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Regulation of rhizosphere microenvironment by rice husk ash for reducing the accumulation of cadmium and arsenic in rice

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ABSTRACT

It is important to reduce Cd and As content in brown rice in contaminated paddy soils. We conducted research on the effects of rice husk ash (RHA) on the Cd and As in the rhizosphere microenvironment (soil, porewater, and iron plaque) and measured the Cd, As, and Si content in rice plants. The main elements in RHA were Si (29.64%) and O (69.17%), which had the maximum adsorption capacity for Cd was 42.49 mg/kg and for As was 18.62 mg/kg. Soil pH and available Si content increased, while soil available Cd and As decreased following application of 0.5%–2% RHA. RHA promote the transformation of Cd to insoluble fraction, while As was transformed from a poorly soluble form to a more active one. RHA reduced Cd content and increased Si content in porewater, and reduced As only at the later rice growth stages. RHA increased the amount of iron plaque, thereby decreasing the Cd content in iron plaque, while increased the As content in it. Cd and inorganic As content in brown rice were decreased, to 0.31 mg/kg and 0.18 mg/kg, respectively. The decrease of Cd in brown rice was due to the decrease of Cd mobility in soil, thereby reducing root accumulation, while the decrease of As in brown rice was affected by the transport from roots to stems. Therefore, RHA can be considered as a safe and efficient *in-situ* remediation amendment for Cd and As co-contaminated paddy soil.

Introduction

Owing to their persistence, tendency to accumulate, and high toxicity, cadmium (Cd) and arsenic (As) in the soil reduce crop quality and yield, irreversibly harm human body via the food chain, and pose a threat to food safety and human health in Asian countries (Dong et al., 2016; Wang et al., 2022). The available Cd in soil exists in the form of a cation (Cd^{2+}), while As exists as arsenate (AsO_4^{3-}) or arsenite (AsO_3^{3-}). Therefore, their environmental chemical behavior is opposite, and it is difficult to reduce their bioavailability at the same time. The methods and materials used to control Cd polluted soil are not suitable for the treatment of As pollution in many cases (Honma et al., 2016). Then, it is critical to find effective methods to reduce the bioavailability of Cd and As in soil and reduce its accumulation in brown rice.

In-situ remediation is one of the remediation technologies used for soil heavy metal pollution. Through a series of

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reactions of amendments for Cd and As, such as adsorption, precipitation, redox, and ion exchange, the speciation of Cd and As in soil changes, and their bioavailability is reduced, thereby reducing their accumulation in agricultural products (Gu et al., 2018). Multiple combinations of amendments have been studied, including the application of composite amendments for iron and alkaline inorganic materials (Zhai et al., 2020), iron and organic materials (Yao et al., 2019), iron and alkaline inorganic and organic materials (Zhang et al., 2020), and alkaline inorganic and organic materials (Gu et al., 2018). Among the inorganic materials, silicon (Si)-containing material has attracted particular research interest, showing good remediation effects (Seyfferth et al., 2016).

Silicon is an important element in plants, and the application of Si can not only promote plant growth and yield, but also alleviate the toxic effects of heavy metals on plants (Adrees et al., 2015; Meharg and Meharg, 2015). Studies have shown that Si can not only affect the available content of Cd and As in the soil by changing the physical and chemical properties of the soil, thereby affecting their absorption and accumulation in rice, but also affect their migration and transportation by affecting the formation of iron plaque on the root surface of rice (Ning et al., 2014; Ma et al., 2015). For example, application of Si fertilizer (mainly composed of Na_2SiO_3) to soil polluted with 3.51 and 0.53 mg/kg Cd was found to reduce Cd content in brown rice (Li et al., 2018). When different concentrations of sodium silicate solution were applied to simulated As-contaminated soil (soil As content was about 60.0 mg/kg) and four genotypes of rice were planted in flooded soil, the As content in roots, straw, and husks of rice decreased by 28.0%–35.0%, 15.0%–35.0%, and 32.0%–57.0%, respectively; The content of dimethylarsonic acid in brown rice also decreased significantly (Wu et al., 2016).

In the soil-rice system, the important factors affecting the uptake and accumulation of Cd and As in rice are their bioavailability and migration in soil, which are influenced by the rhizosphere microenvironment, including porewater and iron plaque on the root surface of rice. Cd and As in porewater are most easily absorbed by plants, and their concentration is an important index reflecting their dissolved state (Khan et al., 2015). He et al. (2020) applied steel slag, a Si-containing material, and significantly reduced the solubility of Cd in soil, while reducing Cd content in porewater by 30.8%. Zhou et al. (2021) found that a high dosage of steel slag could reduce the quantity of exchangeable As in soil, promote the transformation of As from non-specific adsorption or specific adsorption states to an insoluble state, and significantly reduce the amount of As in porewater. Research also showed that the iron plaque had a strong ability to accumulate heavy metals in the soil, playing an important role in the absorption and transportation of heavy metals in rice (Zhang et al., 2018), but the effect of Si on Cd and As in iron plaque needs to be determined.

To date, the Si-rich materials used to remediate soil heavy metal pollution have mainly been obtained from mineral Si, including various silicates, Si fertilizers, pulverized coal slag, and diatomite. Despite good results, large-scale application is costly and can cause secondary pollution to the environment (Haynes, 2014; Liu et al., 2014). The Si content of the rice plant is >10%. Phytogenic Si prepared from rice straw is easily absorbed by rice and does not cause secondary environmental pollution (Xiao et al., 2014). Previous studies have shown that application of rice husk ash (RHA) to the soil can promote the methylation of As and reduce its content in brown rice (Seyfferth et al., 2016; Teasley et al., 2017; Limmer et al., 2018). The main component of RHA is amorphous SiO_2 (Deshmukh et al., 2012), but the energy spectrum and functional group composition and specific surface area of RHA are still unclear. It is very important to identify the effect of phytogenic Si on Cd and As accumulation in rice plants.

In order to explore effective ways to improve the utilization of phytogenic Si, this study prepared a soil amendment using rice husks as a raw material. After aerobic combustion, the rice husk was treated with HNO_3 to obtain RHA, and its structure and morphology were analyzed. By analyzing the changes of related detection indexes in soil, porewater, and iron plaque of the rice rhizosphere microenvironment after RHA application, the study sought to determine: (1) the mechanism of RHA fixation of Cd and As in the rice rhizosphere; (2) the variational tendency of Cd, As, and Si content in rice plant; and (3) the key factors affecting Cd and As accumulation in brown rice. This research developed our understanding of the mechanisms by which RHA decreases Cd and As bioavailability in soil and their accumulation in rice.

1. Materials and methods

1.1. Test materials

The tested soil was taken from a polluted farmland in eastern Hunan (28°29.232′N and 113°87.540′E), which was severely contaminated by Cd and As due to extensive heavy metal mining and smelting over a long time. The basic physical and chemical properties of the soil were: pH 5.63, organic matter (OM), 32.10 g/kg, total Cd, 2.30 mg/kg, total As, 90.45 mg/kg, available Si, 162.16 mg/kg. The tested rice variety (*Oryza sativa* L.) was Huang-hua-zhan (conventional rice), provided by the Hunan Nongfeng Seed Industry Co., Ltd., China. The chemical reagents used in the test were analytical reagent and produced by Sinopharm Chemical Reagent Company, China.

1.2. Preparation of RHA

Husk powder pretreatment. The contents of the heavy metals Pb, Cd, Cr, As, and Hg in the rice husk were not more than 5 mg/kg. The husk was cleaned of impurities and then crushed into powder with particle size $\leq$ 0.85 mm.

Husk powder ignition. The crushed husk powder was evenly spread on the combustion plate (EG35B, Beijing Laibotaike, China), with a thickness of 0.5–1.0 cm. It was then placed in the combustion furnace (KSW-612, Beijing Yongguang, China) for aerobic initial combustion. The combustion furnace temperature was controlled at (200 ± 5)°C, and the powder was burned for 0.5–1.0 hr under aerobic conditions until there was no black smoke.

Husk powder combustion. The combustion furnace continued to heat up to (600 ± 5)°C at a rate of 15°C/min, burning for 1.5–2.0 hr under aerobic conditions. After natural cooling, husk ash with a gray color and no black impurities was obtained. The husk ash was ground through the sieve, making the particle size ≤ 0.425 mm.

Ash removal and modification. Acid removal and modification were carried out at (25 ± 3)°C. Subsequently, 0.01–0.015 mol/L HNO_3 was added to achieve a solid-liquid ratio of 1:20–1:30 (*m*/*V*), and the mixture was stirred for 1 hr at a rate of 100–200 r/min. After stirring, the leaching solution was filtered through a 0.075 mm sieve to retain the solid.

Cleaning and drying. According to the solid-liquid ratio of 1:20–1:100 (*m*/*V*), the ash was washed repeatedly with deionized water to make the solution pH between 6.5 and 7.5. It was then dried at 60–80°C to obtain a constant weight, producing powdered particles with a particle size range of 0.075–0.149 mm.

This product was RHA and the basic physical and chemical properties of RHA were shown in Appendix A Table S1.

1.3. *Pot experiment*

The rice pot experiment was conducted at the campus test site, where the four seasons are distinct, the rain and heat are in the same period, and the environmental conditions are all in a natural state. The planting time was July 15 to October 15, 2020. Each plastic pot (inner diameter 25 cm, height 29 cm) contained 5.0 kg air-dried test soil and one of four RHA application treatments (*W*/*W*, 0, 0.5%, 1%, and 2%); 0 application rate was used as a control (CK); each treatment had three replicates. After RHA was added to each pot, tap water was added. After stirring evenly, the soil was aged naturally for 15 days with a water layer of 2 cm. Before transplanting rice seedlings, basal fertilizers (0.28 g/kg for urea (N), 0.21 g/kg for $(NH_4)_3PO_4$ (P_2O_5), and 0.22 g/kg for K_2CO_3 (K_2O)) were added for 3 days. The test rice seedlings were transplanted with two plants in a pot; these were raised in farmland soil without heavy metal pollution and consisted of seedlings with five leaves in the same growth stage. Submergence cultivation with a 2 cm deep water layer was maintained, and pesticide and basal fertilizer were supplemented according to the growth of the rice.

In the experiment, the porewater acquisition device (MOM-10 cm, Rhizon, the Netherland) was put into the pot before rice transplanting at a depth of 15 cm, and the porewater was extracted with a 0.45 µm membrane anaerobic closed pumping needle.

1.4. *Sample collection and pretreatment*

Rice plants and corresponding rhizosphere soil samples were collected at plant maturity. The rice plants were washed with ultra-pure water, oven-dried at 105°C and then at 70°C to constant weight, and divided into five parts: root, stem, leaf, husk, and brown rice. Their dry weight was measured and they were then crushed to ensure a particle size ≤ 0.149 mm. The soil samples were naturally air-dried, ground through a 2 mm and 0.149 mm nylon sieve, and sealed for storage. Porewater was collected every 15 days and stored in a 4°C refrigerator, and chemical analysis was completed within 24 hr.

1.5. *Physical and chemical analysis*

The morphological structure of materials in the experiment was studied using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (Sigma HD, Zeiss, Germany). The specific surface area and porosity were measured using specific surface area and porosity analyzers (ASAP 2020 Plus 2.00, Micromeritics, USA). Functional groups of the materials were analyzed using a fourier transform infrared spectrometer (FT-IR) (Nicolet iS10, Themro, USA). Elemental analysis of frozen root slices was conducted using a SEM-EDS (Sigma 300, Zeiss, Germany).

Soil pH was measured with a pH meter (PHS-3C, Shanghai REX, China) under a soil water ratio of 1:2.5 (*m*/*V*). Soil-available Cd was tested with 0.11 mol/L HOAc (Rauret et al., 1999), and soil-available As was tested with 1 mol/L NH_4Cl (Rahman et al., 2014). The modified BCR and Wenzel continuous extraction methods were used to classify the fraction of soil Cd and As (Förstner et al., 1983; Rauret et al., 1999). The content of Cd and As in porewater were determined by adding acid to dissolve the heavy metals, which was then diluted and the solution prepared for detection. To measure the content of Cd in rice various tissues, the plants were digested with HNO_3–$HClO_4$ (*V*/*V*, 4:1). To measure the As content, they were digested with 6 mol/L HCl after a dry-ash treatment. The inorganic As (*i*As) content in the brown rice was measured after extraction with 6 mol/L HCl and heating for 18 hr at 60°C in a water bath. The above four methods were presented in reference (Bao, 2000). Furthermore, the iron plaque over the rice root surface was extracted with a sodium citrate-sodium bicarbonate-sodium disulfite reagent (DCB) (Taylor and Crowder, 1983).

The Cd content of the diluted and digested solution from soil, porewater, plant tissues (root, stem, leaf, husk), and iron plaques was detected by an inductively coupled plasma atomic emission spectrometer (ICP 6300, Thermo, USA) and from the brown rice with a graphite furnace atomic absorption spectrophotometer (ICE-3500, Thermo, USA). The As content of the diluted samples of the digestion solution was determined using an atomic fluorescence spectrophotometer (AFS-8220, Beijing Jitian, China).

The Si content in porewater was measured using the molybdenum blue method with UV–visible spectroscopy (UV-1700, Shimadzu, Japan) at 660 nm (Bao, 2000). The available Si in soil was extracted by citric acid-silico-molybdenum blue colorimetry (0.025 mol/L $C_6H_8O_7$ citric acid) (Yu et al., 2016), while for Si analysis of the rice plant, it was digested with $NaOH$–H_2O_2 (Zhang et al., 2018) and measured at 650 nm using UV-visible spectroscopy.

In the analysis process of all samples, national standard material soil (GBW(E)−070009) and rice (GBW 10045, GSB-23) were used for quality control analysis, and blank experiments were conducted at the same time. The recoveries of Cd, As, and Si were 95.2%–105.1%, 98.6%–107.6%, and 94.4%–108.8%, respectively.

1.6. *Statistical analyses*

Data were analyzed using Microsoft Excel 2007 and SPSS 18.0, and the results were shown as mean ± standard deviation

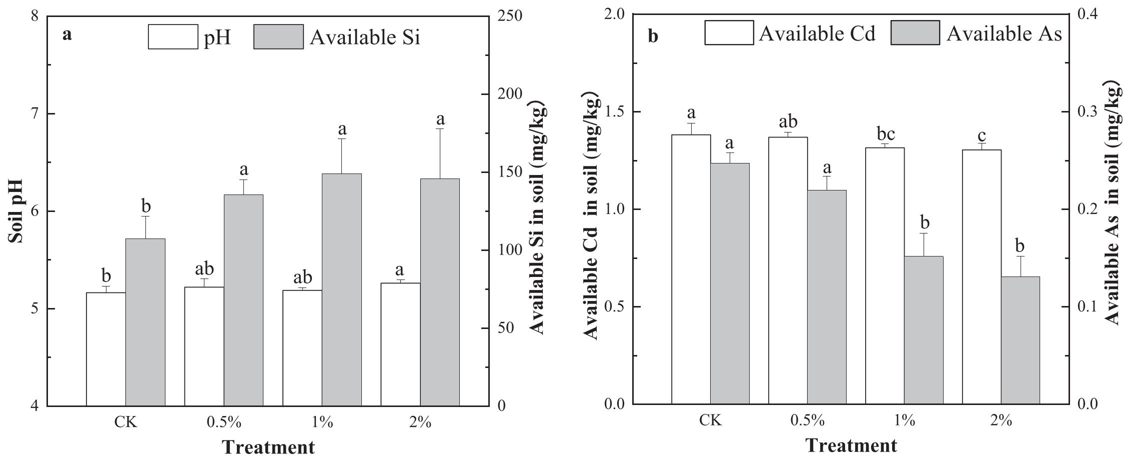

Fig. 1 – Effects of RHA (rice husk ash) application on soil pH, available Si, Cd, and As. Different lowercase letters represent statistically significant differences ($P < 0.05$).

($n = 3$). A one-way ANOVA with Duncan's multiple range test ($P<0.05$) was used to analyze the differences between treatments. Pearson index was used to analyze the correlation of the data. Heat map and Mantel test analysis were completed on the Cloud platform for Xiamen University cooperation. Origin 8.5 was used to prepare figures.

To evaluate the capacity of roots to accumulate Cd and As in soil, the bioaccumulation factor (BCF) was calculated as follows (Wang et al., 2020):

$$BCF_{Cd} = C_{root-Cd}/C_{soil-Cd} \quad (1)$$

$$BCF_{As} = C_{root-As}/C_{soil-As} \quad (2)$$

To examine Cd and As transport capacity from iron plaque to roots and from roots to stems, the translocation factor (TF) was calculated as follows (Wang et al., 2020):

$$TF_{iron\ plaque-Cd} = C_{root-Cd}/C_{iron\ plaque-Cd} \quad (3)$$

$$TF_{iron\ plaque-As} = C_{root-As}/C_{iron\ plaque-As} \quad (4)$$

$$TF_{root-Cd} = C_{stem-Cd}/C_{root-Cd} \quad (5)$$

$$TF_{root-As} = C_{stem-As}/C_{root-As} \quad (6)$$

where, $C_{iron\ plaque\text{-}Cd}$ (mg/kg), $C_{root\text{-}Cd}$ (mg/kg), and $C_{stem\text{-}Cd}$ (mg/kg) are the Cd content in iron plaque, root, and stem, respectively; $C_{iron\ plaque\text{-}As}$, $C_{root\text{-}As}$ (mg/kg), and $C_{stem\text{-}As}$ (mg/kg) are the As content in iron plaque, root, and stem, respectively; $C_{soil\text{-}Cd}$ (mg/kg) and $C_{soil\text{-}As}$ (mg/kg) are the Cd and As content in the corresponding soil.

2. Results

2.1. *Effects of RHA on soil physicochemical properties and available content of CD and as*

Application of RHA increased soil pH and available Si content in the soil and reduced available Cd and As levels (Fig. 1). Compared with the CK treatment, soil pH increased slightly with increasing RHA application, which increased by 0.02–0.10 units, and there was a significant difference ($P<0.05$) at the 2% treatment (Fig. 1a). Soil-available Si content increased by 26.4%–38.6% gradually, and the Si content with 0.5%–2% treatments were significantly different ($P<0.05$) than that with CK treatment. RHA treatment also reduced soil available Cd and As content (Fig. 1b). With RHA application at 0.5%–2%, the amount available Cd and As in rhizosphere soil decreased by 0.9%–5.6% and 11.1%–47.1%, respectively, compared with the CK treatment. The available Cd and As decreased significantly ($P<0.05$) in the 1% and 2% treatments, from 1.38 to 1.30 mg/kg and 0.25 to 0.13 mg/kg, respectively.

2.2. *Effects of RHA application on various fractions of soil CD and AS*

RHA could promote the transformation of Cd to insoluble state in soil, as well as that of insoluble As to obligately adsorbed and non-specifically adsorbed forms (Fig. 2). Compared with the CK treatment, the proportions of acid-extractable Cd (HOAc-Cd), organic bound Cd (Org-Cd), and Fe-Mn bound Cd (Fe/Mn-Cd) decreased with increasing RHA, with a significant difference ($P<0.05$) under the 2% treatment. The proportion of residual Cd (O–Cd) increased from 15.9% to 22.1%, and the difference was significant under the 2% treatment ($P<0.05$) as well. The proportion of non-specifically adsorbed As (NS-As) showed an increasing trend, with a significant ($P<0.05$) increase from 0.6% to 0.8% in the 1% treatment compared with the CK treatment. The proportion of obligately adsorbed As (S-As) also significantly increased ($P<0.05$) from 14.7% to 17.3%. The proportion of amorphous Fe-Al oxide bound As (NqFe/Al-As) showed an increasing trend, although the difference was not significant compared with the CK treatment. The proportion of crystalline Fe-Al oxide combination As (CFe/Al-As) and residual As (O-As) showed a downward trend, and the proportion of O-As under the 2% treatment was significantly different ($P<0.05$).

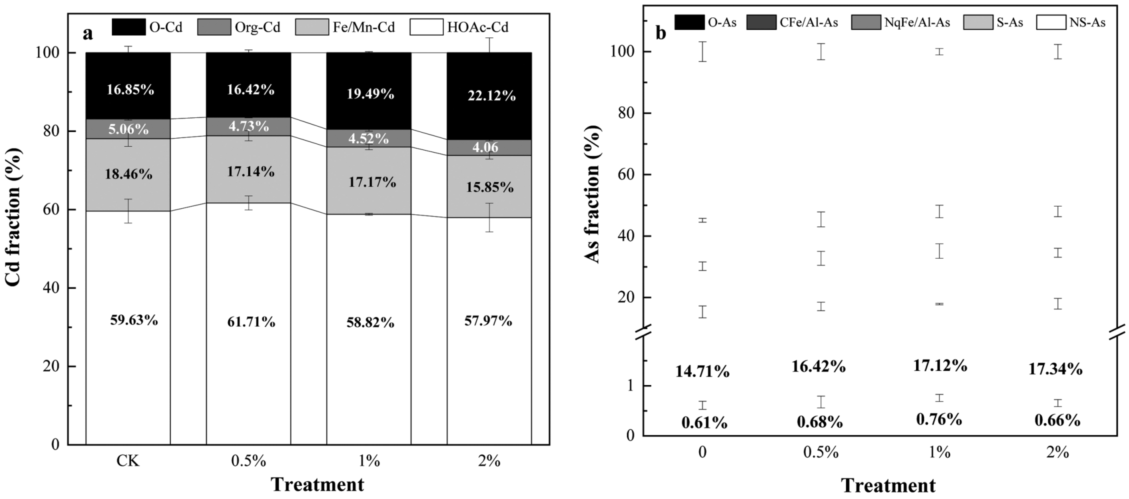

Fig. 2 – Effects of RHA application on various fractions of soil Cd and As. (a) soil Cd fraction, HOAc-Cd: acid-extractable Cd, Fe/Mn-Cd: Fe-Mn bound Cd, Org-Cd: organic bound Cd, O-Cd: residual Cd; (b) soil As fraction, NS-As: non-specifically adsorbed As, S-As: obligately adsorbed As, NqFe/Al-As: amorphous Fe-Al oxide bound As, CFe/Al-As: crystalline Fe-Al oxide combination As, O-As: residual As.

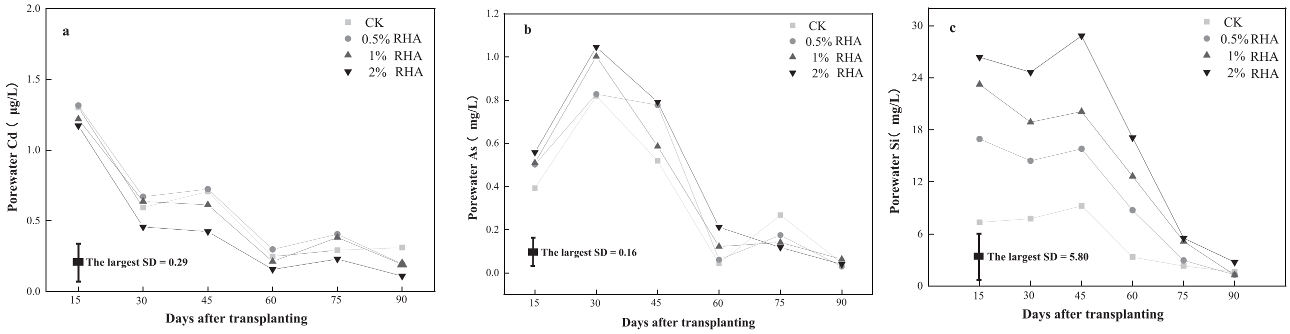

Fig. 3 – The concentration of Cd (a), As (b), and Si (c) in porewater varies with the growth time of rice plant after RHA application.

2.3. *Effects of RHA on the contents of Cd, As, and Si in porewater*

The concentration of Cd, As, and Si in porewater were affected by RHA application as rice growth time lengthened (Fig. 3). The Cd content in porewater decreased gradually from 1.25 to 0.18 μg/L. Compared with the CK treatment, the 2% RHA application significantly ($P<0.05$) reduced the Cd content in porewater on day 15, but there was no significant difference at other detection time points (Fig. 3a). As shown in Fig. 3b, As content in porewater showed an upward trend from day 15 to day 30 and then a significant ($P<0.05$) decrease to day 60. The As content in porewater increased slightly on day 75 and reached its minimum value on day 90. Compared with the CK treatment, the RHA application increased the As content in porewater at the first four monitoring time points, while significantly ($P<0.05$) reducing it from 0.26 to 0.18 mg/L and 0.04 to 0.03 mg/L at the last two monitoring points, respectively. Compared with day 45, the Si content in the porewater decreased by 82.4%, 91.8%, 93.7%, and 90.5% on day 90, respectively (Fig. 3c). The application of RHA significantly ($P<0.05$) increased the Si content in porewater by 69.5%–413.0% compared with the CK treatment, and the increase was at least two times greater at the first five monitoring points. The treatments with 2% RHA resulted in the largest Si content in porewater at each monitoring time point. Therefore, RHA reduced Cd and increased Si content in the porewater, but only reduced As content at the later stage of rice growth.

2.4. *Effect of RHA on the contents of Cd, As, Fe, and Mn in the iron plaque of rice root surface*

The Cd content in iron plaque (DCB-Cd) decreased with increasing RHA application amount (Fig. 4a). DCB-Cd decreased by 8.7%–28.8% after 0.5%–2% application. Under the 2% application, it decreased to its minimum value of 1.67 mg/kg, and there was a significant ($P<0.05$) difference compared with the CK treatment. A large amount of As was accumulated in the iron plaque, with contents in the range of 414.0–551.1 mg/kg, which was much higher than that of the test soil (90.5 mg/kg). The As content in iron plaque (DCB-As) increased by 28.0%–33.1% after the 0.5%–2% RHA application. There were signif-

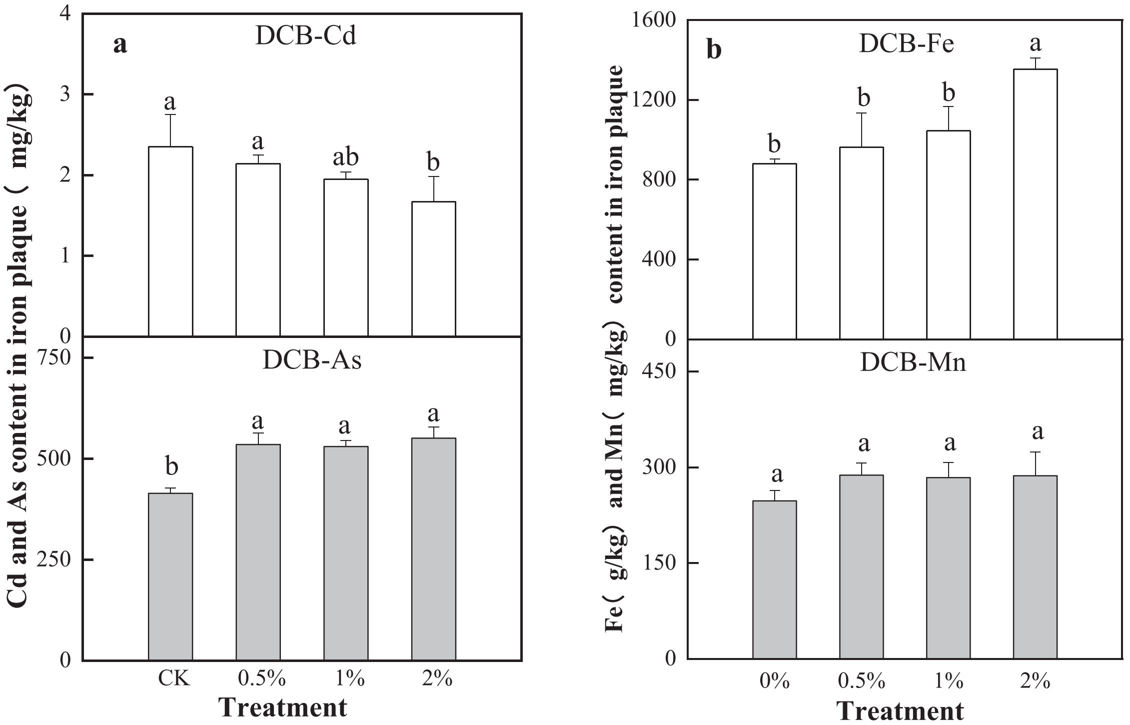

Fig. 4 – Effects of RHA on the content of Cd and As (a), Fe and Mn (b) in the iron plaque. DCB-Cd/As/Fe/Mn means the Cd/As/Fe/Mn content in iron plaque. Different lowercase letters represent statistically significant differences ($P < 0.05$).

icant (P<0.05) differences between RHA and CK treatments, but there was no significant difference among the different RHA treatments. When the RHA application was 0.5%–2%, the Fe content in iron plaque (DCB-Fe) increased by 9.4%–53.7%, although only the 2% treatment had a significant (P<0.05) difference compared to CK treatment (Fig. 4b). The Mn content in iron plaque (DCB-Mn) also increased slightly, but the difference between each treatment and the CK treatment was not significant. Thus, the application of RHA increased the amount of iron plaque on the root surface and concurrently increased the amount of As it contained, but its Cd content was reduced, which indicated that RHA could promote the formation of iron plaque and enhanced its sequestration effect on Cd and As.

2.5. *Effects of rha on absorption and accumulation of Cd, As, and Si in rice tissue*

The application of 0.5%–2% RHA significantly (P<0.05) reduced the Cd content in various tissues of rice plant (Fig. 5a). Compared with the CK treatment, the Cd content in brown rice, husk, stems, leaves, and roots decreased by 36.8%–75.4%, 68.5%–78.4%, 61.1%–83.8%, 37.3%–66.8%, and 34.2%–68.8%, respectively, and the decrease was the largest under the 2% treatment. Moreover, the Cd content in brown rice decreased significantly (P<0.05) from 1.26 mg/kg in the CK treatment to 0.31 mg/kg in the 2% treatment. This is a sharp drop of roughly six times the maximum contaminant level (MCL) of Cd in the Chinese National Food Safety Standards GB2762–2017 for brown rice (0.2 mg/kg).

After RHA application, As content in various tissues of rice plant showed a decreasing trend (Fig. 5b). Compared with the CK treatment, 0.5%–2% RHA treatment significantly (P<0.05) reduced the As content in stems, leaves, and roots by 50.0%–78.8%, 16.8%–82.8%, and 14.9%–38.1%, respectively. When applying 2% RHA, the *i*As content in brown rice decreased to 0.18 mg/kg, which was significantly (P<0.05) lower than CK by 30.8%, and was lower than the MCL of 0.2 mg/kg set in the Chinese National Food Safety Standards GB2762–2017.

The application of RHA increased the Si content in all the tissues of rice plant (Fig. 5c). Compared with the CK treatment, the Si content under the 2% treatment was significantly (P<0.05) higher. Si content in brown rice, husk, stems, leaves, and roots reached their maximum under this treatment, with increases of 29.5%, 16.0%, 49.2%, 43.9%, and 12.1%, respectively.

2.6. *Effects of RHA on translocation of Cd and As in the soil-root system*

According to the results of BCF (Table 1), RHA significantly (P<0.05) reduced the BCF_{Cd} compared with the CK treatment. For As, only the 2% treatment significantly (P<0.05) reduced its BCF_{As}. In addition to this, RHA influenced TF. Compared with CK, the $TF_{iron\ plaque\text{-}Cd}$ and $TF_{root\text{-}Cd}$ showed a decreasing trend with RHA application, and the difference was significant (P<0.05) under the 2% treatment; each RHA treatment significantly (P<0.05) reduced the $TF_{iron\ plaque\text{-}As}$ and $TF_{root\text{-}As}$. This suggested that the application of RHA was able to reduce the Cd bioaccumulation capacity of roots and root-to-stem trans-

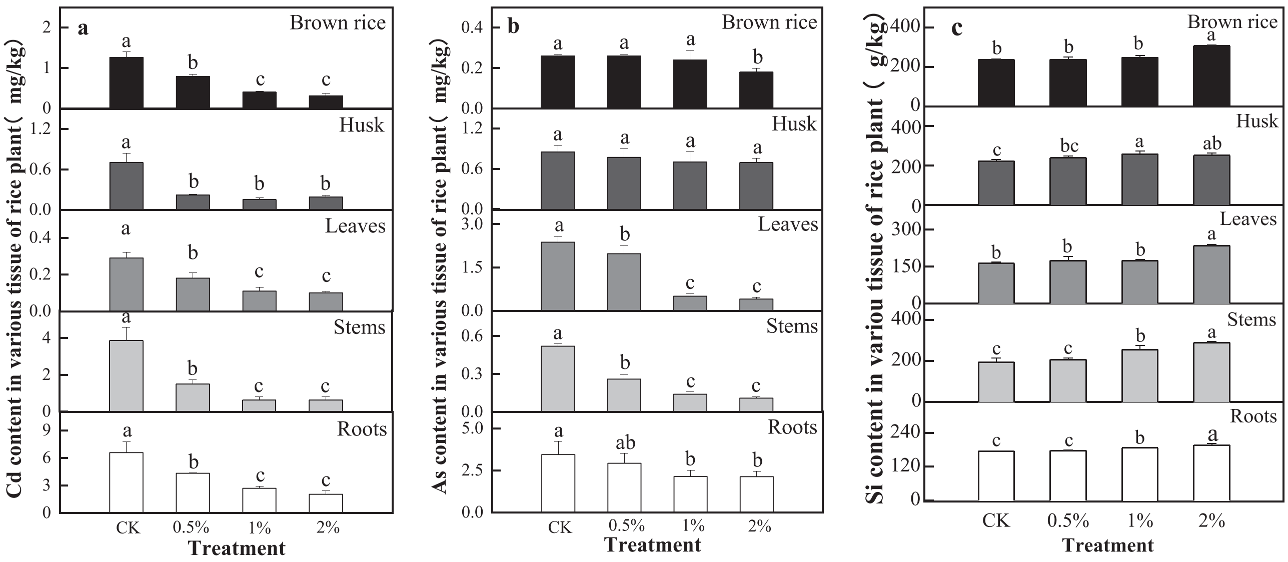

Fig. 5 – Effects of RHA on the content of Cd (a), As (b), and Si (c) in various tissues of rice plant. Different lowercase letters represent statistically significant differences ($P < 0.05$).

Table 1 – Bioaccumulation factor (BCF) of Cd and As from soil to rice roots and translocation factor (TF) of Cd and As from iron plaque to roots and from roots to stems.

Treatment	BCF		TF from iron plaque to roots		TF from roots to stems	
	BCF_{Cd}	BCF_{As}	$TF_{iron\ plaque-Cd}$	$TF_{iron\ plaque-As}$	$TF_{root-Cd}$	$TF_{root-As}$
CK	2.92±0.505a	0.020±0.004a	0.004±0.001a	2.674±0.898a	0.598±0.202a	0.317±0.202a
0.5%	1.93±0.037b	0.011± 0.003ab	0.002± 0.000ab	2.291±0.529b	0.348±0.202b	0.315±0.022a
1%	1.24±0.606c	0.011±0.002ab	0.002±0.000ab	1.616±0.717b	0.297±0.202b	0.163±0.012b
2%	0.92±0.180c	0.011± 0.001b	0.002±0.000b	1.288±0.479b	0.255±0.202b	0.166±0.051b

The data were shown as mean ± SD ($n = 3$). Different lowercase letters represent statistically significant differences ($P < 0.05$).

port, and root-to-stem transport for As was also reduced with RHA application, which fixed it in the iron plaque.

2.7. *Correlation of Cd and As in brown rice with soil and root indexes*

Correlation analysis was performed on soil pH; soil-available Cd, As, and Si content; Cd, As, Fe, and Mn content in iron plaque; Cd, As, and Si content in roots; BCF; and TF. The results showed (Appendix A Fig. S1) that the soil-available Si content was significantly negatively correlated with the BCF_{Cd} and root Cd content, indicating that the increase of available Si was beneficial to reduce the absorption and accumulation of soil Cd by rice roots. It also showed that the root As content was significantly positively correlated with soil-available As, while BCF_{As} was significantly negatively correlated with soil-available Si content. Reducing the available As content and increasing the available Si content in the soil were beneficial to reducing the uptake and accumulation of soil As by the roots. The Mantel test was used to analyze the effects of the above indicators on Cd and As in brown rice. We found that Cd in brown rice was positively correlated with $TF_{iron\ plaque-Cd}$, BCF_{Cd}, soil-available Cd, and root Cd, while As in brown rice had no significant relationship with these indicators.

3. Discussion

3.1. *The mechanism of action of RHA on rhizosphere Cd*

Both SEM and TEM observed that the RHA was tiny spindle-shaped, like the raw rice. TEM exhibited that the fine particles are loosely agglomerated in large quantities with many pores between them (Appendix A Fig. S2). Point spectrum analysis showed that the main elements were Si (29.6%) and O (69.2%). As the particle size meets the requirements of the nanomaterial scale (0.1–100 nm), it can be seen that RHA prepared in the experiment is mainly composed of a large number of loosely aggregated nano-sized SiO_2 particles. FT-IR results showed that RHA contained groups such as -COOH (1700 cm^{-1}), C = C (1480 cm^{-1}), and Si-O-Si (1170 cm^{-1}) (Appendix A Fig. S3). The mechanism of RHA that reduces soil-available Cd and promotes its transformation to an insoluble state involves the C = C group in RHA that can provide a π donor and -COOH functional group, which is conducive to the adsorption and immobilization of Cd (Tan et al., 2022). Concurrently, RHA had a specific surface area of 75.93 m^2/g (Appendix A Table S1) and a certain adsorption capacity for Cd, which was also proved by adsorption experiments (Appendix A Table S2). In addition, RHA could increase the available Si content in the soil (Fig. 1), thereby increasing the content of Si in the porewater of treatment groups. The available Si promoted the chemical reaction of soluble Cd in the soil leading to silicate precipitation, and consequently, Cd moved toward the insoluble state transition; this is also the reason why the Cd content in porewater was lower than that under the CK treatment (Fig. 3). The immobilization of Cd in the soil caused a reduction in Cd adsorbed and immobilized in the iron plaque, resulting in a decrease in the amount accumulated in the root (Fig. 5).

3.2. The mechanism of action of RHA on rhizosphere As

Unlike that for Cd, the effect of Si on As in rice plant is complex and undetermined. Some studies propose that Si can promote the release of As in the solid phase, thereby increasing the absorption and accumulation of As in rice plant (Seyfferth and Fendorf, 2012; Lee et al., 2014), and others have found that Si can reduce the absorption and accumulation of As by competing with it for the transport channel (Tang and Zhao, 2020). In this study, the application of Si transformed the insoluble As in the soil to the more migratory form, which is consistent with the study of Wu et al. (2016). However, it is very interesting that RHA effectively reduced the amount of available As in soil. We believe that the changes in As form are not only related to the competition between Si and As for the adsorption sites to promote the release, but also to the occurrence of As adsorption in RHA itself (Appendix A Table S2), and the fact that RHA can alleviate heavy metal toxicity and promote healthy root growth. This increases radial oxygen loss (ROL) that promotes the formation of iron plaque on rice roots, which fixes available As. Studies have found that ROL can change the various fractions of As and promote non-specific and specific adsorption and amorphous Fe-Al oxide combination with As in rhizosphere soil (Wu et al., 2016). In this study, RHA significantly promoted root growth (Appendix A Table S3). Root tissue became more healthier under RHA application, as demonstrated by elemental analysis of frozen root slices (Appendix A Fig. S4), indicating that RHA alleviates the damage caused by Cd and As to the root system and promotes its growth, which is in turn beneficial to improving ROL. The enhancement of ROL promotes iron plaque formation on the surface of rice roots; as it can strongly adsorb As in soil (Zhang et al., 2018; Wu et al., 2016). This can also be proved by the increase of DCB-Fe and DCB-As (Fig. 4). In addition, RHA also promotes the absorption of nutrient elements by the root system (Appendix A Fig. S4), thereby supporting the formation of iron plaque. The As content in the porewater treated by RHA was lower on day 75 and 90, compared to that under CK treatment; this was due to the adsorption and fixation by the iron plaque. Concurrently, the study found that the key growth stage for As accumulation in rice was the grain filling stage (Deng et al., 2020), which occurred on day 75 and 90, reducing the absorption of As yet further. Therefore, the result suggested that in the rice rhizosphere microenvironment, the RHA can alleviate the harmful effects of Cd and As on rice plant, promote root growth and beneficial to improving ROL, and then increase the amount of iron plaque. As a result, the root As content decreases with the increasing of As amount in the iron plaque.

3.3. Mechanism of RHA on Cd and As accumulation in rice

Silicon is a necessary and beneficial element for rice growth (Li et al., 2014), alleviating abiotic and biological stress (Pontigo et al., 2015; Wang et al., 2017) and improving yield and quality (Liu et al., 2017). Si can alleviate the harmful effects of Cd on rice. In the short-term, Si and Cd form a Si-Cd complex which precipitates on the root cell wall, and this inhibits Cd from entering the cells (Ma et al., 2015; Fan et al., 2016; Cui et al., 2017). In the long term, Si enriched cells reduce Cd content in the cytoplasm through vacuolar compartmentation and stress (Ma et al., 2016). Si application downregulates the expression of the Cd transporters OsNramp5 and OsHMA2 in rice roots (Cui et al., 2017), reducing its absorption and transport. Si application also improves the antioxidant defense ability of cells (Hasanuzzaman et al., 2017; Chen et al., 2019) and alleviates Cd toxicity.

Silicon also mitigates the toxic effects of As because Si ($H_4SiO_4^{\circ}$) and As (AsO_3^{3-}) have similar chemical properties. As enters rice through the Si transport channel proteins Lsi1 and Lsi2 in rice roots and migrates to the vascular bundle through the Si transport channel under the transpiration effect (Ma et al., 2008; Tang and Zhao, 2020). Therefore, increasing the available Si content in soil can significantly inhibit the absorption and transport of As and has been shown to reduce the *i*As content in brown rice (Liu et al., 2014; Meharg and Meharg, 2015). Furthermore, the application of Si also strengthens the function of the antioxidant defense system in rice roots and straw cells. It maintains the balance between the production of intracellular reactive oxygen free radicals and the metabolic system (Seyfferth et al., 2016; Geng et al., 2018), thereby alleviating As stress and promoting the growth of the plant. Elemental analysis of frozen root slices showed that the content of beneficial elements Si and Fe increased, while the presence of the toxic elements Cd and As decreased after 1% RHA application (Appendix A Fig. S4), further confirming the accuracy of the above analysis.

BCF and TF indicate the transport capacity of Cd and As through soil-root, iron plaque-root, and root-stem transport processes, which play crucial roles in differences in Cd and As accumulation in rice. Mantel analysis of Cd and As in brown rice indicated that the reduction of Cd is mainly due to the reduction of Cd mobility in soil caused by the application of RHA, thereby reducing root accumulation. For As, the rhizosphere-related indicators do not have a significant relationship with it (Appendix A Fig. S1). Since the transport of As in rice occurs through the same channel as Si, we tested the correlation between $TF_{root\text{-}As}$ and the As in brown rice content. The Pearson index was 0.586 ($P<0.05$), which indicated that the content of As was affected by transport from roots to stems. Therefore, the reduction of As content in brown rice after RHA application is mainly caused by the reduction of transport from roots to stems.

4. Conclusions

In this study, application of 0.5%–2% RHA increased the available Si content in soil and porewater. RHA application also reduced Cd in the porewater, but only reduced As content at the later stage of rice growth, which alleviated plant damage. It also promoted the formation of iron plaque and enhanced its sequestration effect on Cd and As. RHA fixed Cd in soil, reducing its accumulation in iron plaque and root tissue, thereby reducing its content in the brown rice. RHA reduced the accumulation of As in roots by increasing As content in iron plaque and also reduced As transport from roots to stems, reducing the overall amount in the rice plants. When the RHA application amount was 0.5%–2%, the Cd and As accumulation in rice plants decreased, with Cd content and *i*As reduced by 36.8%−75.4% and 0.30%−30.8%, respectively. In the future, RHA could be used to remediate paddy soil polluted by Cd and As on its own or in combination with other technical solutions. The effects of RHA on ROL and microbial biodiversity also need to be studied.

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.

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.09.005.

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