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Influence of zero-valent iron and rice husk on As and Cd uptake in rice

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Abstract: Zero-valent iron (ZVI) and rice husk (RH) have potential as adsorbents for heavy metals; however, their effects on iron plaque formation and heavy metal uptake by plants are still unclear. In this study, the impacts of ZVI, RH and their combinations on iron plaque formation on the root surface and the uptake of As and Cd by rice plants were investigated. A pot experiment was performed under waterlogged conditions using $As^{(III)}$- or $Cd^{(II)}$-spiked soil. The results showed that ZVI (0.05% or 0.2%) with or without RH significantly increased iron plaque formation and Fe contents in rice plants and pore water. Under As treatment, ZVI (0.05% or 0.2%) without or with RH obviously increased the As content in plaques and reduced the As content in grains by 67% and 66% and 19% and 24%, respectively. The Cd content was markedly increased in iron plaques and reduced in roots, shoots and grains by ZVI and RH. ZVI (0.05% or 0.2%), RH and their combinations reduced the grain Cd content by 61, 62, 60, 68 and 69%. These findings suggest that ZVI is effective in hindering As and Cd uptake by rice with or without RH in paddies contaminated with As or Cd.

Keywords: paddy soil; bioavailability; adsorption; accumulation; translocation

In recent years, heavy metal pollution in farmland has become serious and widespread in China. According to the National Soil Pollution Status Survey Bulletin, arsenic (As) and cadmium (Cd) have a point exceeding rates of 7.0% and 2.7%, respectively, which may pose a serious threat to agricultural production and food safety (Kang et al. 2020). Excessively high levels of As and Cd not only affect the yield and quality of crops but also result in indirect accumulation in the human body through the food chain (Zhao and Wang 2020). Long-term food consumption with excessively high levels of As and Cd will cause prostate cancer, cardiovascular disease and itai-itai disease (Zhao and Wang 2020). Therefore, it is urgent to develop and explore effective methods to limit the accumulation of As and Cd in crops.

Rice is one of the most important staple food crops and feeds billions of people worldwide. Compared with other cereal crops, As and Cd are more easily absorbed by the rice (Zhao and Wang 2020). Therefore, effectively reducing the migration of As and Cd into rice has become a popular research topic. Studies have shown that rice husk (RH) can aid in improving rice growth as a silicon-rich by-product and obviously increase Cd sorption on soils (Seyfferth et al. 2016, Khan et al. 2017), but the impacts of RH incorporation to soil on As and Cd uptake by rice have not been reported. In addition, numerous studies have been performed on the immobilisation of heavy metals in soil by nanoscale zero-valent iron (NZVI), which can effectively reduce the bioavailability of heavy metals and decrease their uptake

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by plants (Xue et al. 2018). Moreover, NZVI can promote the formation of iron plaques on the root surface, which further limits the migration of heavy metals into plants (Hu et al. 2020). In the application of contaminated soil remediation, however, the potential threats are rather poorly recognised. Some studies have shown that NZVI exhibits cellular toxicity and leads to cell death by producing intracellular responsive oxygen species (von Moos and Slaveykova 2014). NZVI can enter cellular organelles and tissues and result in alterations in the morphology and function of cells and tissues (Liu et al. 2013). Microscale ZVI (MZVI) application may be a potentially more environmentally-friendly approach to the immobilisation of As and Cd in farmland and food security; however, current information on the effects of MZVI on As and Cd uptake by rice plants is still limited.

Based on the above considerations, the aims of our study were to explore: (1) Could exogenous MZVI, RH and their combinations decrease the migration of As and Cd in rice?; (2) Could MZVI or RH enhance iron plaque formation to further alter bioavailability and distribution of As and Cd? To explain these questions, a soil culture experiment was designed to investigate the concentrations of As and Cd in rice plants (grain, shoot, root, and iron plaque) exposed to As and Cd *via* exogenous addition of MZVI, RH and their combinations.

MATERIAL AND METHODS

Soil collection and treatments. Paddy soil was collected from Shufang township, Nanping city, Fujian province, and put into a plastic cylinder (3.5 kg soil) after sieving through a 3 mm sieve. Exogenous $NaAsO_2$ (30 mg As/kg) and $Cd(NO_3)_2 \cdot 4\ H_2O$ (3 mg Cd/kg) were spiked into the soil according to our previous study (Xu et al. 2020), and ZVI (0.05% and 0.2%) or RH (5%) was also added to the soil, which was fully stirred and then immersed for 1 month using deionised water. ZVI (100 mesh) was purchased from Nangong Xindun Alloy Spraying Co. Ltd. The RH (collected from Dingjiabao village, Liuerbao town, Liaozhong county, Shenyang city, Liaoning province) was ground and sieved through a 100 mesh sieve. The physicochemical properties of the soil, including pH, electrical conductivity (EC), and the total contents of Cd, As, C and N, were derived from our previous study (Xu et al. 2020). There were a total of 12 different treatments for As or Cd.

Rice seedling cultivation and harvest. Rice seeds (Yongyou 9) were surface-sterilised with 30% (*v/v*) H_2O_2 solution for 15 min, washed thoroughly with deionised water, and then germinated in soil. After 10 days, uniform rice seedlings were selected and transplanted into plastic cylinders. Before transplanting the rice seedlings, pore water was collected once using a rhizon sampler (Rhizosphere Research Products, Wageningen, the Netherlands), which was inserted vertically into the soil and further collected at 30, 60, 90 and 120 days after transplanting. At harvest, the seedlings were divided into roots, shoots and grains. The fresh roots were washed thoroughly with deionised water and incubated in a 100 mL beaker for iron plaque extraction using a modified dithionite-citrate-bicarbonate (DCB) method, according to Xu et al. (2020). After DCB extraction, the fresh roots, shoots and grains were oven-dried at 70 °C to a constant weight. The dried samples were ground and digested, according to Xu et al. (2020).

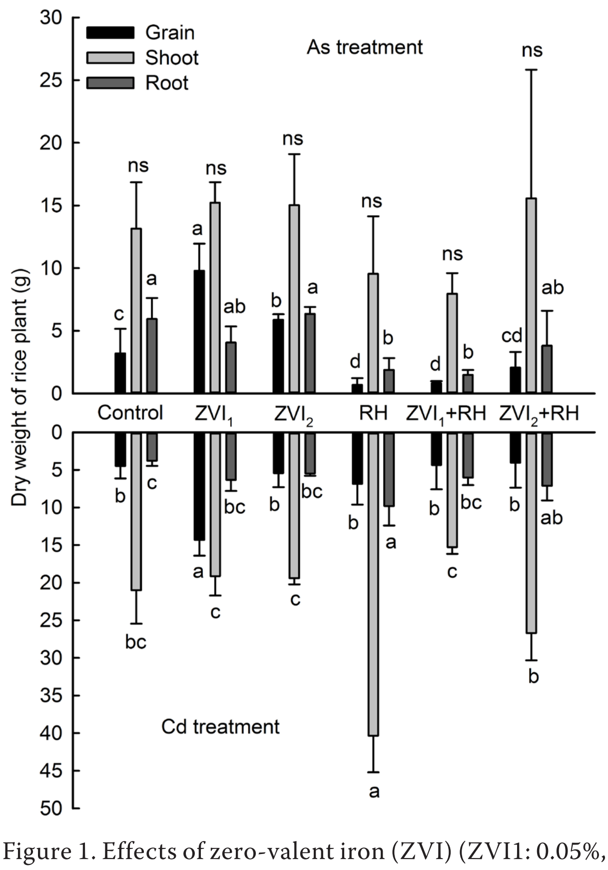

Figure 1. Effects of zero-valent iron (ZVI) (ZVI1: 0.05%, ZVI2: 0.2%), rice husk (RH) (5%) and their combinations on the biomass of root, shoot and grain of rice plant grown in As or Cd spiked soil. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level; ns – not significant

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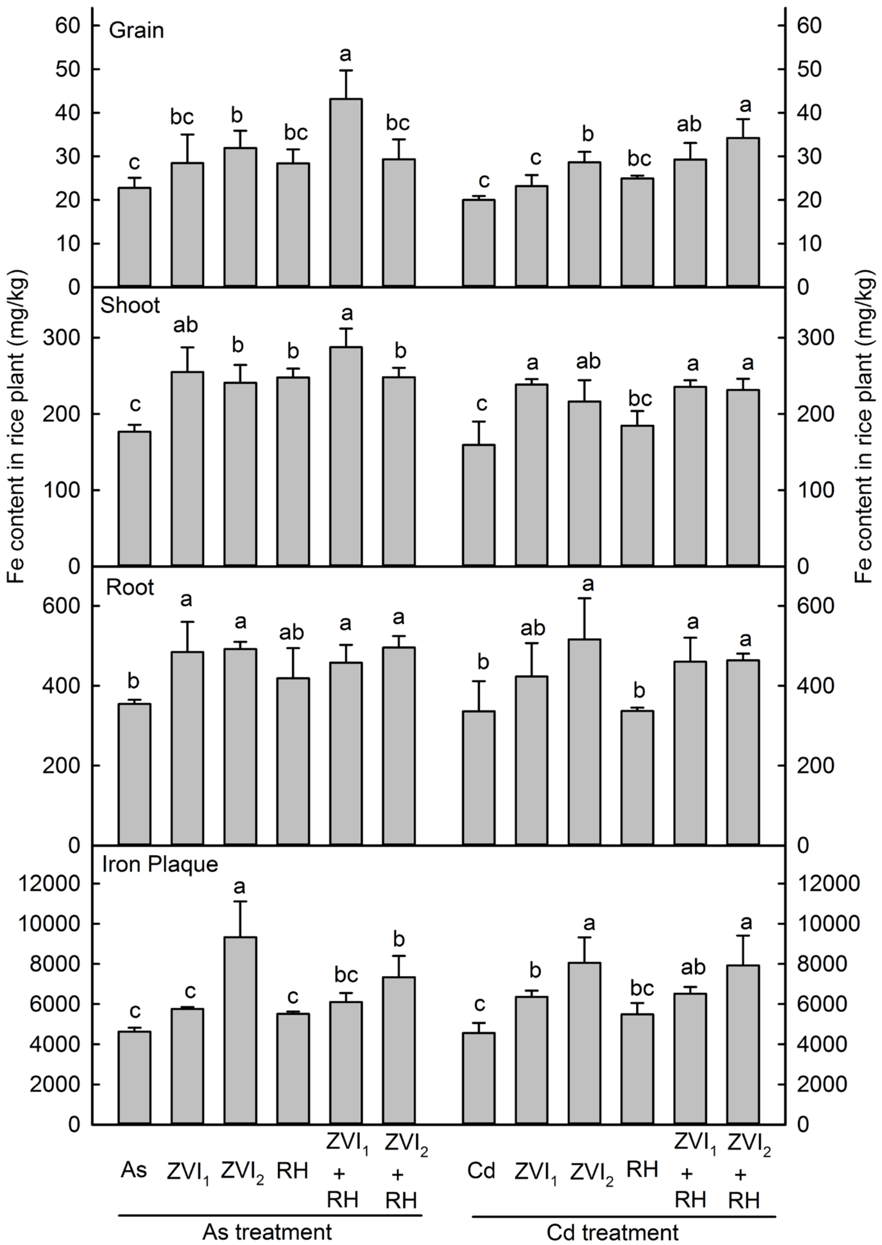

Figure 2. Effects of zero-valent iron (ZVI) (ZVI1: 0.05%, ZVI2: 0.2%), rice husk (RH) (5%) and their combinations on Fe content of iron plaque, root, shoot and grain of rice plant grown in As or Cd spiked soil. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level

Statistical analysis. Data, presented as the means ± standard error (n = 3), were analysed using the least significant difference (*LSD*) at the 5% level. One-way ANOVA was performed using SPSS software (19.0, SPSS, Inc., Chicago, USA) to detect significance (P < 0.05). The effects of ZVI, RH and their combinations on the plant biomass and the contents of Fe, As, and Cd in pore water and rice plants exposed to As or Cd stress were tested.

RESULTS AND DISCUSSION

Influence of ZVI and RH on biomass and the formation of root iron plaques. Under the As a treatment, the biomass of rice grain was significantly increased by ZVI (0.05% and 0.2%) but decreased by the application of RH and RH + ZVI (0.05%) (Figure 1). The biomass of rice shoots was not affected by the application of NZVI, RH or their combinations. The root biomass was markedly decreased by the application of RH and RH + ZVI (0.05%). Under Cd treatment, only 0.05% ZVI addition significantly increased the biomass of rice grains (Figure 1). The biomass of shoots and roots was observably promoted by RH application. A recent report by Liu et al. (2020) showed that the biomass of 30 and 70-day-old rice plants increased with 100 mg/kg NZVI in paddy soil contaminated with pentachlorophenol but de-

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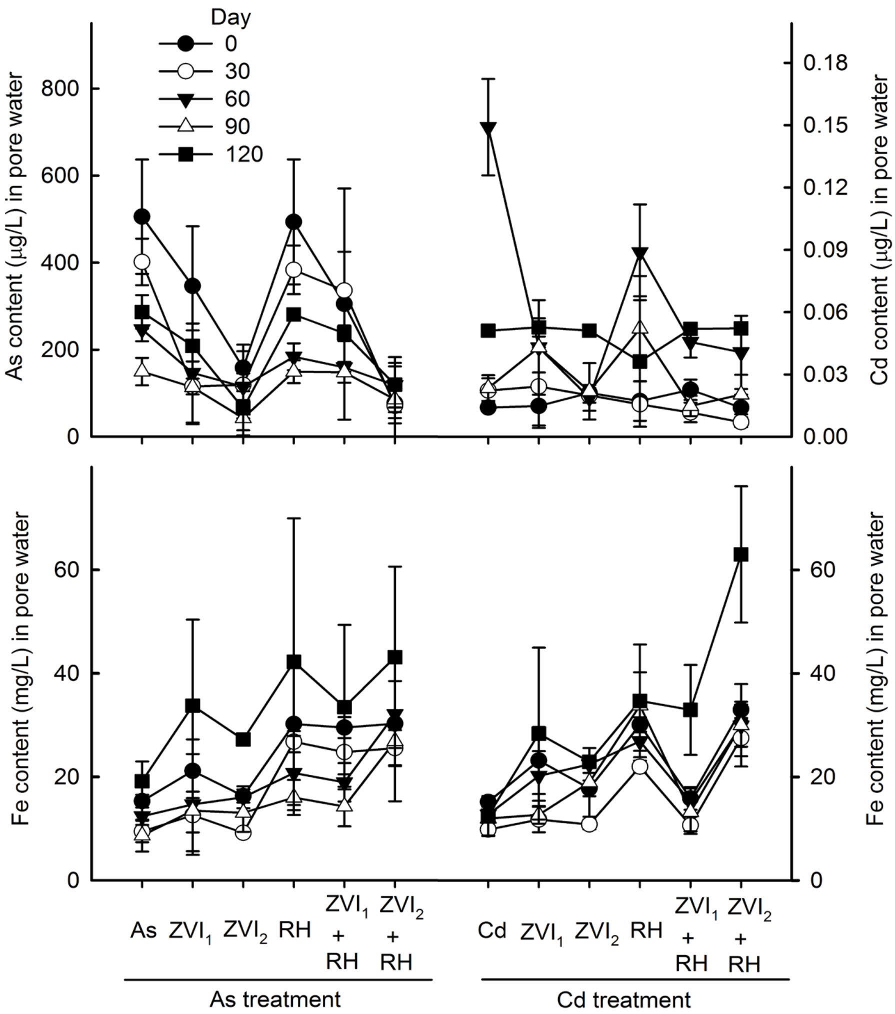

Figure 3. Effects of zero-valent iron (ZVI) (ZVI1: 0.05%, ZVI2: 0.2%), rice husk (RH) (5%) and their combinations on the contents of As, Cd and Fe in pore water in As or Cd spiked soil. Error bars represent the standard error (*n* = 3)

creased with 1 000 mg/kg NZVI. The application of 0.05% and 0.62% ZVI increased the biomass of the shoots and roots of rice plants grown in soil under As and Cd treatments (Qiao et al. 2018, Mlangeni et al. 2020), which suggest that microscale ZVI can promote plant growth. The adsorption of As and Cd by ZVI and RH will reduce their adverse effects on rice growth. Fe and Si could enter the rice to compete with As and Cd, which indirectly alleviated their toxicity. Furthermore, ZVI can affect the biomass by providing Fe^{2+} and enhancing iron plaque formation to decrease the bioavailability of As and Cd (Qiao et al. 2018).

The Fe contents in rice plants were consistent under As or Cd treatment (Figure 2). The application of ZVI (0.05% and 0.2%) and RH + ZVI (0.05% and 0.2%) significantly increased the Fe content in iron plaques, roots, shoots and grains. No significant effect on the Fe content in rice plants was found with RH addition alone. The order of the Fe content in rice plants from highest to lowest was as follows: iron plaques > roots > shoots > grains. Under As or Cd treatment, the application of ZVI (0.05% and 0.2%), RH and their combinations increased the Fe content in pore water, which varied from 8.6 to 63 mg/L (Figure 3). Our result was in line with the findings of Qiao et al. (2018), who showed that ZVI (0.05%) enhanced iron plaque formation in rice plants under As stress, and Hu et al. (2020), who found that the amount of Fe in iron plaques can be increased with more than 0.2% ZVI addition. Liu et al. (2020) showed that NZVI at 100 or 1 000 mg/kg altered root morphology by inducing the formation of iron plaques in rice plants. The increase in Fe content in pore water also explained the enhanced formation of iron plaques under the application of ZVI, RH and their combinations (Figure 3). However, Liu et al. (2020) found that the addition of 100 or 1 000 mg/kg NZVI significantly increased the Fe content in roots and iron plaques, but NZVI at 1 000 mg/kg decreased

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the Fe content in grains, stems and leaves, which indicated a toxic effect on rice plants due to altered micronutrient uptake.

Changes in As and Cd contents in rice plants under ZVI, RH and their combinations. Under As stress, ZVI application significantly promoted the As content in iron plaques but reduced the As content in roots, shoots and grains (Figure 4). No significant difference was found for As content in rice plants with RH addition. The combinations of ZVI and RH markedly increased and decreased the As content in iron plaques and grains, respectively. The As reduction in grains was 67% and 66% under 0.05% and 0.2% ZVI addition and was 19% and 24% under the RH + 0.05% ZVI and RH + 0.2% ZVI treatments, respectively. The Cd content was significantly increased with the application of ZVI (0.05% and 0.2%), RH and their combinations in root iron plaques but decreased in root tissues (Figure 5). The application of 0.2% ZVI with or without RH allowed more Cd to be adsorbed on the root surface. A decrease in shoot Cd content was caused by ZVI (0.05% and 0.2%), and this effect was obvious under RH and its combination with ZVI (0.05% and 0.2%).

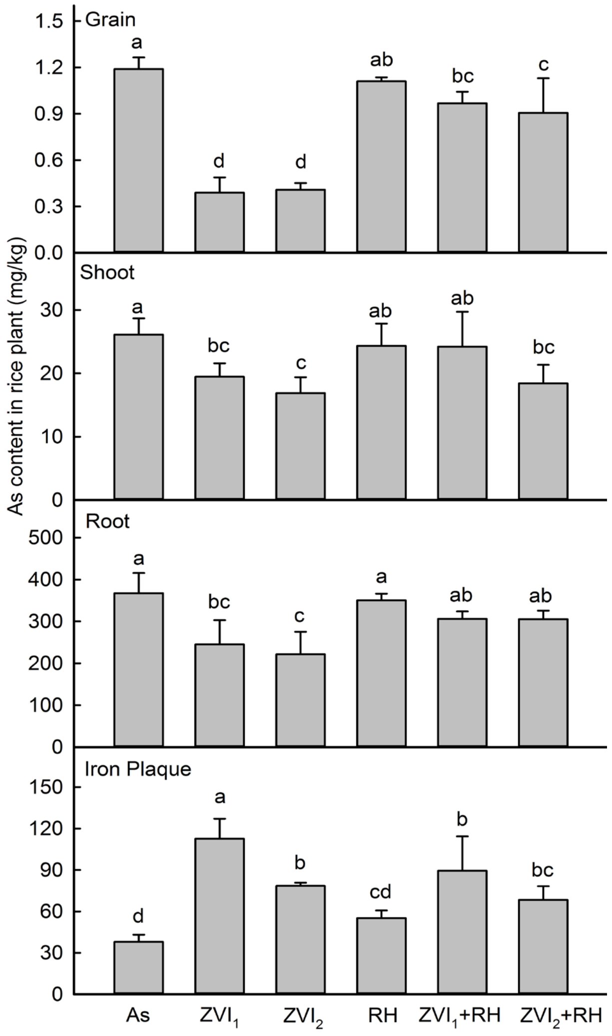

Figure 4. Effects of zero-valent iron (ZVI) (ZVI1: 0.05%, ZVI2: 0.2%), rice husk (RH) (5%) and their combinations on As content of iron plaque, root, shoot and grain of rice plant grown in As-spiked soil. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level

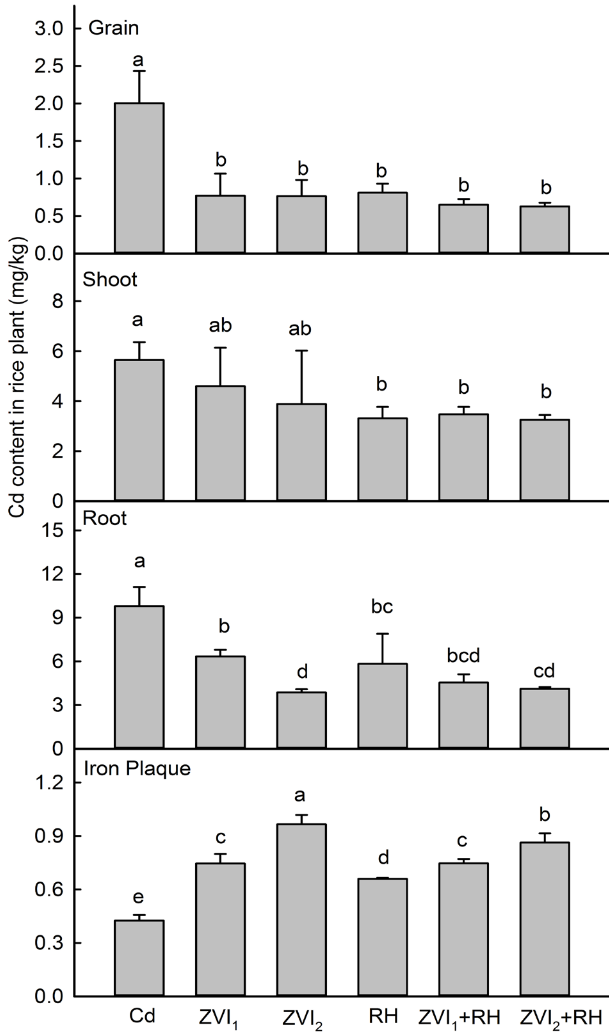

Figure 5. Effects of zero-valent iron (ZVI) (ZVI1: 0.05%, ZVI2: 0.2%), rice husk (RH) (5%) and their combinations on Cd content of iron plaque, root, shoot and grain of rice plant grown in Cd-spiked soil. Error bars represent the standard error (n = 3). Different letters indicate the mean difference is significant among treatments at the 0.05 level

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In comparison with a single Cd treatment, the Cd content in rice grains was markedly reduced with the addition of ZVI (0.05% and 0.2%), RH and their combinations. The application of ZVI (0.05% and 0.2%), RH and their combinations reduced the Cd content in grains by 61, 62, 60, 68 and 69%.

On the one hand, the decrease in the uptake and accumulation of As or Cd is due to the enhanced formation of iron plaques (Liu et al. 2008, Lee et al. 2013), which can adsorb more As or Cd on the root surface and limit their migration into the plant. Lee et al. (2013) showed that root iron plaques had a trapping effect on As, which can restrict its translocation to rice shoots, but this effect was dependent on the rice variety planted. Hu et al. (2020) found that ZVI can immobilise active As in soil and enhance the formation of Fe plaques, which prevents the translocation of As to rice plants. Similar to As, in addition to the adsorption of Cd in the soil, the uptake of Cd was retarded by the promotion of root Fe/Mn plaque formation (Cao et al. 2021). These results were consistent with our results showing a significant negative correlation between the As or Cd content in grains and the Fe content in plaques (Figure 6). Furthermore, Fe sufficiency can significantly reduce Cd concentrations in rice plants by increasing the amount of solution Fe^{2+} to suppress the expression of related Fe-transport genes (Chen et al. 2017).

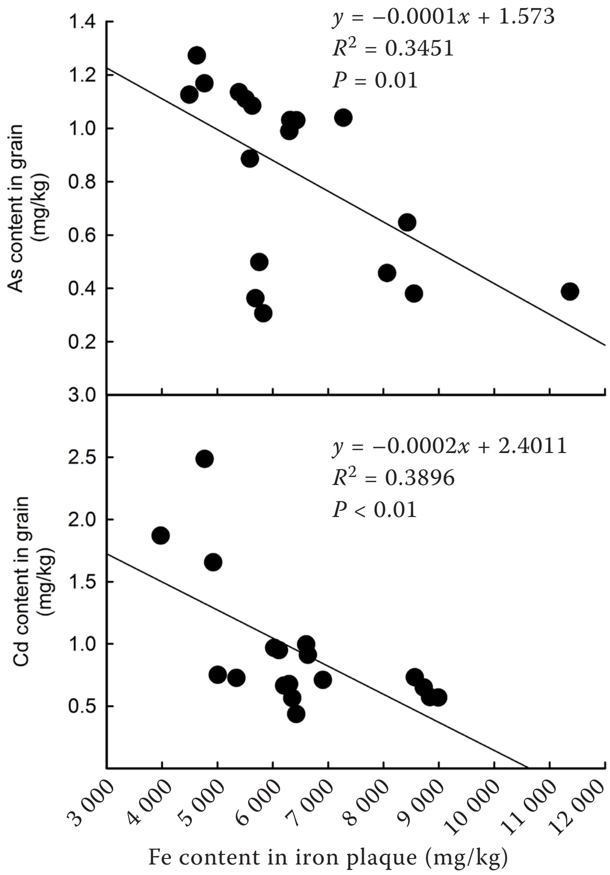

Figure 6. Correlation between contents of Fe in iron plaque and As or Cd in the grain of rice plant grown in As or Cd spiked soil (n = 18)

On the other hand, the addition of ZVI adsorbed As on the surface, resulting in a decrease in pore water As which reduced the As bioavailability in the soil (Figures 3 and 4). The As content in pore water showed a downward trend with increasing ZVI (0.05% and 0.2%) with or without RH and varied from 43 to 506 μg/L (Figure 3). Our result was in line with the results of Mueller et al. (2017), who demonstrated that applying NZVI can reduce As concentrations in soil pore water by over 80%. As an adsorbent, RH exhibited the excellent capacity to absorb Cd and increased Cd sorption by 21–41% onto saturated soil and by 38–54% onto upland soil (Khan et al. 2017), which explained the 59.6% decrease in grain Cd under RH treatment in our present study.

Our results indicated that the ZVI addition can markedly reduce the contents of As and Cd in grains. RH and RH + ZVI show more obvious effects on the decrease of grains Cd content. This study suggests that ZVI and RH could be alternative soil amendments to immobilise As or Cd and decrease their bioavailability in soils and their uptake by rice plants. The long-term effects of ZVI or RH on As or Cd immobilisation in farmland and their translocation in rice in the field require further studies.

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