The Remediation Strategy and Mechanism of Combined Passivation and Foliar Inhibition for Safe Rice Production in Red Paddy Soil Contaminated With Heavy Metals

Fengsong Zhang

Institute of Geographic Sciences and Natural Resources Research CAS: Institute of Geographic Sciences and Natural Resources Research Chinese Academy of Sciences

Lixia He

Taiyuan University of Science and Technology

Guixiang Zhang (✉ zhanggx@tyust.edu.cn)

Taiyuan University of Science and Technology

Zhuo Wei

Louisiana State University

Jiaqi Wang

Institute of Geographic Sciences and Natural Resources Research CAS: Institute of Geographic Sciences and Natural Resources Research Chinese Academy of Sciences

Hongfang Liu

Taiyuan University of Science and Technology

Siyang Wu

Chinese Academy of Environmental Planning

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Abstract

To solve the problem of Cd in rice over the food-safe standard, the present field study was carried out to explore the combined passivators (a mixture of quicklime (Q), polyacrylamide (A), sepiolite (S)) and Si-/Se- containing foliar inhibitors (Si or Se) at low (1) and high (2) application rates were added into the red paddy soil. After harvest the rice, the soil samples were collected to examine the soil properties, bacterial community, and the availability of heavy metals (Cd, Pb, and Cu) in soil. The rice samples were obtained to investigate the accumulation of heavy metals by rice. All of the treatments increased the soil's pH, but the treatments excluding QSe2 decreased the total P and soil organic matter (SOM), which was favourable for the immobilisation of heavy metals in red paddy soil by decreasing 14.29%-42.86% of available Cd, 10.18%-63.17% of available Pb, and 6.95%-36.81% of available Cu. With the increasing application rates, QAS significantly decreased the heavy metals available because of the enhanced immobilisation, while QSi and QSe significantly increased the heavy metals available because of the inhibited plant uptake. After remediation, QA1, QSi2, and QSe2 most effectively decreased the uptake Cd by rice in the present red paddy soil to solve the problem of Cd exceeding the threshold value according to the National Food Safety Standard of China (GB2762-2017). Additionally, the treatments, with the exception of Q1, QA1, QSi1, and QSi2, did not dramatically change the community structure of bacteria at the genus level in soil. Considering the safety and stability of soil, QSe2 was the primary recommendation for remediating Cd-contaminated red paddy soil.

Highlights

- Heavy metal-polluted red soil was remediated by combined passivator and foliar inhibitor
- Increase of pH and decrease of TP and SOM facilitated immobilisation of heavy metals
- The treatments of QA1, QSi2, and QSe2 most effectively decreased rice's uptake of Cd
- For safe utilisation of red soil, QSe2 was primary recommended for its remediation

Introduction

Soil contaminated with heavy metals is a serious worldwide environmental issue (Selvi et al. 2019). Heavy metals pollute about $2.35 \times 10^{12} \text{ m}^2$ of the world's cultivated land (Bermudez et al., 2012). According to China's first national soil pollution survey, 19.4% of farmland sites exceeded the threshold of heavy metals in soil (MLRPRC 2014). The accumulation of heavy metals (i.e., cadmium (Cd), lead (Pb), and cuprum (Cu)) in rice due to soil pollution has become a topic of great public concern (Zhao et al. 2020; Hussain et al. 2020). This is especially true for red paddy soil as the strong acidity led to the high activity of heavy metals and easy uptake by rice (Chen et al. 2019). Therefore, the safe utilisation of red soil contaminated by heavy metal is facing a great challenge (Chen et al. 2019; Bolan et al. 2014).

Passivators have wide applicability to reduce the bioavailability of various heavy metals in soils through adsorption, complexation, and precipitation (Huang et al. 2018; Hussain et al. 2020; Zhao et al. 2020). For

example, quicklime (Q)—one of the most commonly used inorganic passivators—can effectively elevate soil pH and thus effectively passivate the heavy metals in soil (Huang et al. 2018). However, long-term heavy use of lime may cause soil particles to cement together, reduce soil porosity, and decrease the diversity of the microbial community (Song et al. 2017; Jing et al. 2021). Recently, application of quicklime combined with clay minerals such as sepiolite (S) and polymer such as polyacrylamide (A) has received widespread attention, showing a better remediation effect than single quicklime and avoiding the hardening of soils that repeat application of quicklime into soil can cause (Chen et al. 2019). For example, polyacrylamide can interact with clay minerals in soil, and enhance the adsorption affinity of heavy metals in order to decrease rice's uptake of heavy metals (Fijalkowska et al. 2021).

Furthermore, a combination of passivators and foliar inhibitors effective strategy for safe utilisation of heavy metals-contaminated soil (Tang et al. 2020; Xue et al. 2019). For example, foliar Si-/Se- containing inhibitors were used to reduce enrichment and transport of heavy metals in rice (Li et al. 2020a; Wang et al. 2020). The foliar application of Si- or Se-containing inhibitors reduced Cd accumulation in grains by antagonising heavy metals (Wang et al. 2020). Specifically, Si can improve plants' Cd tolerance by regulating heavy metal transport proteases, improving antioxidant ability, and downregulating transcriptions of Cd transporter-related genes (Li et al. 2020a; Wang et al. 2020). The Se can alleviate Cd toxicity by preventing oxidative stress, regulating photosynthesis, and protecting photosynthetic systems (Li et al. 2020a). It can also retard Cd and Pb translocation to rice grains by upregulating the Se-binding protein (Wang et al. 2020). Combined lime application and Se-containing foliar inhibitor significantly decreased the Cd content in husked rice as compared with the single lime application (Xue et al. 2019). At present, most studies focus on the barrier effect that foliar inhibitors play in the uptake of Cd by rice when spraying them in paddy fields, while few other heavy metals are involved (Zhao et al. 2020; Li et al. 2020a). Additionally, studies on the combination of passivators and foliar fertilisation are still scarce, especially in acidic red soil studies. Furthermore, there is a knowledge gap on the changes in soil properties, particularly in the shift of community structure of microorganisms in red paddy soil after application of combined passivators and foliar inhibitors.

Therefore, the aims of this study were to: (1) explore the effects of combined passivators and foliar Si- or Se-containing foliar inhibitors on the availability and uptake of heavy metals by rice in red paddy soil, (2) elucidate the effects of soil remediation practice on physicochemical properties and community structure of bacteria, and (3) identify the practical strategy for safe utilisation of red paddy soil contaminated with heavy metals. The results of this study will helpful provide insight into the immobilisation mechanism of heavy metals in red paddy soil by combined passivators and foliar Si- or Se- containing foliar inhibitors, which will be beneficial for the safe utilisation of such soil.

Materials And Methods

Soil and Chemicals

The passivators used include polyacrylamide, sepiolite, and quicklime. Quicklime ($\text{CaO} > 98\%$) and polyacrylamide (nonionic) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. The sepiolite ($\text{SiO}_2 > 50\%$, $\text{MgO} > 20\%$) was purchased from Shanghai Macklin Biochemical Co., Ltd. All passivators were passed through a 200-mesh sieve before applying for laboratory and field experiments. Jiangsu AgraForUm soil remediation Co., Ltd provided the foliar Si- or Se-inhibitors.

The soil used in screening components of combined passivators is the same as the soil in the field experiment. The surface soil was collected from the heavy metal-contaminated site in Bishan Village, Taihe County, as described in Section 2.3.1. The tested soil was acid red soil. Basic physical and chemical properties: total phosphorus (TP): $462.67 \text{ mg}\cdot\text{kg}^{-1}$, total nitrogen (TN): $2.47 \text{ g}\cdot\text{kg}^{-1}$, soil organic matter (SOM): $24.37 \text{ g}\cdot\text{kg}^{-1}$, cation exchange capacity (CEC): $10.40 \text{ cmol}\cdot\text{kg}^{-1}$, and pH 4.58. The concentrations of total Cd, Pb, and Cu in soil were $0.28 \text{ mg}\cdot\text{kg}^{-1}$, $36.33 \text{ mg}\cdot\text{kg}^{-1}$, and $29.92 \text{ mg}\cdot\text{kg}^{-1}$, respectively. The rocks, gravel, and branches were removed from soils. The air-dried soils were grounded to pass through a 2 mm aperture sieve. Soil pH, SOM, CEC, TN, and TP were analysed according to Lu (2000).

Laboratory Screening Experiment

The laboratory screening experiment was conducted to investigate the passivators' effect for immobilisation of heavy metal in red soil (Table S1). The bioavailability of Cd, Pb, and Cu in soils was determined in soil treated with different kinds of passivators (Fig. S1). Briefly, each passivator was applied to 100 g of soil at the ratio of 5% in a 200 mL round box and then well mixed. The mixture in each round box was flooded with distilled water. The weighing method was adopted to maintain the amount of distilled water in each round box. The samples without passivator were set as the control. After 7 d, all the samples were dried in a drying oven at 60°C . The dried soils were passed through a sieve (2 mm). There were three parallel samples for each treatment.

Field Experiment

This study started in July 2020 in red paddy soil in Bishan Village, Taihe County, Ji 'an City, Jiangxi Province, China ($114^\circ 57' - 115^\circ 20' \text{E}$, $26^\circ 27' - 26^\circ 59' \text{N}$). In May 2020, the grid method was used for controlling the distribution of points and layout of surface soil sampling points: 0.67 ha of demonstration farmland was divided into grids of $25 \text{ m} \times 25 \text{ m}$. A mixed surface soil sample was taken from each grid with a depth of 0-0.2 m, and there were 11 surface soil samples in total. The concentration of Cd in the soil exceeded the standard in China (Soil environmental quality. Risk control standard for soil contamination of agricultural land. GB 15618-2018). Moreover, heavy metals were significantly enriched in the cultivated rice. The concentration of Cd in rice exceeded the threshold value according to the National Food Safety Standard of China (GB2762-2017) (data not provided).

According to the results of laboratory screening experiment (Fig. S1), the effective combined passivators and foliar inhibitors were a single quicklime (Q1 and Q2), a mixture of quicklime and polyacrylamide (QA1 and QA2), a mixture of quicklime and sepiolite (QS1 and QS2), a mixture of quicklime, polyacrylamide,

and sepiolite (QAS1 and QAS2), a mixture of quicklime and Si (QSi1 and QSi2), and a mixture of quicklime and Se (QSe1 and QSe2). The different number after the abbreviation for each combined passivator and foliar inhibitor represents their different application rate (Table S2). There were thirteen kinds of treatments in the field experiment: (1) no combined passivator and foliar inhibitor (CK); (2) single quicklime, Q1; (3) Q2; (4) quicklime and polyacrylamide, QA1; (5) QA2; (6) quicklime and sepiolite, QS1; (7) QS2; (8) quicklime, polyacrylamide, and sepiolite, QAS1; (9) QAS2; (10) quicklime and foliar Si-inhibitors, QSi1; (11) QSi2; (12) quicklime and foliar Se-inhibitors, QSe1; and (13) QSe2. After the combined passivators applied to the surface of the paddy soil were fully mixed with the ploughing soil, rice planting was carried out. Foliar Si- or Se-inhibitors were sprayed twice at the jointing stage and filling stage. A hybrid *indica* cultivar named *Tianyouhuazhan* was planted on July 16, 2020, and harvested on November 1 of the same year.

Sampling And Determination

Five surface soils (0-20 cm) were taken from each grid and mixed into one sample. The three replicated soil samples were collected for each grid. All of the collected soil samples were air-dried at ambient temperature. The rocks, gravel, and branches were removed from the soils. The dried soil was sieved through a 2 mm nylon mesh for further analysis. At the ripening stage, the rice of each plot was collected with three replicates at the location corresponding to the soil sample. All rice samples were ground for further analysis.

To determine the total content of heavy metals in soil and rice, the samples were digested with HF-HNO₃-HClO₄ and HNO₃, respectively. Total amount of Cd in soil was determined by Graphite Furnace Atomic Absorption Spectrometry (PinAAcle 900Z, PerkinElmer, 2015), while total Pb and Cu were determined by Flame Atomic Absorption Spectrometry (PinAAcle 900T, PerkinElmer, 2015). The contents of Cd, Pb, Cu, and Se in rice were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, 7900, Agilent, 2009) (GB5009.268 - 2016). The bioavailable Cd, Pb, and Cu in the soil were extracted by diethylenetriamine pentaacetic acid-calcium chloride-triethanolamine (DTPA-CaCl₂-TEA) buffer solution (pH = 7.3 ± 0.2) (Vrinceanu et al. 2019). The contents of Cd, Pb, and Cu in the extract were determined by Atomic Absorption Spectrometer (AAS, Z-2300, Hitachi, 2008). During the experiment, the soil composition analysis standard substance GBW07405 (GSS-5) and the biological component analysis standard substance (citrus leaves) GBW10020 (GSB-11) were used to control the analytical quality. The total recoveries of Cd, Pb, and Cu in soil and rice samples were 95%-105%.

Data analysis

Statistical analyses were conducted using SPSS 23 software. The statistical difference between the results from different plots were analysed at the significance level of $p < 0.05$ using the one-way ANOVA and Duncan's at the 5% probability level analysis. Polychoric correlation coefficient analysis was used to compare the average values. Statistical significance was determined at 95% confidence interval with the significance level at $p < 0.05$. The "out-of-bag" method was used for Random Forest variable importance

scoring. We used R (version 3.6.1) to determine the Random Forest importance ranking, which provided feedback for indicator (%IncMSE). The larger the calculated value, the greater its importance.

The bioconcentration factors (BCF), as defined in the following equation (Zhang et al. 2017):

$$BCF = \frac{C_{Rice}}{C_{Soil}} \times 100\%$$

where C_{Rice} is the concentration of heavy metal in rice grains, and C_{Soil} is the concentration of total heavy metal in soil.

Results And Discussion

Effects of combined passivators and foliar inhibitors on soil properties

The application of combined passivators and foliar inhibitors into the red paddy soil generally significantly changed the soil's chemical properties (Table 1). All of the combined passivators and foliar inhibitors increased the soil pH as compared with the control ($p < 0.05$ for most of the treatments). Application of quicklime into acidic soil could effectively increase the soil pH because of the decrease in exchangeable acidic cations (Muhammad et al. 2020). Regarding other studied parameters of soil properties, the combined passivators and foliar inhibitors generally decreased TN, TP, and SOM in paddy soil as compared with the control ($p < 0.05$ for most of the treatments). Although the loss of TN and TP in soil could not be excluded, another important reason for the decrease in TN and TP contents after application of the combined passivators was attributed to rice's promotion of nitrogen and phosphorus utilisation (Watson et al. 2016; Cao et al. 2018; Mohammadi and Shariatmadari 2020). Although there was the introduction of organic components in some combined passivators, the decrease in SOM in all the treatments as compared with the control indicated that the combined passivators, especially the inorganic components, enhanced the mineralisation of soil organic carbon (Paradelo et al. 2015; Neto et al. 2021; Wu et al. 2021). The different from the aforementioned soil properties, most of the combined passivators had little influence on CEC in the paddy soil studied ($p > 0.05$ with few exceptions).

The significantly negative correlation between TP and soil pH ($p < 0.05$, Fig. 1) was most likely due to the increase in use efficiency of phosphorus by rice with increasing soil pH (Muhammad et al. 2020; Zhao et al. 2020). A significantly positive correlation was observed between the concentrations of SOM and TN in paddy soil ($p < 0.001$, Fig. 1). The consistent variation trend of TN and SOM was most likely due to the fact that TN in soil was largely related to the balance of accumulation and decomposition of SOM (Groffman et al. 2001).

Effects of combined passivators and foliar inhibitors on the availability of heavy metals in red paddy soil

The combined passivators and foliar inhibitors decreased the available Cd, Pb, and Cu by 14.29%-42.86% (except for QSi2), 10.18%-63.17%, and 6.95%-36.81% (except for QSi2) as compared with the control, respectively ($p < 0.05$, with the exception of very few cases; Table 2). Others have also reported the decrease in the availability of heavy metals in soils with the incorporation of passivators (He et al. 2021). For a given treatment, the trend of the concentration of available heavy metals was $Pb > Cu > Cd$, which was most likely related to their total contents in soil. From the perspective of application rates of combined passivators, the increase of Q1, QA1, and QS1 to Q2, QA2, and QS2 did not significantly change the available heavy metals in soil ($p > 0.05$, with the exception of Cd in Q1 and Q2; Table 2 and Table S2). Therefore, when combined with the quicklime, polyacrylamide, and sepiolite (QAS), the significant decrease of available heavy metals in soil with the increase of QAS1 to QAS2 ($p < 0.05$; Table 2 and Table S2) was most likely due to the interaction of polyacrylamide and sepiolite as they enhanced the immobilisation of heavy metals. Unlike the changes in available heavy metals in QAS-treated soils, the available heavy metals in the soils treated with QSi2 and QSe2 were significantly higher than those in the soils treated with QSi1 and QSe1 ($p < 0.05$; Table 2 and Table S2) (Li et al. 2020a; Wang et al. 2020). This observation was primarily attributed to the fact that foliar Si- or Se-inhibitors prevented the uptake of heavy metals by rice, resulting in the relative high concentration of available heavy metals in soil (Wang et al. 2020; Hussain et al. 2020).

The correlations between the available heavy metals in red paddy soil and soil properties are presented in Fig. 1. The soils treated with the combination of quicklime and foliar inhibitor were excluded in this correlation analysis due to its different mechanism on heavy metals. As seen in Fig.1, there were significant negative correlations between available heavy metals and soil pH ($p < 0.05$ for Cd and Pb, and $p < 0.01$ for Cu), indicating the important role of increasing soil pH on the immobilisation of heavy metals in red paddy soil (Vrinceanu et al. 2019; Mohamed et al. 2015). The increase of soil pH after adding alkaline substances such as quicklime and sepiolite into acidic soil contributed to the release of hydroxyl ions during hydrolysis, thereby promoting the conversion of heavy metal cations to precipitates and residual fraction (Huang et al. 2019). Additionally, soil pH was also suggested as related to the adsorption capacity of soil colloid to heavy metals. It has been reported that the adsorption capacity of soil colloid to heavy metals increases with the rise of soil pH (Liu et al. 2017). The concentrations of available heavy metals were significantly positively correlated with SOM ($p < 0.001$ for Cd, and $p < 0.01$ for Pb and Cu), which indicates the important role of decreasing SOM on the immobilisation of heavy metals in soil (Liu et al. 2015). The effect of SOM on heavy metals could be due to bidentate sites where the SOM-binding sites controlled the release of heavy metals from soils (Liu et al. 2019). The significantly positive correlations between the available heavy metals ($p < 0.05$ for Cd, and $p < 0.01$ for Cu) and TP indicated the positive role of combined passivators in the stabilisation of heavy metals in red paddy soil studied, which was attributable to the formation of insoluble phosphate compounds through the reaction of water-soluble phosphate with heavy metal cations in soil (Jin et al. 2019; Li et al. 2020a; Li et al. 2021).

These results indicate that the change in available heavy metals in soil was not only affected by soil pH, but also by other soil properties such as SOM and TP (Song et al. 2017).

The results from the rank of factors on available heavy metals in soil (Fig. 2) indicated that the dominant factors for available heavy metals were different depending on the types of the heavy metals. The first two dominant factors regulating the available Cd, Pb, and Cu were TN and TP, CEC and TP, and TP and SOM. Evidently, TP was one of the key factors on the three available heavy metals studied.

Effects of combined passivators and foliar inhibitors on the uptake of heavy metals by rice

For heavy metals-contaminated farmland soil, although the concentrations of total and available heavy metals in red paddy soil should not be ignored, the more critically important issue was to prevent and control rice's uptake of heavy metals in order to ensure food safety. From Table 2, the effect of the given combined passivators and foliar inhibitors on the uptake of heavy metals by rice was related to the types of heavy metals. Specifically, the combined passivators and foliar inhibitors significantly decreased rice's uptake of Cd by 22%-93% as compared with the control ($p < 0.05$ excluding QS2), but generally had little influence on rice's uptake of Pb ($p > 0.05$ excluding QA1 and QSe2). The variation of Cu in rice depended on the types and application rates of combined passivators and foliar inhibitors. For example, QA1, QSi1, QSi2, and QSe2 significantly decreased the uptake of Cu in rice ($p < 0.05$), while others had little influence on ($p > 0.05$) or significantly increased ($p < 0.05$) the uptake of Cu by rice.

The significantly positive correlation between available Cd in red paddy soil treated with combined passivators (excluding foliar Si- or Se-inhibitors) and the uptake of Cd by rice ($p < 0.01$) indicated that the decrease of available Cd in red paddy soil studied was one of the effective approaches to control the uptake of Cd (Khan et al. 2017; Jing et al. 2019; Lei et al. 2020; Yang et al. 2020; Huang et al. 2019). Unlike the observation of Cd, there was a significant negative correlation between available Pb in soil and the rice's uptake of Pb ($p < 0.05$), indicating that rice cannot uptake a part of chemical-extractable Pb (Zhang et al. 2016). There was no significant correlation between available Cu in soil and the uptake of Cu by rice ($p > 0.05$). Although the similar reason to the case of Pb could not be ruled out, the reason for finding Cu was most likely due to the fact that exogenous Cu was introduced into soil at varying degrees depending on the amount of Cu in different types and dosages of combined passivators (Table S2 and Table S4). Similar fluctuations were also observed after applying chicken or swine manure into soil (Wan et al. 2020).

With the increasing application dosage of QSi1 and QSe1 to QSi2 and QSe2, the uptake of heavy metals significantly decreased ($p < 0.05$ excluding Pb and Cu in soil treated with QSi). This observation was most likely due to the fact that Si and Se effectively inhibited the uptake of heavy metals because the increasing dosage of quicklime significantly increased their uptake ($p < 0.05$ with the exception of Pb; Table 2) (Li et al. 2020a; Wang et al. 2020). Foliar sprays of Si and Se can inhibit the transportation of

heavy metals from stem to rice or reduce the bio-concentration factor of Cd in the roots of rice (Li et al. 2020a; Guo et al. 2021). The increase of Si or Se with the increasing application dosage of QSi or QSe further confirmed the antagonism of Si and Se with heavy metals in rice.

The results from the rank of factors on uptake of heavy metals by rice (Fig. 2) indicates that the amount of available Cd in soil primary controlled its uptake. This finding further confirmed that the decrease of available Cd in red paddy soil was one of the effective approaches to control the uptake of Cd, as mentioned in Section 3.3. The main factors affecting the uptake of Pb and Cu were pH and CEC, respectively. The result indicates that soil chemical properties in the acidic red paddy soil studied played a more important role in affecting the uptake of Pb and Cu by rice as compared with the concentration of available heavy metals assessed by chemical extraction.

The variation trend of the BCF of heavy metals was similar to that of heavy metal uptake (Table S3 and Table 2). The BCF of Cd in the control was as high as 87.39%, indicating that the rice under natural conditions in this study showed a superb uptake of Cd. The combined passivators and foliar inhibitors significantly decreased the BCF of Cd ($p < 0.05$ excluding QS2), indicating that combined passivators and foliar inhibitors effectively inhibited the transport of Cd from soil to rice. However, the combined passivators and foliar inhibitors had little influence on the BCF of Pb ($p > 0.05$ excluding QA1 and QSe2), indicating that the Pb translocation rate from soil to rice has not changed. This finding was different from a previous study that reported that the combination application of passivators and foliar inhibitors could suppress the formation of Fe/Mn plaques on rice roots and uptake by rice (Li et al. 2020b). The BCF of Cu increased significantly after applying Q2, QS, QAS, and QSe1 ($p < 0.05$), indicating the increased Cu translocation rate from soil to rice. Therefore, the combined passivators and foliar inhibitors should be used carefully for the safe utilisation of Cu-contaminated acidic red soil.

Microbial community response to the addition of combined passivators and foliar inhibitors into red paddy soil

The combined passivators and foliar inhibitors had little influence on the richness (ACE and Chao index) and diversity (Shannon and Simpson index) of bacteria in red paddy soil studied ($p > 0.05$, Fig. S2). However, combined passivators and foliar inhibitors had influence on the bacterial community at the phylum and genus level. The most abundant phylum of microbes found were *Proteobacteria* (15%–23%) and *Chloroflexi* (14%–25%), followed by *Acidobacteria* (11%–17%) and *Actinobacteria* (8%–12%) (Fig. 3). This result is in agreement with Wang et al. (2021) and Wen et al. (2020), who also found *Proteobacteria* and *Acidobacteria* as dominant phyla in soils in southern China and acidic red loam soil contaminated by heavy metals. As compared with the CK, the decrease in the relative abundances of *Acidobacteria* in most treatments was due to the increase in soil pH that was detrimental to the survival of *Acidobacteria* (Wang et al. 2021; Wan et al. 2020; Debnath et al. 2016). At the genus level, the top 53 genera of bacteria were analysed by bistercluster analysis (Fig. 4). The clustering results from different treatments, indicating that the genera of bacteria in the CK were similar to those in the soils treated with

most of the treatments, with the exception of Q1, QA1, QSi1, and QSi2, indicating that most of the treatments did not dramatically change the genus composition of bacteria in the red paddy soil studied. The high similarity of genera of bacteria in soil treated with Q1, QA1, QSi1, and QSi2, which was evidently separated with the other treatment groups. This result indicated that bacteria at the genus level were more sensitive to the quicklime, polyacrylamide, and foliar Si-inhibitors (Liang et al. 2021; Watson et al. 2016).

The application of inorganic and/or organic passivators into soil influenced the composition of the bacterial community via the changes of soil physicochemical properties (Feng et al. 2020; Wang et al. 2021). The redundancy analysis (RDA) was used to examine the relationships between the soil physicochemical properties and the genera of bacteria (Fig. 5). The first two axes for the community composition of bacteria accounted for 23.21% and 13.43% of the total variations, respectively. At the genera level, many bacteria, such as *Subgroup_18_norank*, *Vicinamibacterales_uncultured*, and *Subgroup_17_norank*, each belonging to the phylum of *Acidobacteria*, had a negative correlation with the CEC but a positive correlation with TP. *Xanthobacteraceae_uncultured*, *Xanthobacteraceae_unclassified*, and *SC-I-84_norank* belonging to the phylum of *Proteobacteria* had a positive correlation with the CEC, but had a negative correlation with TP. In addition, pH had a positive correlation with *RBG-13-54-9_norank*, *SBR1031_norank*, and *Anaerolineaceae_uncultured* belonging to the phylum of *Chloroflexi*. These observations indicated that the changes in soil physical-chemical properties influenced the bacterial community structure at the genus level (Ren et al. 2018; Wang et al. 2021).

Fig. 5 displays the correlation between availability of heavy metals and genera of bacteria in paddy soil. The available heavy metals in red paddy soil studied were negatively correlated with the abundance of *Thermodesulfovibronia_uncultured*, indicating the positive role of this genus in the stabilisation of heavy metals. The microbes in the genus of *Thermodesulfovibronia* can reduce SO_4^{2-} to S^{2-} , facilitating the formation of sulfide precipitation (Jiang et al. 2021). However, the available heavy metals are positively correlated with the abundance of *Subgroup_17_norank*, *Vicinamibacterales_uncultured*, and *Subgroup_18_norank* belonging to the phylum of *Acidobacteria*. The phylum of *Acidobacteria* is an acidophilic bacteria that survives better in an acidic environment and thereby has the ability to promote the release of heavy metal cations (Wang et al. 2021; Wen et al. 2020). The available Cd and Cu in red paddy soil studied is negatively correlated with the abundance of *Xanthobacteraceae_uncultured*, *Xanthobacteraceae_unclassified*, and *SC-I-84_norank* belonging to the phylum of *Proteobacteria*, which was similar to the results from Luo et al. (2019) who reported that *Proteobacteria* was sensitive to Cd. These results indicate that adding combined passivators might indirectly affect the heavy metals in the soil by changing the soil bacterial community at the genus levels.

Agricultural and environmental implications

The results in this study showed that applying combined passivators and foliar inhibitors (except for QS2) into the red paddy soil studied could reduce the concentrations of Cd in rice to permitted food-safe levels ($< 0.2 \text{ mg kg}^{-1}$), according to National Food Safety Standard of China (GB2762-2017) (MLRPRC 2014). Additionally, the application of combined passivators and foliar inhibitors did not lead to the other heavy metals being present in excess of standards. Among the combined passivators and foliar inhibitors, QA1, QSi2, and QSe2 showed the best effect for decrease in the uptake of Cd by rice in the red paddy soil studied. After taking the safety and stability of soil structure and function into consideration (Table 1 and Fig. 3), QSe2 ($1875 \text{ kg} \cdot \text{ha}^{-1}$ Quicklime + $250 \text{ mL} \cdot \text{mu}^{-1}$ foliar selenium inhibitors) was the primary recommendation for remediating the heavy metals-contaminated red paddy soil, particularly for remediation of Cd-contaminated red paddy soil.

This study provided an efficient, inexpensive, and easy-to-operate strategy for remediating heavy metals-contaminated red soil in a paddy field. However, different from traditional knowledge, it found that foliar inhibitors may also affect soil's physical-chemical properties and soil bacterial community. Some combined passivators decreased the concentrations of available Cu in soil, but increased Cu uptake in rice. In addition, the change of bacterial community at the genus levels may benefit the stabilisation of heavy metals in red paddy soil. In the future, it is necessary to determine the pathways or mechanisms by which foliar inhibitors affect soil chemistry and microbial structure. Future monitoring is necessary to explore the long-term effects of combined passivators on the safe utilisation of heavy metals-contaminated red paddy soil. Additionally, further studies are needed to investigate the change mechanism of soil physical and chemical properties and to elucidate the interaction mechanism between heavy metals and microorganisms.

Conclusion

Most of the combined passivators and foliar inhibitors increased soil pH, and generally decreased TN, TP, and SOM in paddy soil. Except for QSi2 (combined quicklime and foliar Si-inhibitors), the treatments decreased the soil Cd, Pb, and Cu available. Soil pH, TP, TN, and SOM played an important role in the immobilisation of heavy metals in red paddy soil. The treatments significantly decreased rice's uptake of Cd, which was mainly due to the decrease of available Cd in red paddy soil. Moreover, Si and Se effectively inhibited the uptake of heavy metals. Bacteria at the genus level were relatively sensitive to the quicklime, polyacrylamide, and foliar Si-inhibitors. The shift of bacterial community structure indirectly affected the soil available heavy metals. Except QS2, the concentrations of heavy metals in rice in other treatments were lower than the standards (GB2762-2017 National Food Safety Standard of China). Considering the safety and stability of soil, QSe2 (combined quicklime and foliar Se-inhibitors) was the primary recommendation for remediating the heavy metals-contaminated red paddy soil.

Declarations

Author contribution Fengsong Zhang: Methodology, Original draft preparation, and Funding, Lixia He: Experiment, Data analysis and Graphic production, Guixiang Zhang: Supervision, Writing, Reviewing and

Editing, Zhuo Wei: Reviewing and Editing, Jiaqi Wang: Experiment, Hongfang Liu: Determination of heavy metals, Siyang Wu: Reviewing and Editing

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Data availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval This study was approved by the ethics committee of Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, China.

Consent to participate All the listed authors agree.

Consent to publish Not applicable.

Conflict of interest We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Tables

Table 1 Effect of combined passivators and foliar Si-/Se- containing inhibitors on soil properties. (TP: total phosphorus, TN: total nitrogen, SOC: soil organic carbon, CEC: cation exchange capacity. Different letters above columns indicate significant difference at $p < 0.05$ between treatments at the stage (Tukey, replicate N=3).)

	pH	TP (mg/kg)	TN (g/kg)	SOM (g/kg)	CEC (cmol(+)/kg)
CK	4.58 ± 0.08 a	462.67 ± 18.50 e	2.47 ± 0.13 f	27.25 ± 1.34 gh	10.40 ± 0.46 cd
Q1	4.93 ± 0.10 ab	383.67 ± 43.47 bc	1.74 ± 0.03 a	19.03 ± 0.70 ab	10.00 ± 1.10 bcd
Q2	5.68 ± 0.80 c	441.67 ± 48.95 cde	2.17 ± 0.07 cde	22.47 ± 0.95 de	9.96 ± 0.21 bcd
QA1	5.29 ± 0.22 bc	344.67 ± 39.11 ab	1.96 ± 0.18 abc	19.35 ± 0.78 bc	10.07 ± 0.57 cd
QA2	5.05 ± 0.17 abc	395.33 ± 20.74 bcd	2.41 ± 0.07 ef	23.90 ± 0.72 ef	10.53 ± 0.72 cd
QS1	5.05 ± 0.16 abc	429.67 ± 33.65 cde	2.07 ± 0.04 bcd	21.07 ± 0.59 cd	8.72 ± 0.68 ab
QS2	5.25 ± 0.15 bc	319.67 ± 19.60 a	2.47 ± 0.12 f	25.43 ± 1.12 fg	9.46 ± 0.43 abc
QAS1	5.39 ± 0.60 bc	317.50 ± 12.02 a	2.27 ± 0.18 def	22.95 ± 0.49 de	10.90 ± 0.10 d
QAS2	5.02 ± 0.52 ab	350.33 ± 48.99 ab	1.88 ± 0.14 ab	18.75 ± 2.47 ab	9.36 ± 0.59 abc
QSi1	5.54 ± 0.24 bc	340.33 ± 8.08 ab	2.03 ± 0.05 bcd	21.47 ± 1.76 d	10.21 ± 0.84 cd
QSi2	5.43 ± 0.36 bc	452.00 ± 7.07 de	1.94 ± 0.26 abc	17.20 ± 0.14 a	10.53 ± 1.12 cd
QSe1	5.06 ± 0.07 abc	354.00 ± 29.46 ab	2.36 ± 0.28 ef	22.65 ± 2.05 de	8.66 ± 0.89 a
QSe2	5.55 ± 0.11 bc	399.67 ± 38.73 bcd	2.77 ± 0.19 g	28.00 ± 1.84 h	8.58 ± 0.31 a

Table 2 The contents of available Cd, Pb and Cu in soils and the total contents of Cd, Pb, Cu, and Se in rice. (Cd_{SA}, Pb_{SA} and Cu_{SA} stand for the available heavy metals in the soil. Cd_{RT}, Pb_{RT} and Cu_{RT} stand for the total heavy metals in the rice.

	Cd _{SA} (mg/kg)	Pb _{SA} (mg/kg)	Cu _{SA} (mg/kg)	Cd _{RT} (mg/kg)	Pb _{RT} (mg/kg)	Cu _{RT} (mg/kg)	Se _{RT} (mg/kg)
CK	0.21 ± 0.00 h	6.68 ± 0.42 f	4.89 ± 0.36 e	0.24 ± 0.01 e	0.03 ± 0.02bc	1.86 ± 0.20 cd	0.20 ± 0.00 b
Q1	0.13 ± 0.01 abc	5.11 ± 0.07 bcd	3.77 ± 0.22 bc	0.02 ± 0.00 a	0.04 ± 0.02c	1.78 ± 0.25 cd	0.03 ± 0.00 a
Q2	0.18 ± 0.03 g	5.06 ± 0.46 bcd	4.16 ± 0.23 cd	0.06 ± 0.02 b	0.04 ± 0.01bc	2.38 ± 0.06 ef	0.03 ± 0.01 a
QA1	0.16 ± 0.02 ef	5.89 ± 0.48 def	3.65 ± 0.50 abc	0.02 ± 0.00 a	ND a	1.10 ± 0.11 a	0.03 ± 0.00 a
QA2	0.16 ± 0.00 def	5.75 ± 0.28 cde	4.15 ± 0.27 cd	0.10 ± 0.01 c	0.04 ± 0.00 bc	2.11 ± 0.30 de	0.03 ± 0.00 a
QS1	0.17 ± 0.00 fg	6.00 ± 0.49 ef	4.55 ± 0.29 de	0.18 ± 0.03 d	0.05 ± 0.01 c	2.57 ± 0.28 fg	0.04 ± 0.01 a
QS2	0.18 ± 0.01 g	5.63 ± 0.71 cde	4.12 ± 0.55 cd	0.26 ± 0.03 f	0.03 ± 0.01 b	2.39 ± 0.19 ef	0.06 ± 0.00 a
QAS1	0.15 ± 0.01 cde	5.33 ± 0.46 cde	3.74 ± 0.25 bc	0.19 ± 0.02 d	0.05 ± 0.01 c	2.58 ± 0.36 fg	0.04 ± 0.01 a
QAS2	0.13 ± 0.00 ab	3.46 ± 0.53 a	3.09 ± 0.14 a	0.06 ± 0.00 b	0.04 ± 0.01 c	2.64 ± 0.11 fg	0.04 ± 0.00 a
QSi1	0.14 ± 0.01 bcd	4.91 ± 0.23 bc	3.76 ± 0.10 bc	0.07 ± 0.00 b	0.04 ± 0.01 c	1.55 ± 0.10 bc	0.03 ± 0.00 a
QSi2	0.35 ± 0.00 i	5.85 ± 0.72 def	5.74 ± 0.50 f	0.04 ± 0.00 a	0.04 ± 0.01 bc	1.22 ± 0.04 ab	0.04 ± 0.01 a
QSe1	0.12 ± 0.01 a	4.43 ± 0.07 b	3.35 ± 0.34 ab	0.19 ± 0.01 d	0.05 ± 0.01 c	2.78 ± 0.27 g	0.49 ± 0.04 c
QSe2	0.18 ± 0.01 g	5.53 ± 0.75 cde	4.23 ± 0.24 cd	0.04 ± 0.01 a	ND a	1.26 ± 0.09 ab	1.10 ± 0.07 d

Different letters above columns indicate significant difference at $p < 0.05$ between treatments at the stage (Tukey, replicate N=3).)

ND: not detected.

Figures

Figure 1

Pearson's correlation analysis of soil physicochemical properties, availability of heavy metals and heavy metals in rice. (Cd_{SA}, Pb_{SA} and Cu_{SA} stand for the available heavy metals in the soil. Cd_{RT}, Pb_{RT} and Cu_{RT} stand for the total heavy metals in the rice. Se_{RT} stand for the Se in the rice. * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$.)

Figure 2

Sorting importance of random forest variables. (A: Importance ranking of factors affecting Cd_{SA}; B: Importance ranking of factors affecting Pb_{SA}; C: Importance ranking of factors affecting Cu_{SA}; D: Importance ranking of factors affecting Cd_{RT}; E: Importance ranking of factors affecting Pb_{RT} and F: Importance ranking of factors affecting Cu_{RT}. Cd_{SA}, Pb_{SA} and Cu_{SA} stand for the available heavy metals in the soil. Cd_{RT}, Pb_{RT} and Cu_{RT} stand for the total heavy metals in the rice. Se_{RT} stand for the Se in the rice. TP: total phosphorus, TN: total nitrogen, SOM: soil organic carbon, CEC: cation exchange capacity.)

Figure 3

Effects of combined passivators and foliar inhibitors on the composition and diversity of the soil microbial community: Multiple comparison bar chart.

Figure 4

Heat map of the relative abundances of the predominant genera.

Figure 5

Redundancy analyses (RDA) of the correlations between soil environmental variables and microbial community composition. (CdSA, PbSA and CuSA stand for the available heavy metals in the soil. CdRT, PbRT and CuRT stand for the total heavy metals in the rice. SeRT stand for the Se in the rice. * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$.)

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