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Coupling effects of Fe(II) and $CaCO_3$ application on cadmium uptake and accumulation in rice (*Oryza sativa* L.)

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Summary

Excessive cadmium (Cd) in rice, caused by Cd pollution of farmlands, poses a serious threat to human health. In this study, a pot experiment was conducted to investigate the effects of two doses of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), two types of Fe(II) (EDTA-Fe(II) and $FeSO_4$; 0.14 g Fe kg^{-1}), and their combined application on the uptake and accumulation of Cd in rice plants grown in Cd-contaminated acidic soil. The results revealed that $FeSO_4$ significantly increased rice grain biomass, whereas the other treatments had no significant effects. Further, the addition of EDTA-Fe(II) or $FeSO_4$ significantly enhanced iron plaque formation on the root surface and increased the Fe content in the rice plants and porewater. Compared to the control, $CaCO_3$ addition weakened the formation of iron plaque and reduced the Fe concentration in the porewater and root tissue, stems and leaves, whereas the Fe concentration in brown rice and the husks remained unaffected. Combined application of $CaCO_3$ and Fe(II) significantly promoted the formation of iron plaque and increased the Fe concentration in brown rice. However, the Cd concentration in the iron plaque was reduced by $CaCO_3$ addition but increased by Fe(II) treatment. Notably, all treatments reduced the Cd concentration in all rice plant tissues. The application of Ca_1, Ca_2, EDTA-Fe(II), $FeSO_4$, Ca_1+EDTA-Fe(II), Ca_1+$FeSO_4$, Ca_2+EDTA-Fe(II) and Ca_2+$FeSO_4$ significantly reduced the Cd concentration in brown rice by 69%, 63%, 51%, 60%, 46%, 39%, 38%, and 29%, respectively. These results indicate that the application of $CaCO_3$, EDTA-Fe(II)/$FeSO_4$, or their combination can effectively reduce Cd accumulation and translocation in rice plants.

Keywords: *Oryza sativa* L., Cadmium translocation, Calcium carbonate, Ferrous ion, Iron plaque

Introduction

Rapid industrial progress has exacerbated the frequent occurrence of environmental problems. The results of the 2014 China Soil Pollution Survey Bulletin showed that the heavy metal Cd pollution of cultivated soils is relatively serious (Duan et al., 2016). Cd is an element not required by plants and highly toxic to crops and humans. It affects crop growth and yield and, can be ingested by humans through the food chain (Chen et al., 2018); the health problems caused by Cd pollution are of widespread concern (Clemens et al., 2013).

As the main crop in China, rice cultivation has been facing heavy metal pollution, particularly Cd contamination, which has caused significant food safety and public health concerns, especially in its southern region (Hu et al., 2016). Therefore, there is an urgent need to explore effective methods to reduce the migration and accumulation of Cd in rice. Iron plaque, a reddish-brown iron oxide adhesive plaque, forms on the root surfaces of wetland plants, a strategy wetland plants have evolved to adapt to unfavorable anaerobic environments (Fu et al., 2016). The formation of iron plaque not only maintains the normal growth of rice plants in anaerobic environments, but to some extent also aids the adsorption and migration reduction of heavy metals into rice (Zandi et al., 2023). Iron plaque, an amphoteric colloidal iron oxide, can adsorb anions and cations from the environment (Tai et al., 2018). It can immobilize Cd on the root surfaces, thereby reducing the migration of Cd to the aboveground parts of rice plants (Fu et al., 2018; Liu et al., 2008; Siddique et al., 2021a; Siddique et al., 2021b; Zhang et al., 2019). Numerous studies investigating this mechanism were based on solution or soil culture experiments conducted during the rice seedling stage. However, little is known about the effect of an enhanced the formation of iron plaque on the accumulation of Cd in rice grains. Examining rice planted on farmlands with mild to moderate Cd pollution is of great practical significance for effectively reducing the accumulation of Cd in grains, maintaining it below the national standard limit. Many previous studies have used $FeSO_4$ to induce the formation of iron plaque to investigate its influence on the absorption and migration of heavy metals. Moreover, recent research has shown that EDTA-Fe(II) also has the potential to limit Cd migration into rice through the formation of EDTA-Cd and the promotion of root cell wall chelation (Xu et al., 2010; Zhang et al., 2022). However, its effect on iron plaque formation and Cd enrichment in rice grains remains unclear, especially compared to the application of $FeSO_4$.

$CaCO_3$, an acidic soil conditioner, is widely used in agriculture. Its application can reduce the bioavailability of heavy metals in the soil and decrease the Cd content in rice to some extent (He et al., 2021). However, the use of $CaCO_3$ can also cause problems such as a deterioration of the soil properties and a temporally decreasing effectiveness in suppressing the bioavailability of heavy metals (Huang et al., 2019). Application of exogenous Fe combined with $CaCO_3$ may inhibit Cd while avoiding these issues. An appropriate increase in acidic soil pH with an exogenous Fe(II) supply can promote the formation of iron plaque (Zhang et al., 2019). However, the effects of combined use of Fe(II) and $CaCO_3$ on Cd translocation and accumulation in rice under Cd stress remain unclear. After the application of Fe(II) to soil, a small amount of Fe(II) is absorbed and utilized by plants, and other Fe(II) ions can form iron plaque on the root surface or crystalline/amorphous iron oxides in the soil (Liu et al., 2006). These iron oxides can play a pivotal role in limiting soil-available Cd, even after the rice harvest or when planting rice in the next season, overcoming the disadvantages $CaCO_3$ has when applied alone.

Based on the above considerations, we aimed to investigate the following: (1) the influence of two types of Fe(II) ($FeSO_4$ and EDTA-Fe(II)) on iron plaque formation and Cd uptake and accumulation in rice, and (2) the combined effects of $CaCO_3$ and Fe(II) on Cd translocation and enrichment in rice.

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Materials and methods

Soil preparation and experimental design

The test soil was obtained from a paddy field in Jian'ou City, Fujian Province, China. After the soil was removed, it was dried, ground, crushed with a mallet, and mixed evenly using a 1 cm sieve. The soil pH and organic matter content were 6.4 and 11.2 g kg^{-1}, respectively. The Cd content of the soil was 0.41 mg kg^{-1}. The percentages of sand, loam, and clay type soils were 45.2%, 48.2%, and 6.3%, respectively. 3.5 kg of screened soil was placed in a plastic bucket (height: 21 cm; bottom diameter: 18 cm; top diameter: 22.5 cm) and 2 g kg^{-1} (Ca$_1$) or 10 g kg^{-1} (Ca$_2$) of $CaCO_3$ were added to the soil as the $CaCO_3$ treatments. The soil was mixed to maintain a 3 cm flooded layer for 30 days. 1 mL $Cd(NO_3)_2{\cdot}4H_2O$ (3.5 mg Cd mL^{-1}) solution was added to the bucket to stably maintain the equilibrium for 60 days. The uniform rice seedlings were then transferred to a bucket. For EDTA-Fe (II) or $FeSO_4$ treatment, 300 mL EDTA-Fe (II) or $FeSO_4$ solution (0.49 g Fe) were added to the bucket to achieve an added Fe content of 0.0025 mol kg^{-1} (0.14 g Fe kg^{-1}) in soil once the rice plant had grown for 4 weeks. Based on our previous research, this amount of exogenous Fe addition will not have a negative impact on rice growth (Yu et al., 2021). Equal amounts of $CaCO_3$ (Ca$_1$ or Ca$_2$) and Fe(II) (EDTA-Fe (II) or $FeSO_4$) were used for the combined $CaCO_3$ and Fe(II) treatment.

Rice seeds (Yongyou 9) were soaked in 30% H_2O_2 for 15 min and washed thoroughly with deionized water. Subsequently, the seeds were germinated in clean quartz sand. After 10 days, uniform rice seedlings were grown in a 1/5 strength Hoagland nutrient solution, which was changed every three days. The pH was adjusted to 5.5. After 14 days, uniformly growing rice seedlings were transplanted into plastic buckets for growth. There were a total of nine treatments: (1) control, (2) Ca$_1$, (3) Ca$_2$, (4) EDTA-Fe(II), (5) $FeSO_4$, (6) Ca$_1$+EDTA-Fe(II), (7) Ca$_1$+$FeSO_4$, (8) Ca$_2$+EDTA-Fe(II), and (9) Ca$_2$+$FeSO_4$. Each treatment was repeated three times. Rhizon samplers were inserted into the potting soil from one side at a depth of 15 cm and an angle of 45° (one sampler per pot) prior to rice transplanting. Subsequently, pore water samples (20 mL) were collected from the rhizosphere soil at various time points: 0 day (before rice transplanting), and then 30, 60, 90, and 120 days after rice transplanting. Immediately upon collection, each sample was transferred to a plastic tube containing 0.4 mL of superior grade pure HNO_3 and stored for further analysis.

Plant harvest and treatments

When the rice was harvested, the roots and aerial parts were washed with deionized water, and the roots, stems, leaves, and grains were separated using clean scissors. Rice roots with the reddish brown iron plaque were placed in a beaker and extracted with a dithionite-citrate-bicarbonate (DCB) extraction solution at 25 °C for 1 h according to Yu et al. (2021). In detail, roots washed with deionized water were soaked in an 80 mL DCB mixture including 0.03 mol L^{-1} sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), 0.125 mol L^{-1} sodium bicarbonate ($NaHCO_3$), and 1.2 g sodium dithionite ($Na_2S_2O_4$). After 1 h, the rice roots were then washed three times with deionized water, and the DCB extract solution was transferred to a volumetric flask and brought to a volume of 200 mL. The rice root, stem, leaf, and grain samples were placed in a 60 °C oven for 72 h. Then, the rice grains were divided into brown rice and husks using a sheller, and the dry weight of each part of the rice plant was measured. All plant samples were digested after crushing according to our previously described method (Yu et al., 2021). In detail, 0.2 g of powdered sample was accurately measured and placed into a Teflon vessel. Subsequently, 8 mL superior grade pure HNO_3 was added into the vessel. After 12 h, 1 mL superior grade pure H_2O_2 (30%, v/v) was added into the vessel for digestion using a microwave digestion system (MARS6; CEM, USA), following a specific protocol: raising the temperature to 110 °C in 15 min, holding it for 30 min, and then allowing it to cool for 20 min until it reached room temperature. The digested liquid was then transferred to a volumetric flask and brought to a volume of 100 mL. All liquid samples were filtered with a 0.45 µM aqueous phase membrane filter and stored in plastic bottles for subsequent analysis. The Cd, Fe, Mn, Cu and Zn concentrations in these liquid samples were determined using inductively coupled plasma mass spectrometry (ICP-MS, NexION 300 ×; Perkin Elmer, NY). Furthermore, following the harvest, the soil pH was measured in a 5:1 water/soil suspension using a pH meter (Mettler Toledo, Switzerland). The Cd translocation factor (TF) in rice plant at the mature stage was calculated as follows:

$TF_{grain/husk}$ = Cd concentration in grain/ Cd concentration in husk; $TF_{husk/leaf}$ = Cd concentration in husk/ Cd concentration in leaf; $TF_{leaf/stem}$ = Cd concentration in leaf/ Cd concentration in stem; $TF_{stem/root}$ = Cd concentration in stem/ Cd concentration in root; $TF_{root/iron\ plaque}$ = Cd concentration in root/ Cd concentration in iron plaque.

Statistical analysis

Data are presented as the mean ± SD (n = 3) and were analyzed using the least significant difference (LSD) at the 5% level. One-way analysis of variance (ANOVA) was performed using SPSS (19.0, SPSS, Inc., Chicago, IL, USA) to detect significant ($p < 0.05$) effects of $CaCO_3$, $FeSO_4$, EDTA-Fe(II), and $CaCO_3$+ $FeSO_4$/EDTA-Fe(II) treatment on rice plant biomass, soil pH, and the Cd, Fe, Mn, Cu, and Zn concentrations in the brown rice, husks, leaves, stems, roots, iron plaque, and pore water samples.

Results

Rice plant growth under $CaCO_3$ or Fe(II) addition

At the tillering stage, all treatments increased the leaf biomass by 15-60% (Fig. S1). Further, the application of Ca$_1$ and $FeSO_4$ significantly increased stem biomass by 21.7% and 26.8%, respectively (Fig. S1),

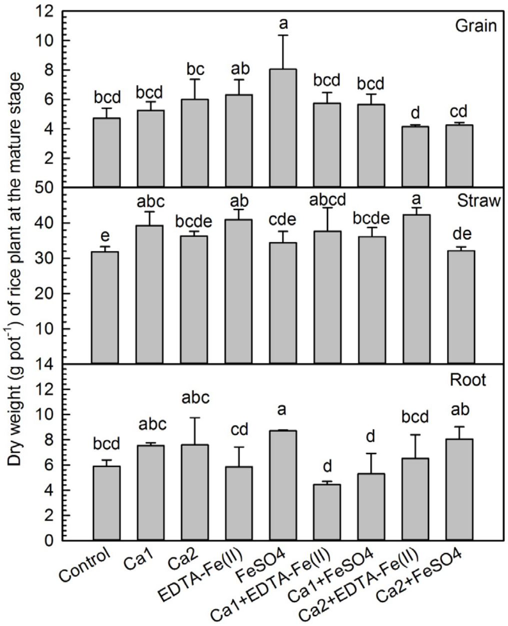

Fig. 1: Effects of $CaCO_3$ (Ca$_1$: 2 g kg^{-1}, Ca$_2$: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on biomass of grain, straw and root of rice plant grown in Cd-contaminated acidic soil at the mature stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

and the combination of Ca_2 and $FeSO_4$ increased the biomass of the rice roots by 67.4% (Fig. S1). At the mature stage, the biomass of the rice grains and roots was significantly increased after $FeSO_4$ treatment, but unaffected by the other treatments (Fig. 1). $FeSO_4$ increased the grain and root biomass by 70.6% ($p = 0.001$) and 47.8% ($p = 0.014$), respectively (Fig. 1). The application of Ca_1, EDTA-Fe(II), and the combination of EDTA-Fe(II) and $CaCO_3$ (Ca_1 and Ca_2) significantly increased straw biomass by 18.3-33.1% (Fig. 1).

Fe distribution in iron plaque and plant tissues

At the tillering stage, the Fe concentration in the iron plaque on the root surfaces was enhanced by the application of Fe(II) (EDTA-Fe(II) and $FeSO_4$) (Fig. S2). The addition of Ca_2 and EDTA-Fe(II) markedly reduced and increased the Fe concentrations in the root tissue, respectively (Fig. S2). The Fe concentrations in the stems and leaves were reduced by $CaCO_3$ (Ca_1 and Ca_2) and Ca_2+Fe(II) (Fig. S2). At the mature stage, the application of Fe(II) and the combination of $CaCO_3$ and Fe(II) significantly increased the Fe concentration in iron plaque by 32.5-67.6% (Fig. 2). The Fe concentrations in the root tissues, stems, and leaves were significantly reduced by $CaCO_3$ treatment (Fig. 2), and the application of Ca_1+$FeSO_4$ and Ca_2+Fe(II) decreased the Fe concentration in the root tissue (Fig. 2). The Fe concentration in the husks and brown rice was enhanced by Fe(II) treatment and the combination of $CaCO_3$ and Fe(II), but unaffected by $CaCO_3$ treatment (Fig. 2).

Concentrations and translocation of Cd in rice plants

Compared with the control, the Cd concentration at the tillering stage was significantly increased in the iron plaque and decreased in the root tissue after EDTA-Fe(II) treatment (Fig. S3). However, the Cd concentration in the stems was significantly reduced by $CaCO_3$ (Ca_1 and Ca_2), Fe(II) (EDTA-Fe(II), and $FeSO_4$), and their combinations (except Ca_1+EDTA-Fe(II) and Ca_2+$FeSO_4$) (Fig. S3). The application of $CaCO_3$ and the combination of $CaCO_3$ and Fe(II) (except for

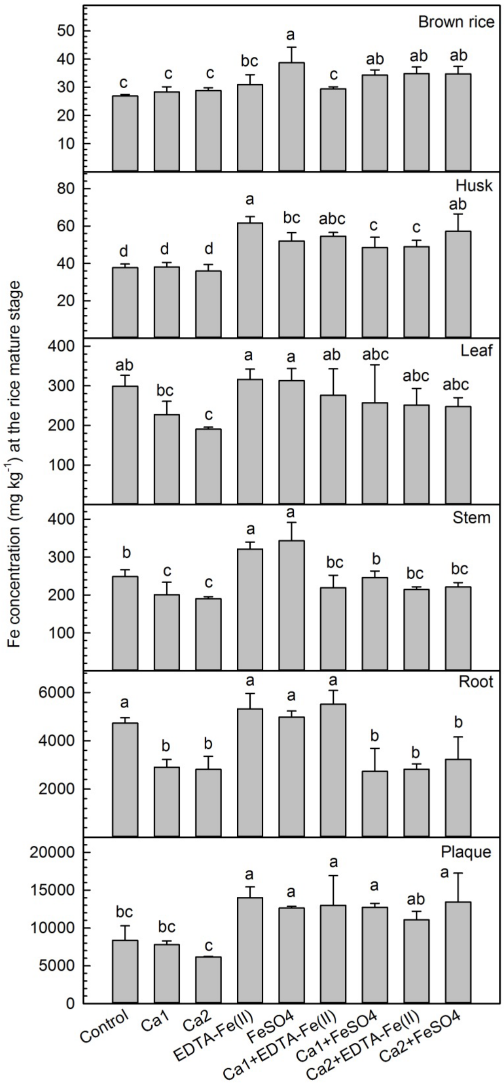

Fig. 2: Effects of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on Fe concentration in brown rice, husk, leaf, stem, root and iron plaque of rice plant grown in Cd-contaminated acidic soil at the mature stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

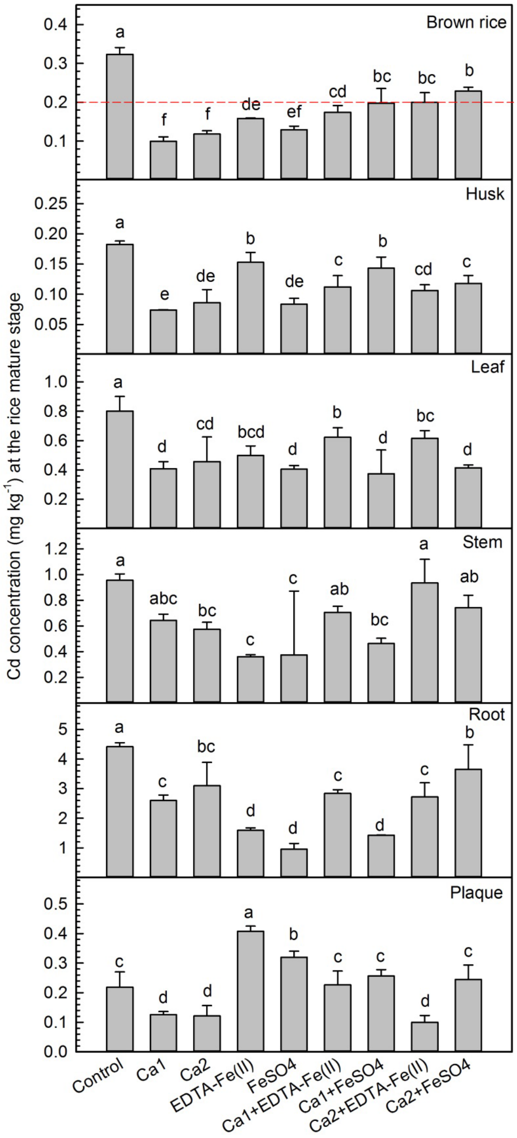

Fig. 3: Effects of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on Cd concentration in brown rice, husk, leaf, stem, root and iron plaque of rice plant grown in Cd-contaminated acidic soil at the mature stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

Ca$_1$+EDTA-Fe(II)) significantly reduced the Cd concentration in the leaves (Fig. S3). At the mature stage, the Cd concentration in the iron plaque was significantly decreased after CaCO$_3$ and Ca$_2$+EDTA-Fe(II) but increased after EDTA-Fe(II) or FeSO$_4$ (Fig. 3) treatment. Compared with the control, all treatments markedly decreased the Cd concentrations in the root tissues, leaves, husks, and brown rice (Fig. 3). Specifically, the Cd concentrations in the brown rice were reduced by 69%, 63%, 51%, 60%, 46%, 39%, 38%, and 29% after Ca$_1$, Ca$_2$, EDTA-Fe(II), FeSO$_4$, Ca$_1$+EDTA-Fe(II), Ca$_1$+FeSO$_4$, Ca$_2$+EDTA-Fe(II), and Ca$_2$+FeSO$_4$ treatment, respectively (Fig. 3). The $\text{TF}_{\text{grain/husk}}$ after the application of Ca$_1$, EDTA-Fe(II), and Ca$_1$+EDTA-Fe(II)/FeSO$_4$ was significantly lower than that of the control (Fig.4). Moreover, compared to the control, the $\text{TF}_{\text{root/iron plaque}}$ after EDTA-Fe(II), FeSO$_4$, Ca$_1$+EDTA-Fe(II)/FeSO$_4$ treatment was significantly lower (Fig. 4).

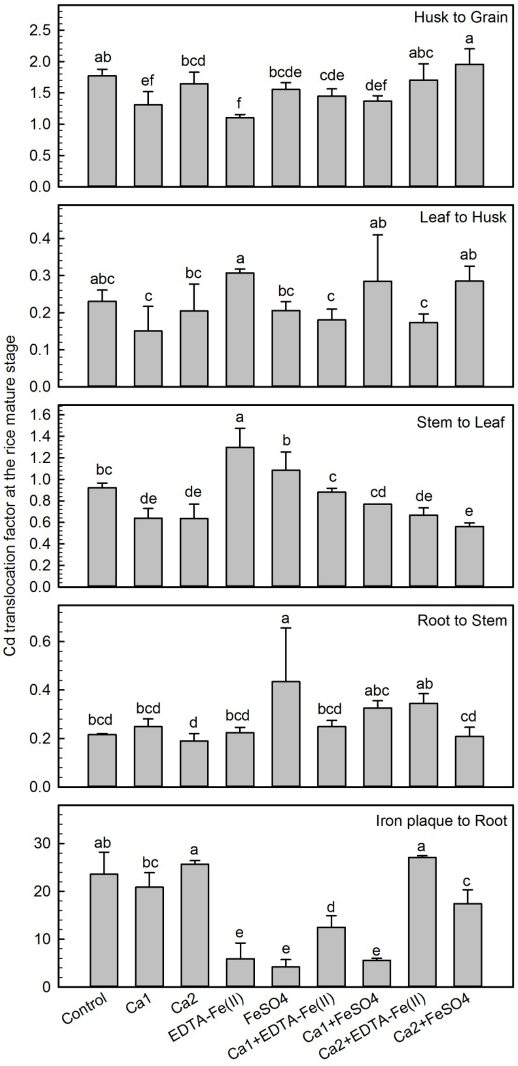

Fig. 4: Cd translocation factor from iron plaque to root, root to stem, stem to leaf, leaf to husk, and husk to grain of rice plant grown in Cd-contaminated acidic soil at the mature stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

Change of soil pH and Cd/Fe concentration in pore water

Soil pH decreased after rice harvest compared to before rice planting (Fig. 5). All treatments significantly increased soil pH by 0.71-1.65 in comparison with the control group (Fig. 5). From 0 to 120 days, the pH of the pore water gradually decreased (Fig. S4). On day 120, the pH of the pore water in all treatments was higher than that of the control treatment (Fig. S4). After the rice harvest, all treatments had significantly increased the soil pH compared to the control (Fig. 5). The concentrations of Cd and Fe in the pore water exhibited a gradual downward trend from day 0 to 120 (Fig. 6 and 7). The application of CaCO$_3$ and the combination of CaCO$_3$ and Fe(II) significantly decreased the pore water Cd concentration after 90 and 120 days, respectively (Fig. 6). Additionally, compared with the control, the application of CaCO$_3$ decreased the Fe concentration in the pore water (Fig. 7). Notably, EDTA-Fe(II) treatment maintained the highest Fe concentration in the pore water from days 30 to 120 (Fig. 7).

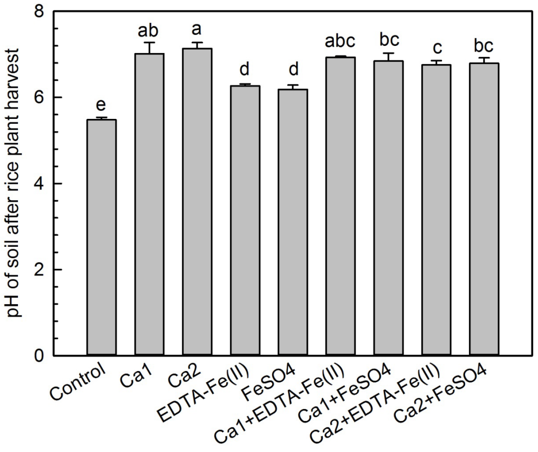

Fig. 5: Effects of CaCO$_3$ (Ca$_1$: 2 g kg^{-1}, Ca$_2$: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and FeSO$_4$: 0.14 g Fe kg^{-1}) and their combinations on soil pH after rice harvest. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

Mn, Cu and Zn and their correlation with Cd in rice plants

The Mn concentrations in the iron plaque and root tissue were reduced by the application of CaCO$_3$ and Fe(II). However, Ca$_2$ and Ca$_2$+Fe(II) (EDTA-Fe(II) and FeSO$_4$) treatment promoted the Mn concentrations in the stems and leaves (Fig. S5). All treatments (CaCO$_3$, Fe(II), and their combinations) significantly increased the Mn concentration in the brown rice compared to the control. The Cu concentrations in the iron plaque and root tissues were reduced by CaCO$_3$ and Fe(II) application (Fig. S6), and those in the stems and leaves were reduced by EDTA-Fe(II) and increased by Ca$_2$+EDTA-Fe(II) treatment. Compared to the control, the Cu concentration in the brown rice was increased by Ca$_2$ and Ca$_2$+Fe(II) and decreased by Fe(II) and Ca$_1$+Fe(II) treatment. Lastly, all treatments significantly decreased the Zn concentration in the iron plaque but did not affect that in the brown rice (Fig. S7).

The Cd concentration in the brown rice was significantly negatively correlated with that of Mn in the brown rice and leaves after CaCO$_3$ treatment (Tab. S1). However, that in brown rice was significantly positively correlated with the concentrations of Fe in the leaves and stems; Fe, Mn, Cu, and Zn in the root tissue; and Cu and Zn in the iron plaque (Tab. S1). After treatment with EDTA-Fe(II) and FeSO$_4$, a significant negative correlation was found between the Cd concentration in the brown rice, that of Fe in the brown rice and stems, and that of Mn in the husks (Tab. S1).

Discussion

Influence of Ca or Fe addition on rice biomass and Fe content

Our findings are similar to those of Huang et al. (2018), who re-

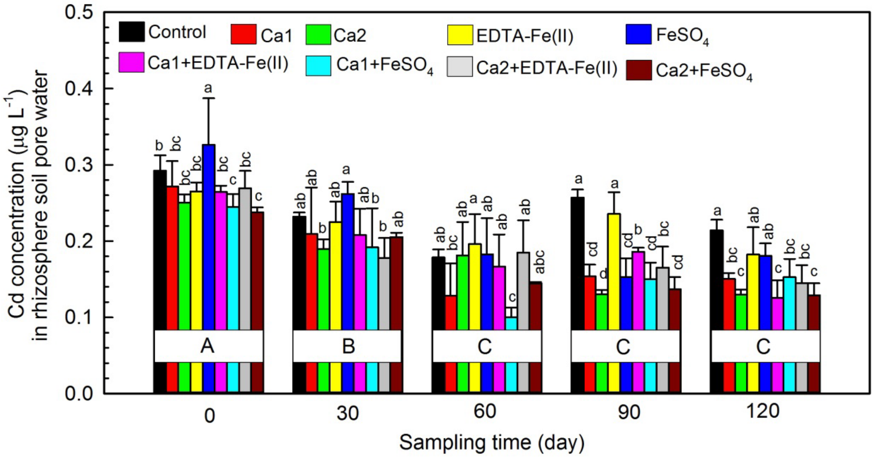

Fig. 6: Effects of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on Cd concentration in rhizosphere soil pore water. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

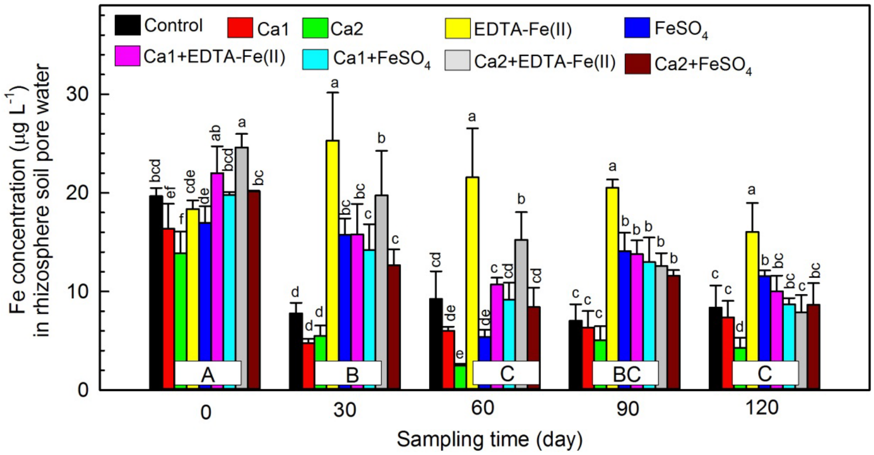

Fig. 7: Effects of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on Fe concentration in rhizosphere soil pore water. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

ported $FeSO_4$ (112 kg ha^{-1} Fe) significantly increased the rice yield by 6.3%. LI et al. (2022) also found that the application of $FeSO_4$ as a basal fertilizer markedly increased the grain yield by 9.2%. Further, the application of EDTA-Fe(II) at 26, 53, and 80 mg kg^{-1} significantly increased maize yield by 20.34%, 30.45%, and 20.84%, respectively (ZHANG et al., 2021). However, the application of high Fe concentrations reduces the biomass of rice plants exposed to heavy metals because of Fe toxicity (LIU et al., 2008; XU et al., 2018). Therefore, an appropriate amount of Fe should be applied to the soil according to actual needs. For acidic soil with light or moderate Cd pollution, the recommended amount of lime is 75-250 kg mu^{-1} $year^{-1}$ (approximately 0.5-1.667 g kg^{-1} $year^{-1}$) (TANG et al., 2019). In the present study, the application of Ca_1 and Ca_2 had no significant effect on rice grain yield. These results are consistent with a study by CHEN et al. (2018), who found that the yield of rice plants under 0.67 mg kg^{-1} Cd at a soil pH of 5.5 was not significantly affected by limestone treatment (1, 2 and 3.33 g kg^{-1}). However, REHMAN et al. (2017) showed that 1 g kg^{-1} limestone increased the rice yield at a soil pH of 7.23-7.54. These differences may be related to factors such as heavy metal exposure and soil pH.

The formation of iron plaque is primarily affected by two factors: radial oxygen loss and the Fe^{2+}content in the rhizosphere microenvironment of plants (ZANDI et al., 2023). Our tillering stage experiment showed that treatment with $CaCO_3$ alone did not significantly affect the amount of iron plaque on the root surfaces of the rice plants but reduced it during the mature stage. On one hand, this may be related to the radial oxygen loss rates in rice plants at different growth stages. MEI et al. (2020) showed that the radial oxygen loss rate in rice roots during the booting stage was higher than that during the seedling and tillering stages, which likely led to varying amounts of iron plaque formation. On the other hand, the reason for this difference may be related to the decrease in Fe^{2+} content in higher-pH environments. Addition of $CaCO_3$ increased the soil pH and thus decreased the available Fe by inducing Fe^{2+} precipitation (CRAVOTTA III and TRAHAN, 1999), which led to a decrease in the Fe content in the pore water. Our results are consistent with those of ZENG et al. (2022) and GUO et al. (2018), who found that the application of oyster shell powder and Ca passivators can reduce the Fe content in pore water by increasing the soil pH. The increased content of Fe in the pore water and root surfaces suggested that Fe(II) or $CaCO_3$+Fe(II) enhanced the formation of iron plaque on the rice plants (Fig. 2 and 3). In fact, the increase in soil pH caused by the Ca passivator was inhibited over time (WANG et al., 2015), and an exogenous Fe(II) supply provides a potential source for promoting the formation of iron plaque.

Coupling effects of Ca and Fe on Cd in rice

Our results showed that the significant increase in soil pH induced by $CaCO_3$ addition and the Fe concentration in rice plants induced by exogenous Fe(II) addition were the two main factors that reduced the Cd concentration in brown rice (Fig. 2, 4, and 6). Ca passivators have a long history of improving acidic soils by increasing soil pH and improving plant growth conditions (CHINTALA et al., 2014). The application of Ca-activating agents to increase soil pH leads to a decrease in available Cd, which is mainly attributed to the formation of $CdCO_3$ (ALI et al., 2022). After removing organic matter, soil containing $CaCO_3$ has a higher adsorption capacity and lower Cd resolution than soil without (ZHAO et al., 2014). SUN et al. (2022) showed that $FeSO_4$ addition reduced soil pH at the rice seedling stage but increased it during the rice harvest period. Further, soil pH significantly increased with the addition of EDTA-Fe (II) (26 and 53 mg kg^{-1} Fe) (ZHANG et al., 2021).

The decrease in the Cd content of the pore water may be attributed to its conversion to $CdCO_3$ and the adsorption of Cd by limestone (LEE et al., 2018; LIM et al., 2013). The application of EDTA-Fe(II) induced the formation of EDTA-Cd, with plant roots absorbing less EDTA-Cd than free Cd (CHEN et al., 2017). However, the opposite result was found by ZHANG et al. (2021), who found that EDTA could increase the Cd content in maize. This may be because the Cd availability in arid and flooded soils differs. Furthermore, iron plaque formation can immobilize Cd on the surface of rice plants but not maize, an upland crop that cannot form iron plaques. In addition, EDTA-Fe(II)-treated root cell walls provide more Cd-binding sites and reduce the mobility of Cd in the xylem (ZHANG et al., 2022). Further, EDTA-Fe(II) and $FeSO_4$ restrict the migration of Cd into rice plants through ion channel competition, thus reducing the Cd content in rice plants (ZHANG et al., 2022). The application of EDTA-Fe(II) and $FeSO_4$ also promotes the formation of iron plaque, thus trapping more Cd on the root surface and reducing the migration of Cd into rice plants (CHEN et al., 2017; HUANG et al., 2018; IRSHAD et al., 2020).

Our experiments showed that the combined application of $CaCO_3$ and Fe(II) did not yield the best Cd reduction effect compared to the addition of only $CaCO_3$ or Fe(II). This may be attributed to the fact that the $CaCO_3$ dosage was not optimal in this study. DONG et al. (2016) showed that five different concentrations of lime treatment could significantly reduce the Pb content in rice grains; however, only treatment with 32 g kg^{-1} lime significantly reduced the Cd. Furthermore, the rice genotype may be another influencing factor. KHALIQ et al. (2019) found no significant difference in Cd reduction of rice (Taigeng8) after the application of 1.5 and 3 g kg^{-1} limestone. However, the Cd content in the grains of three other genotypes (Huyou2, Hanyou737, Yixiang2292) showed a significant decreasing trend with increasing limestone dosage (KHALIQ et al., 2019). Notably, the combined application of exogenous Fe(II) and low-dose $CaCO_3$ (2 g kg^{-1}) reduced the husk-to-grain translocation of Cd in rice, indicating the potential of the combined application of $CaCO_3$ and Fe(II) in inhibiting soil Cd availability and Cd migration into rice. The effect of the combined application of $CaCO_3$ and Fe(II) on limiting Cd enrichment in rice plants should be confirmed by further long-term experiments. WU et al. (2016) showed that the application of a Ca passivator significantly reduced the Pb content in brown rice in the first season but significantly increased it in the second and third seasons. Moreover, the effect of $CaCO_3$ alone on reducing Cd in brown rice in the first season was better than that under a $CaCO_3$+Mn treatment, but the opposite effect was observed in the second season (FANG et al., 2021).

Competition between micronutrients and Cd in rice

Our results indicated that a decrease in Cd content in rice grains was significantly negatively correlated with the Mn content in grains when treated only with $CaCO_3$ and the Fe content in grains treated only with Fe(II). Studies have shown that plants can resist Cd toxicity and reduce their Cd content by improving the absorption of nutrient elements. Cd enters plants through nutrient transport channels, resulting in ion competition between Cd and Fe, Mn, Cu, or Zn (QIN et al., 2020). Exogenous Fe addition can reduce Cd uptake by *Arabidopsis*, while Fe deficiency promotes the enrichment of Cd in peanuts (HE et al., 2017; SU et al., 2014). Both Mn and Zn are antagonists of Cd and can prevent it from entering plants (SARWAR et al., 2015; WANG et al., 2018). CHANG et al. (2020b) showed that OsNRAMP1 and Nramp5 are transporters of both Cd and Mn in root tissue, suggesting competition between Cd and Mn during the transport process. Appropriately increasing the intake of Mn into rice can thus limit the absorption and enrichment of Cd. CHANG et al. (2020a) pointed out that overexpression of *OsNRAMP5* increased Cd and Mn uptake into the roots and resulted in a 49-94% decrease in Cd content in rice grains. These studies provide a guide for appropriate trace element supplementation of rice during the critical heading period through exogenous foliar or basal application to limit the translocation and accumulation of Cd in rice. The concentration of Cd markedly declines with the addition of Cu to rice shoots and roots (CUI et al., 2008). However, elevated Cd levels in the soil will in turn inhibit the uptake of these micronutrients by the plants; a balance between Cd and these mineral elements exists. Through this competitive relationship, researchers are attempting to determine ways to ensure the availability of mineral nutrients required by plants while effectively reducing Cd uptake. Going forward, we aim to assess the effects of this composite treatment on soil Cd availability and uptake by rice by increasing the micronutrient content in the rice via application of Ca or Fe(II).

Conclusion

Our study revealed that EDTA-Fe(II) or $FeSO_4$ treatment both enhanced the formation of iron plaque on the root surfaces, and that the two types of Fe(II) did not lead to significant differences in the amount of iron plaque formed. Further, the application of EDTA-Fe(II) and $FeSO_4$ significantly reduced the uptake and accumulation of Cd into the rice plants. Both doses of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10 g kg^{-1}) significantly reduced the Cd content in the brown rice to below the national standard limit (0.2 mg kg^{-1}) (GB, 2762–2017, China). Notably, application of only $CaCO_3$ weakened the formation of iron plaque and reduced the adsorption of Cd by the plaque. Compared with only Fe(II) treatment, the combined application of Fe(II) and $CaCO_3$ did not significantly affect iron plaque formation, but reduced its Cd content. Further, combined application of $CaCO_3$ and Fe(II) markedly reduced the Cd content of the rice plants, and Fe(II) and low-dose $CaCO_3$ (2 g kg^{-1}) treatment reduced the husk-to-grain translocation of Cd in rice. Further investigation is needed to test the long-term effects of the combined application of $CaCO_3$ and Fe(II) with or without a micronutrient supply on Cd interception and uptake by rice plants.

Acknowledgements

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Conflict of interest

No potential conflict of interest was reported by the authors.

References

ALI, A., LI, M., SU, J.F., LI, Y.F., WANG, Z., BAI, Y.H., ALI, E.F., SHAHEEN, S.M., 2022: Brevundimonas diminuta isolated from mines polluted soil immobilized cadmium (Cd^{2+}) and zinc (Zn^{2+}) through calcium carbonate precipitation: Microscopic and spectroscopic investigations. Sci. Total Environ. 813. DOI: 10.1016/j.scitotenv.2021.152668

Chang, J.D., Huang, S., Konishi, N., Wang, P., Chen, J., Huang, X.Y., Ma, J.F., Zhao, F.J., 2020a: Overexpression of the manganese/cadmium transporter *OsNRAMP5* reduces cadmium accumulation in rice grain. J. Exp. Bot. 71, 5705-5715. DOI: 10.1093/jxb/eraa287

Chang, J.D., Huang, S., Yamaji, N., Zhang, W., Ma, J.F., Zhao, F.J., 2020b: OsNRAMP1 transporter contributes to cadmium and manganese uptake in rice. Plant Cell Environ. 43, 2476-2491. DOI: 10.1111/pce.13843

Chen, H., Zhang, W., Yang, X., Wang, P., McGrath, S.P., Zhao, F.J., 2018: Effective methods to reduce cadmium accumulation in rice grain. Chemosphere 207, 699-707. DOI: 10.1016/j.chemosphere.2018.05.143

Chen, Z., Tang, Y.T., Yao, A.J., Cao, J., Wu, Z.H., Peng, Z.R., Wang, S.Z., Xiao, S., Baker, A.J.M., Qiu, R.L., 2017: Mitigation of Cd accumulation in paddy rice (*Oryza sativa* L.) by Fe fertilization. Environ. Pollut. 231, 549-559. DOI: 10.1016/j.envpol.2017.08.055

Chintala, R., Mollinedo, J., Schumacher, T.E., Malo, D.D., Julson, J.L., 2014: Effect of biochar on chemical properties of acidic soil. Arch. Agron. Soil Sci. 60, 393-404. DOI: 10.1080/03650340.2013.789870

Clemens, S., Aarts, M.G., Thomine, S., Verbruggen, N., 2013: Plant science: the key to preventing slow cadmium poisoning. Trends Plant Sci. 18, 92-99. DOI: 10.1016/j.tplants.2012.08.003

Cravotta III, C.A., Trahan, M.K., 1999: Limestone drains to increase pH and remove dissolved metals from acidic mine drainage. Appl. Geochem. 14, 581-606. DOI: 10.1016/S0883-2927(98)00066-3

Cui, Y., Zhang, X., Zhu, Y., 2008: Does copper reduce cadmium uptake by different rice genotypes? J. Environ. Sci.-China 20, 332-338. DOI: 10.1016/S1001-0742(08)60052-2

Dong, H., Tang, S., Ye, S., Zhao, M., Li, H., Wang, G., 2016: Effect of lime on the transfer of Cd and Pb in the soil-rice cultivation system and their accumulation in the rice grains (in Chinese). Journal of Safety and Environment 16, 226-231. DOI: 10.13637/j.issn.1009-6094.2016.02.044

Duan, Q., Lee, J., Liu, Y., Chen, H., Hu, H., 2016: Distribution of heavy metal pollution in surface soil samples in China: a graphical review. Bull. Environ. Contam. Toxicol. 97, 303-309. DOI: 10.1007/s00128-016-1857-9

Fang, X., Wang, J., Chen, H., Christl, I., Wang, P., Kretzschmar, R., Zhao, F.J., 2021: Two-year and multi-site field trials to evaluate soil amendments for controlling cadmium accumulation in rice grain. Environ. Pollut. 289, 117918. DOI: 10.1016/j.envpol.2021.117918

Fu, Y.-Q., Yang, X.-J., Ye, Z.-H., Shen, H., 2016: Identification, separation and component analysis of reddish brown and non-reddish brown iron plaque on rice (*Oryza sativa*) root surface. Plant Soil 402, 277-290. DOI: 10.1007/s11104-016-2802-8

Fu, Y., Yang, X., Shen, H., 2018: Root iron plaque alleviates cadmium toxicity to rice (*Oryza sativa*) seedlings. Ecotox Environ Safe 161, 534-541. DOI: 10.1016/j.ecoenv.2018.06.015

Guo, J.X., Li, Y.Y., Hu, C., Zhou, S., Xu, H., Zhang, Q.J., Wang, G., 2018: Ca-containing amendments to reduce the absorption and translocation of Pb in rice plants. Sci, Total Environ, 637, 971-979. DOI: 10.1016/j.scitotenv.2018.05.100

He, L.L., Huang, D.Y., Zhang, Q., Zhu, H.H., Xu, C., Li, B., Zhu, Q.H., 2021: Meta-analysis of the effects of liming on soil pH and cadmium accumulation in crops. Ecotoxicol. Environ. Saf. 223, 112621. DOI: 10.1016/j.ecoenv.2021.112621

He, X.L., Fan, S.K., Zhu, J., Guan, M.Y., Liu, X.X., Zhang, Y.S., Jin, C.W., 2017: Iron supply prevents Cd uptake in Arabidopsis by inhibiting *IRT1* expression and favoring competition between Fe and Cd uptake. Plant Soil 416, 453-462. DOI: 10.1007/s11104-017-3232-y

Hu, Y., Cheng, H., Tao, S., 2016: The challenges and solutions for cadmium-contaminated rice in China: a critical review. Environ. Int. 92-93, 515-532. DOI: 10.1016/j.envint.2016.04.042

Huang, B., Li, Z., Li, D., Yuan, Z., Nie, X., Huang, J., Zhou, Y., 2019: Effect of moisture condition on the immobilization of Cd in red paddy soil using passivators. Environmental Technology 40, 2705-2714. DOI: 10.1080/09593330.2018.1449900

Huang, G.X., Ding, C.F., Hu, Z.Y., Cui, C.H., Zhang, T.L., Wang, X.X., 2018: Topdressing iron fertilizer coupled with pre-immobilization in acidic paddy fields reduced cadmium uptake by rice (*Oryza sativa* L.). Sci. Total Environ. 636, 1040-1047. DOI: 10.1016/j.scitotenv.2018.04.369

Irshad, M.K., Chen, C., Noman, A., Ibrahim, M., Adeel, M., Shang, J.Y., 2020: Goethite-modified biochar restricts the mobility and transfer of cadmium in soil-rice system. Chemosphere 242. DOI: 10.1016/j.chemosphere.2019.125152

Khaliq, M.A., Khan Tarin, M.W., Jingxia, G., Yanhui, C., Guo, W., 2019: Soil liming effects on CH$_4$, N$_2$O emission and Cd, Pb accumulation in upland and paddy rice. Environ Pollut 248, 408-420. DOI: 10.1016/j.envpol.2019.02.036

Lee, H.H., Kim, S.Y., Owens, V.N., Park, S., Kim, J., Hong, C.O., 2018: How does oyster shell immobilize cadmium? Arch. Environ. Con. Tox. 74, 114-120. DOI: 10.1007/s00244-017-0453-2

Li, T.T., Li, J.Y., Zhan, X., Wang, X.L., He, B., Cao, F.S., Liao, C.J., Yu, Y.F., Zhang, Z.Y., Zhang, J.H., Li, B., Chen, J.C., Li, H., Zhu, Z.Q., Wei, Y.Y., Hu, J.M., 2022: Application of exogenous iron alters the microbial community structure and reduces the accumulation ofcadmium and arsenic in rice (*Oryza sativa* L.). Nanomaterials-Basel 12. DOI: 10.3390/nano12081311

Lim, J.E., Ahmad, M., Lee, S.S., Shope, C.L., Hashimoto, Y., Kim, K.R., Usman, A.R.A., Yang, J.E., Ok, Y.S., 2013: Effects of lime-based waste materials on immobilization and phytoavailability of cadmium and lead in contaminated soil. Clean-Soil Air Water 41, 1235-1241. DOI: 10.1002/clen.201200169

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. Sci. Total Environ. 394, 361-368. DOI: 10.1016/j.scitotenv.2008.02.004

Liu, W.J., Zhu, Y.G., Hu, Y., Williams, P.N., Gault, A.G., Meharg, A.A., Charnock, J.M., Smith, F.A., 2006: Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (*Oryza sativa* L.). Environ. Sci. Technol. 40, 5730-5736. DOI: 10.1021/es060800v

Mei, X., Li, Q., Wang, H., Fang, H., Chen, H., Chen, X., Yang, Y., Rizwan, M., Ye, Z., 2020: Effects of cultivars, water regimes, and growth stages on cadmium accumulation in rice with different radial oxygen loss. Plant Soil 453, 529-543. DOI: 10.1007/s11104-020-04634-w

Qin, S.Y., Liu, H.G., Nie, Z.J., Rengel, Z., Gao, W., Li, C., Zhao, P., 2020: Toxicity of cadmium and its competition with mineral nutrients for uptake by plants: A review. Pedosphere 30, 168-180. DOI: 10.1016/S1002-0160(20)60002-9

Rehman, M.Z.u., Khalid, H., Akmal, F., Ali, S., Rizwan, M., Qayyum, M.F., Iqbal, M., Khalid, M.U., Azhar, M., 2017: Effect of limestone, lignite and biochar applied alone and combined on cadmium uptake in wheat and rice under rotation in an effluent irrigated field. Environ. Pollut. 227, 560-568. DOI: 10.1016/j.envpol.2017.05.003

Sarwar, N., Ishaq, W., Farid, G., Shaheen, M.R., Imran, M., Geng, M., Hussain, S., 2015: Zinc-cadmium interactions: Impact on wheat physiology and mineral acquisition. Ecotoxicol. Environ. Saf. 122, 528-536. DOI: 10.1016/j.ecoenv.2015.09.011

Siddique, A.B., Rahman, M.M., Islam, M.R., Naidu, R., 2021a: Varietal variation and formation of iron plaques on cadmium accumulation in rice seedling. Environmental Advances 5, 100075. DOI: 10.1016/j.envadv.2021.100075

Siddique, A.B., Rahman, M.M., Islam, M.R., Shehzad, M.T., Nath, B., Naidu, R., 2021b: Influence of iron plaque on accumulation and translocation of cadmium by rice seedlings. Sustainability 13, 10307. DOI: 10.3390/su131810307

Su, Y., Liu, J.L., Lu, Z.W., Wang, X.M., Zhang, Z., Shi, G.R., 2014: Effects of iron deficiency on subcellular distribution and chemical forms of cadmium in peanut roots in relation to its translocation. Environ. Exp. Bot. 97, 40-48. DOI: 10.1016/j.envexpbot.2013.10.001

Sun, X.X., Zhu, J., Tao, R.P., Xiong, H.X., Xu, Y.Q., 2022: Effect of exogenous iron on soil Cd accumulation of rice (in Chinese). Journal of Yangzhou University 25, 74-78. DOI: 10.19411/j.1007-824x.2022.01.014

Tai, Y., Tam, N.F.-Y., Wang, R., Yang, Y., Lin, J., Wang, J., Yang, Y., Li, L., Sun, Y., 2018: Iron plaque formation on wetland-plant roots accelerates removal of water-borne antibiotics. Plant Soil 433, 323-338. DOI: 10.1007/s11104-018-3843-y

Tang, L., Hamid, Y., Zehra, A., Sahito, Z.A., He, Z., Hussain, B., Gurajala, H.K., Yang, X., 2019: Characterization of fava bean (*Vicia faba* L.) genotypes for phytoremediation of cadmium and lead co-contaminated soils coupled with agro-production. Ecotox. Environ. Saf. 171, 190-198. DOI: 10.1016/j.ecoenv.2018.12.083

Wang, M., Yang, Y., Chen, W., 2018: Manganese, zinc, and pH affect cadmium accumulation in rice grain under field conditions in southern China. J. Environ. Qual. 47, 306-311. DOI: 10.2134/jeq2017.06.0237

Wang, X., Tang, C., Mahony, S., Baldock, J.A., Butterly, C.R., 2015: Factors affecting the measurement of soil pH buffer capacity: approaches to optimize the methods. Eur. J. Soil Sci. 66, 53-64. DOI: 10.1111/ejss.12195

Wu, Y.J., Zhou, H., Zou, Z.J., Zhu, W., Yang, W.T., Peng, P.Q., Zeng, M., Liao, B.H., 2016: A three-year in-situ study on the persistence of a combined amendment (limestone+sepiolite) for remedying paddy soil polluted with heavy metals. Ecotoxicol. Environ. Saf. 130, 163-170. DOI: 10.1016/j.ecoenv.2016.04.018

Xu, B., Wang, F., Zhang, Q.H., Lan, Q.Q., Liu, C.Y., Guo, X., Cai, Q.Y., Chen, Y.H., Wang, G., Ding, J., 2018: Influence of iron plaque on the uptake and accumulation of chromium by rice (*Oryza sativa* L.) seedlings: Insights from hydroponic and soil cultivation. Ecotox. Environ. Safe. 162, 51-58. DOI: 10.1016/j.ecoenv.2018.06.063

Xu, W., Li, Y., He, J., Ma, Q., Zhang, X., Chen, G., Wang, H., Zhang, H., 2010: Cd uptake in rice cultivars treated with organic acids and EDTA. J. Environ. Sci. 22, 441-447. DOI: 10.1016/s1001-0742(09)60127-3

Yu, J., Guo, X., Luo, Z., Ding, J., Xu, B., Chen, H., Zheng, C., Li, Y., Chen, Y., Wang, G., 2021: Do brassinosteroids and iron plaque affect the accumulation of As and Cd in rice (*Oryza sativa* L.)? Environ. Technol. Inno. 23, 101660. DOI: 10.1016/j.eti.2021.101660

Zandi, P., Yang, J., Darma, A., Bloem, E., Xia, X., Wang, Y., Li, Q., Schnug, E., 2023: Iron plaque formation, characteristics, and its role as a barrier and/or facilitator to heavy metal uptake in hydrophyte rice (*Oryza sativa* L.). Environ. Geochem. Hlth. 45, 525-559. DOI: 10.1007/s10653-022-01246-4

Zeng, T., Guo, J.X., Li, Y.Y., Wang, G., 2022: Oyster shell amendment reduces cadmium and lead availability and uptake by rice in contaminated paddy soil. Environ. Sci. Pollut. R. 29, 44582-44596. DOI: 10.1007/s11356-022-18727-9

Zhang, Q., Chen, H., Xu, C., Zhu, H., Zhu, Q., 2019: Heavy metal uptake in rice is regulated by pH-dependent iron plaque formation and the expression of the metal transporter genes. Environ. Exp. Bot. 162, 392-398. DOI: 10.1016/j.envexpbot.2019.03.004

Zhang, Q., Huang, D., Xu, C., Zhu, H., Feng, R.-W., Zhu, Q., 2022: Fe fortification limits rice Cd accumulation by promoting root cell wall chelation and reducing the mobility of Cd in xylem. Ecotox. Environ. Safe. 240, 113700. DOI: 10.1016/j.ecoenv.2022.113700

Zhang, X., Zhang, P., Hu, Y.M., Liu, Y.Q., Feng, S.S., Guo, D.W., Dang, X.L., 2021: Immobilization of cadmium in soil and improved iron concentration and grain yields of maize (*Zea mays* L.) by chelated iron amendments. Environ. Sci. Pollut. R. 28, 53161-53170. DOI: 10.1007/s11356-021-14523-z

Zhao, X., Jiang, T., Du, B., 2014: Effect of organic matter and calcium carbonate on behaviors of cadmium adsorption-desorption on/from purple paddy soils. Chemosphere 99, 41-48. DOI: 10.1016/j.chemosphere.2013.09.030

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Supplementary material

Tab. S1: Pearson correlation coefficient between the concentrations of Fe, Mn, Cu, and Zn and Cd in different rice plant tissues.

	grain-Cd[a]	**grain-Cd[b]**	**grain-Cd[c]**
grain-Mn	-0.809**	-0.637	-0.357
grain-Cu	-0.346	0.436	0.085
grain-Zn	-0.377	0.139	-0.071
grain-Fe	-0.641	-0.777*	-0.166
husk-Mn	-0.224	-0.692*	0.140
husk-Cu	-0.662	-0.609	0.072
husk-Zn	0.229	0.466	0.411
husk-Fe	0.132	-0.467	-0.570*
leaf-Mn	-0.748*	-0.464	-0.261
leaf-Cu	-0.461	0.360	-0.526*
leaf-Zn	0.285	0.161	-0.769***
leaf-Fe	0.817**	-0.466	-0.186
stem-Mn	-0.577	-0.581	-0.351
stem-Cu	0.498	0.906***	-0.074
stem-Zn	0.155	-0.027	-0.468
stem-Fe	0.753*	-0.675*	0.352
root-Mn	0.947***	0.576	0.695**
root-Cu	0.825**	0.778*	0.526*
root-Zn	0.909***	0.698*	0.512
root-Fe	0.915***	0.154	0.097
plaque-Mn	0.321	0.201	0.111
plaque-Cu	0.815**	-0.069	-0.189
plaque-Zn	0.717*	0.743*	0.510
plaque-Fe	0.035	-0.486	-0.535*

*Signifcant correlation at $p < 0.05$, **Signifcant correlation at $p < 0.01$, *** Signifcant correlation at $p < 0.001$. [a] means the application of single CaCO$_3$ (control, Ca$_1$ and Ca$_2$). [b] means the application of single Fe(II) (control, EDTA-Fe(II) and FeSO$_4$). [c] means the combinations of CaCO$_3$ and Fe(II) (control, Ca$_1$+EDTA-Fe(II), Ca$_1$+FeSO$_4$, Ca$_2$+EDTA-Fe(II), Ca$_2$+FeSO$_4$).

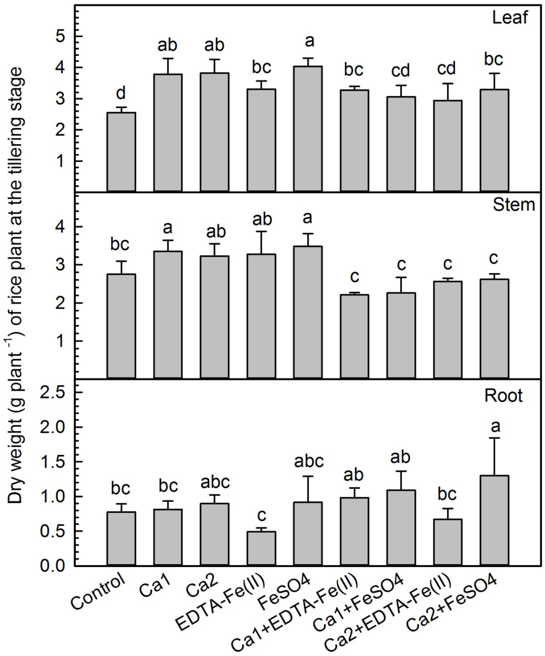

Fig. S1: Effects of CaCO$_3$ (Ca$_1$: 2 g kg^{-1}, Ca$_2$: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and FeSO$_4$: 0.14 g Fe kg^{-1}) and their combinations on biomass of leaf, stem and root of rice plant grown in Cd-contaminated acidic soil at the tillering stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

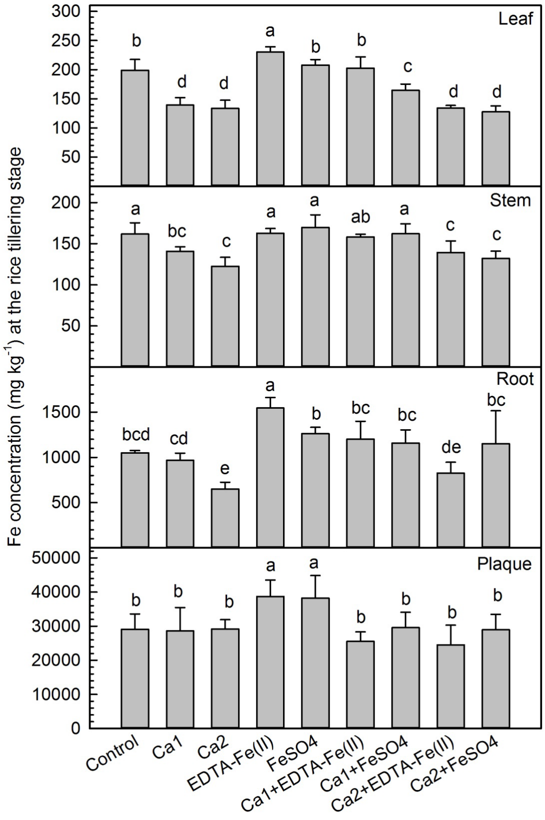

Fig. S2: Effects of CaCO$_3$ (Ca$_1$: 2 g kg^{-1}, Ca$_2$: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and FeSO$_4$: 0.14 g Fe kg^{-1}) and their combinations on Fe concentration in leaf, stem, root and iron plaque of rice plant grown in Cd-contaminated acidic soil at the tillering stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

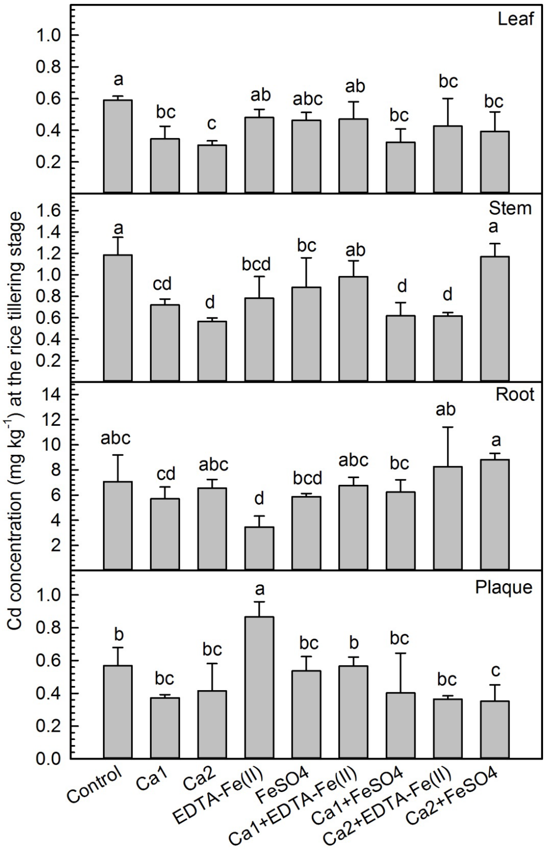

Fig. S3: Effects of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on Cd concentration in leaf, stem, root and iron plaque of rice plant grown in Cd-contaminated acidic soil at the tillering stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

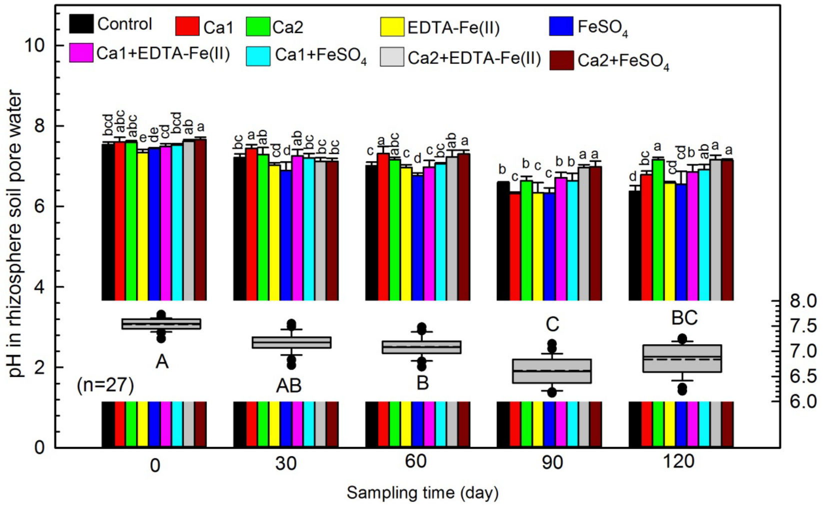

Fig. S4: Effects of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on the pH in rhizosphere soil porewater. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

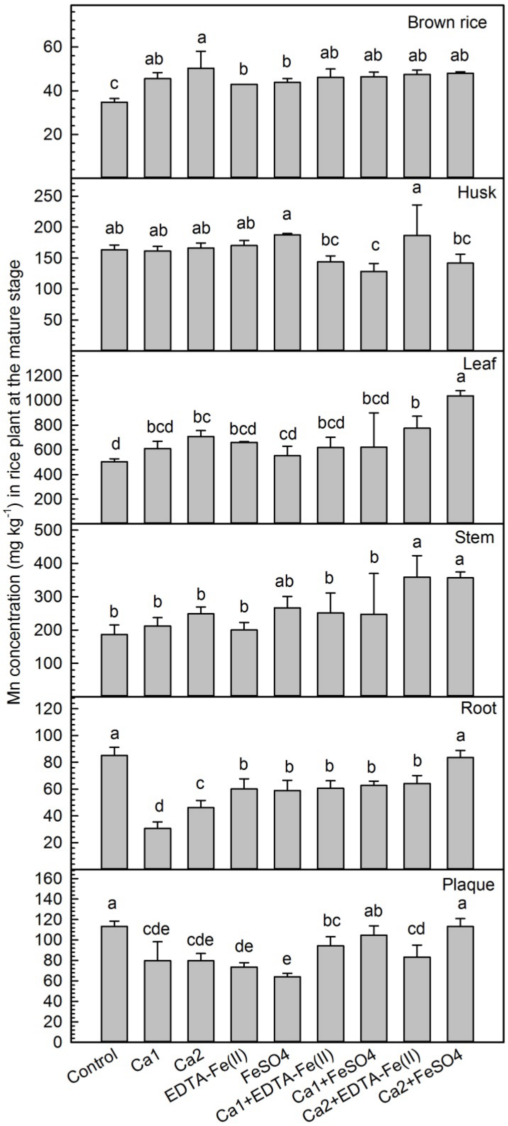

Fig. S5: Effects of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on Mn concentration in brown rice, husk, leaf, stem, root and iron plaque of rice plant grown in Cd-contaminated acidic soil at the mature stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

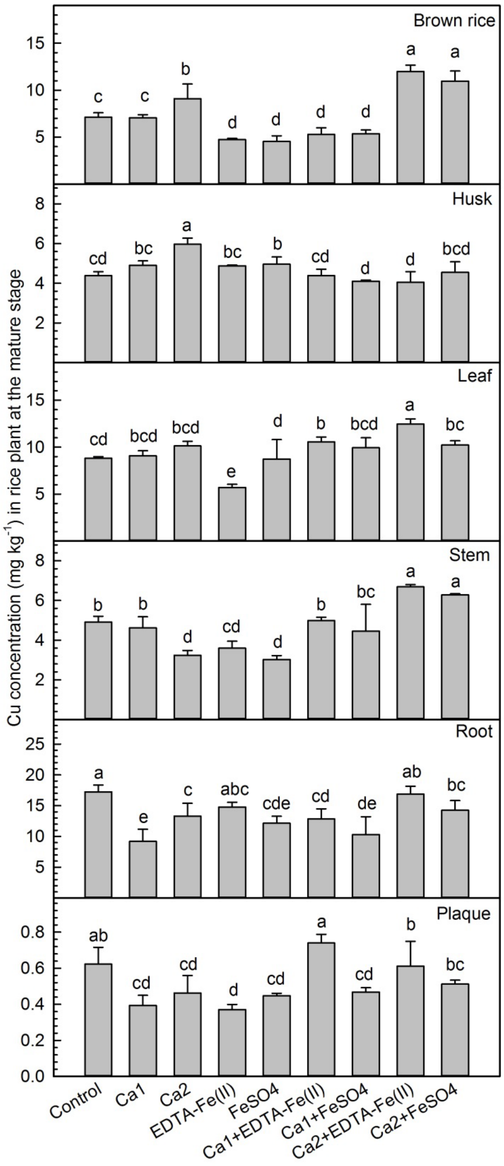

Fig. S6: Effects of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on Cu concentration in brown rice, husk, leaf, stem, root and iron plaque of rice plant grown in Cd-contaminated acidic soil at the mature stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.

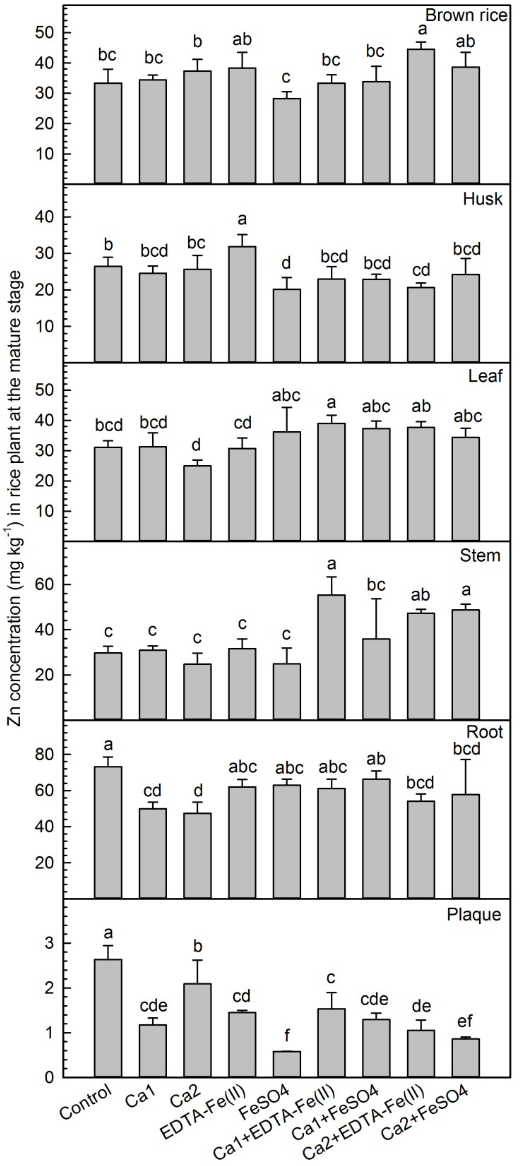

Fig. S7: Effects of $CaCO_3$ (Ca_1: 2 g kg^{-1}, Ca_2: 10g kg^{-1}), Fe(II) (EDTA-Fe(II) and $FeSO_4$: 0.14 g Fe kg^{-1}) and their combinations on Zn concentration in brown rice, husk, leaf, stem, root and iron plaque of rice plant grown in Cd-contaminated acidic soil at the mature stage. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level.