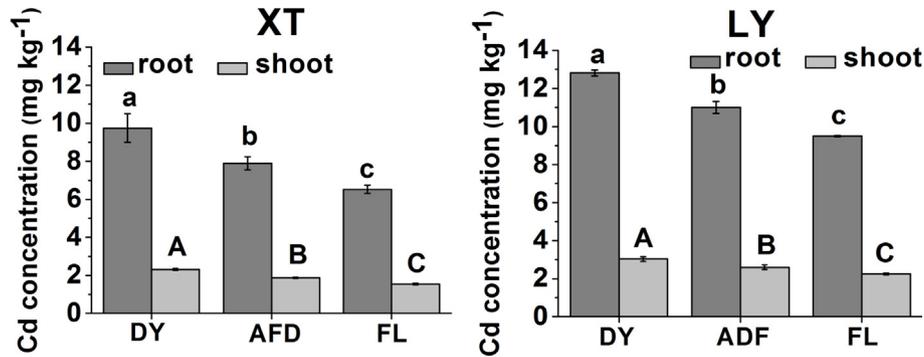


Fig. 5. Cd concentrations in rice roots and shoots under different pe + pH conditions. Different lowercase letters above columns indicate significant differences ($P < 0.05$) in Cd concentrations in roots among treatments. Different capital letters within treatments denote significant differences ($P < 0.05$) in Cd concentrations in shoots.

variations in Cd concentration in rice shoot under different pe + pH conditions were also observed. Treatments ranked in the following order based on Cd concentration: DY > AWD > FL. Clearly, the highest accumulation of Cd in rice plants was observed in DY treatment. For one reason, the concentration of DTPA-Cd in soil in DY treatment was highest (Fig. 3), which favored Cd uptake by rice plants. For another reason, there were the least formation of IP (DCB-Fe) and Cd sequestration on IP (DCB-Cd) in DY treatment (Fig. 4). Similarly, soil pe + pH also affected Cd concentration. Cd concentration at pe + pH close to 11 in rice shoots were greater by 14.1%–19.0% at pe + pH near 8 and by 25.7%–33.1% at pe + pH nearly 4. As Cd translocation from root to the shoot can be driven by mass flow due to leaf transpiration, it should also be taken into account in future research that the differences in leaf transpiration under various water conditions may also be related to Cd accumulation in leaves (Liu et al., 2010).

3.5. Outcomes of Fe and Cd in rhizosphere soil under various pe + pH conditions

The relationship among the measured variables in soil and rice plants was analyzed by Pearson correlation test as shown in Table 2. A significantly negative correlation was observed between pe + pH value and the concentration of amorphous Fe oxides in soil ($R^2 = -0.896, P < 0.01$). The concentration of amorphous iron oxides was positively associated with Fe/Mn–Cd ($R^2 = 0.835, P < 0.01$) and negatively correlated with DTPA-Cd ($R^2 = -0.759, P < 0.01$). This result further confirmed that the low pe + pH condition could increase the amount of amorphous Fe oxides in soil, further to immobilize more Cd bound to Fe/Mn oxides and thus reduce Cd availability in soil. Furthermore, soil pe + pH value was clearly negatively associated with the concentrations of DCB-Fe and DCB-Cd ($R^2 = -0.487, P < 0.05, R^2 = -0.575, P < 0.05$), suggesting that low

pe + pH levels favored IP formation and Cd sequestering on the IP. More importantly, Cd concentration in rice shoots displayed a strong and positive association with soil DTPA-Cd content ($R^2 = 0.954, P < 0.01$) and a negative correlation with DCB-Cd ($R^2 = -0.701, P < 0.05$). This result indicates that both the decreased soil Cd bioavailability and the increased Cd sequestration in IP were responsible for the reduced Cd phytotoxicity to rice plants.

We proposed a model in Fig. 6 to demonstrate the effect of iron

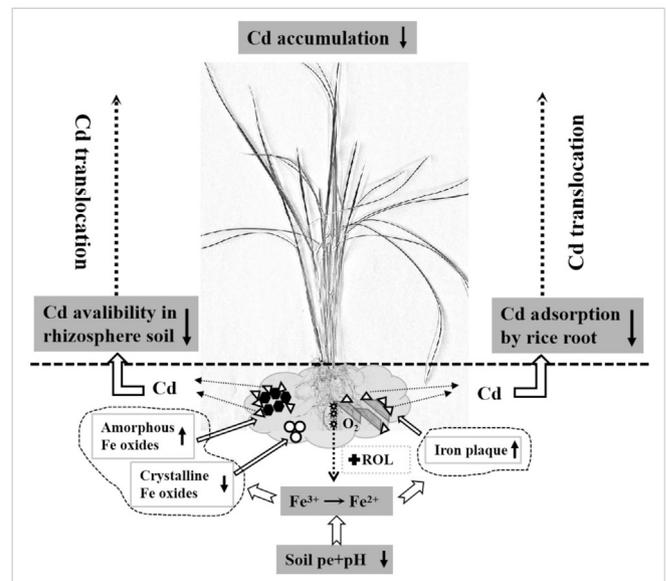


Fig. 6. Proposed model of mechanisms of iron oxides affecting Cd accumulation in rice plants from the rhizosphere at low pe + pH levels.

Table 2
Correlation coefficients (R) between soil pe + pH and the contents of crystalline and amorphous iron oxides (g kg^{-1}) in soils and the concentrations of Fe and Cd in iron plaque (g kg^{-1}); between the contents of amorphous iron oxides (g kg^{-1}) and the amounts of Fe/Mn oxide-bound Cd and DTPA-extractable Cd (mg kg^{-1}) in soils; and between Cd accumulation in rice shoots (mg kg^{-1}) and DTPA-extractable Cd concentrations (mg kg^{-1}) in soils and Cd concentration (mg kg^{-1}) in iron plaque. (* $P < 0.05$, ** $P < 0.01$).

Correlations between pe + pH in soil and following parameters (n = 36)			
Fec	Feo	DCB-Fe	DCB-Cd
0.771**	-0.896**	-0.487*	-0.575*
Correlations between Feo in soil and following parameters (n = 36)			
Fe/Mn–Cd		DTPA-Cd	
0.835**		-0.759**	
Correlations between Cd in rice plants and following parameters (n = 36)			
DCB-Cd		DTPA-Cd	
-0.701*		0.954**	

oxides on Cd accumulation in rice plants under altered pe + pH conditions in the paddy soil. Two primary mechanisms are responsible for the decreased Cd concentration in rice plants corresponding to low pe + pH levels. First, the decreased concentration of iron oxides as a consequence of low pe + pH condition caused more amorphous iron oxide in the rhizosphere soil. Accordingly, the amorphous Fe oxides displayed high affinity of Cd and reduced Cd availability in the rhizosphere soil. Second, IP was developed and attached onto rice root surface because oxygen released from rice roots oxidized Fe²⁺; the Cd sequestered by IP hindered Cd absorption by rice roots. Therefore, the absorption and accumulation of Cd in rice plants decreased at low pe + pH levels.

4. Conclusions

The variation of pe + pH values in the paddy rhizosphere soil greatly influenced the transformation of soil iron oxides and the development of IP on rice roots, thereby affecting Cd phytoavailability. Compared to DY treatment (pe + pH close to 11), the low pe + pH (4.29–8.62) conditions in soil facilitated the transformation of crystalline iron oxides into amorphous forms, in which the amorphous forms could immobilize more Cd and reduce soil Cd availability by 15.3%–33.9%. IP formation resulted in 2.3–3.04-fold more Cd by attachment to root surface. Both the decreased soil Cd bioavailability and the increased Cd sequestration in IP under low pe + pH conditions were responsible for the reduced Cd phytotoxicity to rice plants as indicated by the Pearson correlation test. The information reported in this paper will be useful for developing strategies to reduce rice grain Cd accumulation using water management and understanding the potential mechanisms.

Credit author statement

Shanshan Li: Investigation, Data curation, Writing- Original draft preparation; **Shibao Chen:** Conceptualization, Methodology; **Meng Wang:** Conceptualization, Validation, Writing-Reviewing and Editing; **Xiaoqin Lei:** Software, Validation; **Han Zheng:** Software, Validation; **Xiaoyi Sun:** Validation, Software; **Lifu Wang:** Validation, Software; **Yun Han:** Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was financially supported by the Natural Science Foundation of China [41877387], National Key Research and Development Program of China [2018YFD0800700 & 2016YFD0800707] and Fundamental Research Funds for Central Non-profit Scientific Institution [1610132020025 & 1610132020027].

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