



Simultaneous alleviation of cadmium and arsenic accumulation in rice by applying zero-valent iron and biochar to contaminated paddy soils



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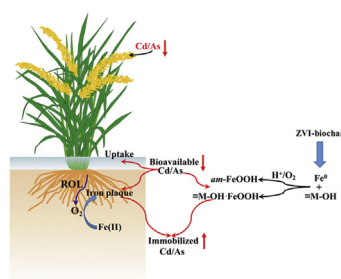
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HIGHLIGHTS

- The single zero valent iron amendment decreased the bioavailability of As.
- The single biochar amendment reduced the bioavailability of Cd.
- The ZVI-biochar amendments simultaneously reduced Cd and As bioavailability.
- The Fe, Cd, and As in plaque increased significantly in the ZVI-biochar amendments.
- The ZVI-biochar amendments increased the amorphous Fe oxides in soils.

GRAPHICAL ABSTRACT



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ABSTRACT

The fates of cadmium (Cd) and arsenic (As) in paddy fields are generally opposite; thus, the inconsistent transformation of Cd and As poses large challenges for their remediation. In this study, the impacts of zero valent iron (ZVI) and/or biochar amendments on Cd and As bioavailability were examined in pot trials with rice. Comparison with the untreated soil, both Cd and As accumulation in different rice tissues decreased significantly in the ZVI-biochar amendments and the Cd and As accumulation in rice decreased with increasing ZVI contents. In particular, the concentrations of Cd ($0.15 \pm 0.01 \text{ mg kg}^{-1}$) and As ($0.17 \pm 0.01 \text{ mg kg}^{-1}$) in rice grains were decreased by 93% and 61% relative to the untreated soil, respectively. A sequential extraction analysis indicated that with increasing Fe ratios in the ZVI-biochar mixtures, bioavailable Cd and As decreased, and the immobilized Cd and As increased. Furthermore, high levels of Fe, Cd, and As were detected in Fe plaque of the ZVI-biochar amendments in comparison with the single biochar or single ZVI amendments. The ZVI-biochar mixture may have a synergistic effect that simultaneously reduces Cd and As bioavailability by increasing the formation of amorphous Fe and Fe plaque for Cd and As immobilization. The single ZVI amendment significantly decreased As bioavailability, while the single biochar amendment significantly reduced the bioavailability of Cd compared

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with the combined amendments. Hence, using a ZVI-biochar mixture as a soil amendment could be a promising strategy for safely-utilizing Cd and As co-contaminated sites in the future.

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1. Introduction

The contamination of metals and metalloids (Cd and As) in paddy soils has become a serious concern that threatens food safety and human health due to rapid urbanization and rapid development of mining and metal processing, especially in southern China and other Southeast Asian countries (Liu et al., 2010; Wang et al., 2003; Yu et al., 2016b). Rice can efficiently accumulate Cd and As into grains, and the dietary intake of rice has become a major source of Cd and As in the human body (Li et al., 2011; Mondal and Polya, 2008; Tsukahara et al., 2003). Therefore, for Cd and As co-contaminated paddy fields, the simultaneous decrease in the bioavailability of Cd and As is needed urgently. However, the geochemical behavior of Cd and As in paddy soil is basically opposite. In paddy fields, the flooding and drainage cycles can change the soil conditions substantially and concomitantly affect the fate of As and Cd in soil. Under flooded conditions, the As adsorbed to iron (Fe) oxides can be released into solution when Fe(III) oxides are reductively reduced, thus increasing As bioavailability (Oremland and Stolz, 2005; Yu et al., 2016a). Conversely, Cd bioavailability may decrease in flooded soil due to the increase in pH or to precipitation with the reduced sulfur (Barrett and McBride, 2007; Bostick et al., 2000). In addition, in the rhizosphere environment, the Fe(II) resulting from the reduction of Fe(III) oxides can also be oxidized to Fe plaque on root surface, partly limiting the entry of Cd into root by adsorption (Lux et al., 2011). It is also reported that Cd and Fe share the same uptake pathway in rice plants, and high levels of Fe(II) in the rice rhizosphere may reduce Cd uptake by competing for metal transporters (Morrissey and Guerinet, 2009; Nakanishi et al., 2006). Given the opposite geochemical behavior of Cd and As in paddy soil, the remediation of Cd and As poses huge challenges in co-contaminated paddy fields, and the soil amendments should be carefully selected to immobilize both Cd and As in co-contaminated sites.

Compared with conventional remediation options, such as soil excavation and dumping, *ex situ* and *in situ* soil washing/flushing, etc., chemical stabilization and phytostabilization have shown to be less destructive alternatives (Komarek et al., 2013). Chemical stabilization, i.e., the *in situ* immobilization of metals through sorption or precipitation reactions induced by soil amendments, has gained considerable interest in recent years for the potential in reducing metals mobility and bioavailability in the amended soil (Komarek et al., 2013; Zanuzzi et al., 2013). Although phytostabilization or combined chemical and phytostabilization has also been used as a cost-effective and environmentally sustainable method for stabilizing and reclaiming mine lands (Tordoff et al., 2000; Wong, 2003; Zanuzzi et al., 2013), for a successful plant growth, it is still necessary to reduce the availability of metals and their toxicity and neutralize soil acidity (Pichtel and Salt, 1998). Typically, lime and compost are commonly selected and employed as soil amendments to reduce the *in situ* metals mobilization and minimize plant uptake (Bolan and Duraisamy, 2003; Bolan et al., 2004; Kumpiene, 2010; Komarek et al., 2013). Biochar is a carbon-rich solid material produced from pyrolysis of biomass under low oxygen conditions (Lehmann and Joseph, 2009). Due to the high carbon content in biochar and its chemical stability in the environment, application of biochar to soil has potential use for long-term carbon

sequestration (Lehmann, 2007). A few recent studies have also demonstrated that biochar has great potential for the retention of heavy metals in soils (Beesley et al., 2010; Uchimiya et al., 2010; Fellet et al., 2011; Karami et al., 2011). Therefore, the application of biochar can potentially provide a new solution for remediation of the heavy metals contaminated soils. Zero-valent iron (ZVI) is generally considered as an excellent metallic material for environmental remediation because of its high reducing ability, low operational cost and generation (Chen et al., 2008). However, the investigation regarding with application of biochar and ZVI for the remediation of Cd and As contaminated paddy field is still lacking. To assess the real mobility of Cd and As in paddy soil, as a consequence, a chemical stabilization option using biochar and ZVI as soil amendments was selected.

Using biochar as soil amendments to decrease metals bioavailability has been widely reported (Zhang et al., 2013). For instance, the addition of orchard prune-derived biochar to mine tailing contaminated with heavy metal significantly reduced the bioavailability of Cd, Pb and Zn (Fellet et al., 2011). Similarly, applying biochar derived from wood or sewage sludge to Cd-contaminated soil also highly reduced bioavailable Cd in the soil (Beesley and Marmiroli, 2011; Méndez et al., 2012; Namgay et al., 2010). Biochar can reduce the As content in plant tissues by weakly retaining As on its surface (Beesley and Marmiroli, 2011; Namgay et al., 2010). The possible mechanisms of heavy metals stabilization after application of biochar to contaminated soils can be attributed to (i) ion exchange: biochar typically has a high pH and cation exchange capacity and metal ions in soil can exchange with Ca^{2+} , Mg^{2+} , and other cations associated with biochar leading to surface innersphere complex or co-precipitation with biochar; (ii) ligand complexation: the functional groups on biochar such as carboxyl, alcoholic hydroxyl or phenolic hydroxyl groups can occur complexation reaction with heavy metal by the electrostatic attraction and inner-sphere specific surface complexations; and (iii) chemical or physical sorption and surface precipitation (Lu et al., 2012; Zhang et al., 2013). However, it has also been reported that biochar amendments can enhance As reduction, resulting in the high As toxicity (Chen et al., 2016). Thus, for Cd and As co-contaminated paddy fields, the application of biochar can effectively immobilize Cd but might increase the bioavailability of As.

It was recently reported that the Fe amendments (such as ferrous iron, iron powder, and amorphous iron-(hydr)oxide) can reduce As uptake in paddy rice (Matsumoto et al., 2016) because an increase in iron (hydr)oxides in soil can regulate the mobility and bioavailability of As species via reductive dissolution or mineralization processes (Mitsunobu et al., 2006; Ultra et al., 2009; Rahman et al., 2013). Zero-valent iron (ZVI) has shown unique roles in immobilizing As in contaminated soil because its corrosion products have high adsorption and incorporation capacities for As (Giménez et al., 2007; Wang et al., 2014; Zhou et al., 2014). However, compared with other Fe amendments such as Fe(II) and Fe(III) sulfate, the use of ZVI may increase the soil pH (Kumpiene et al., 2006), which is favorable for Cd immobilization but may also lead to the enhancement of As bioavailability (Hartley et al., 2004; Yu et al., 2017). Therefore, for co-contaminated sites with As and Cd, the single ZVI amendment may lead to uncertain consequences for

both the As and Cd bioavailability.

Comparison with the single biochar or single ZVI amendments, it seems that a combined amendment may be a good choice for treating co-contaminated sites. In fact, it has been reported that the application of biochar and ZVI amendments to soils contaminated with metals, metalloids and organics significantly reduced both Cu and As leaching (Sneath et al., 2013). However, knowledge of the combined amendment of biochar and ZVI and its potential impacts on Cd and As bioavailability in co-contaminated paddy soil is still very limited. Thus, the aims of this study were: (i) to investigate the potential impacts of ZVI and biochar amendment on the mobility and bioavailability of Cd and As in paddy soil by a combined amendment of ZVI and biochar in a pot trial with rice; and (ii) to determine the correlation between the Fe ratio and the remediating effect of the Fe-biochar mixture through preparing different pre-mixed ZVI-biochar composites with varying Fe ratios.

2. Materials and methods

2.1. Soil sampling and characterization

The soil was collected from an As and Cd co-contaminated paddy field around an abandoned mine in Renhua, Guangdong Province, China, which belongs to a typical region of red soils. The soil sample was randomly taken from 3 to 5 sampling points in a field and was mixed to form a composite sample. Upon arrival at the lab, coarse roots were carefully removed from the freshly collected soil sample. Then the soil was air-dried and passed through a 2-mm sieve before the pot trial. Soil pH was measured in deionized water (soil to water ratio of 1:2.5) after shaking for 1 h. The cation exchange capacity (CEC) was assessed by leaching the soil with 1 M ammonium acetate at pH 7 and then 1 M KCl (Chapman, 1965). Soil organic C (TOC) was determined using a EuroVector EA3000 CHE elemental analyzer (EuroVector, Milan, Italy). The contents of total soil Fe and Cd were determined after digestion of the soil using HF–HClO₄–HCl (Lambrechts et al., 2011). The contents of total soil As was determined by acid digestion (HNO₃, HCl and HF) (Larios et al., 2012). Then, the samples were filtered through 0.45- μ m membrane filters. The Fe, As and Cd concentrations resulting from acid extractions were analyzing using inductively coupled plasma atomic emission spectrometry (ICP-AES) (PerkinElmer, Inc. America), hydrogen generation-atomic fluorescence spectrometer (HG-AFS, SA-10, Beijing Titan Instruments Co. Ltd, China) and graphite furnace atomic absorption spectrometer (ZEEnit 60, Analytik Jena, Germany), respectively. Soil electrical conductivity (EC) was measured with digital FieldScout SoilStik electrical conductivity meter in a 1:2.5 (soil to solution) ratio. Concentrations of available Cd and As fractions in soil were determined by a sequential extraction scheme according to the procedures described by Tessier et al. (1979) and Wenzel et al. (2001), respectively. The basic chemical properties of the soil are shown in Table S11.

Biochar was prepared from oil palm fibers, sourced from Malaysia. After being air-dried, the oil palm fibers were charred at 700 °C for 4 h in a muffle furnace under a N₂ atmosphere. Once cooled, the biochar was ground to pass through a 100-mesh sieve and stored in a drying oven before analysis of characteristics and elemental composition (Table S12), and Fourier transform infrared spectroscopy (FT-IR) (Fig. S11). Fine sized ZVI particles (100-mesh) were purchased from the Aladdin Chemical Company, China, and used as received. The ZVI-biochar composites were prepared by fully mixing 100 g biochar and different ratios of ZVI (0.5, 1.0, 2.5, and 5.0 g, respectively). After thorough mixing, the composites were passed through a 100-mesh sieve (aperture: 0.147 mm) to obtain a fine uniform powder and stored in sealed containers until

required. The oversized ZVI-biochar composites were collected and ground again until all the composites could pass through the sieve. In total, four ZVI-biochar composites containing 0.5%, 1.0%, 2.5%, 5.0% Fe were prepared in this study.

2.2. Pot experiments

For the rice pot trial, seven treatments with three replicates for each treatment were carried out. The mixtures of each treatment were placed into plastic pots at a rate of 10 kg soil per pot (height: 25 cm, diameter: 8.5 cm). Treatments were applied on a dry weight basis; the 1% w/w biochar treatment was equivalent to 100 g biochar per pot. The treatments were: the untreated paddy soil (Ctr), 1% biochar (C), 1% ZVI-biochar with 0.5% Fe (0.5 Fe–C), 1% ZVI-biochar with 1% Fe (1.0 Fe–C), 1% ZVI-biochar with 2.5% Fe (2.5 Fe–C), 1% ZVI-biochar with 5% Fe (5.0 Fe–C), and 0.05% ZVI (Fe). Details about the pot experiments design are listed in Table 1.

The seeds of rice cultivar (*Oryza sativa* L.) used in this study were from the cultivar UU 128. After the surface was sterilized in 30% H₂O₂ for 10 min and then washed thoroughly with deionized water more than ten times, seeds were germinated in a 25 °C thermostat incubator (Yiheng MGC-300H, Shanghai). Approximately three weeks later, rice seedlings with uniform size were transplanted into the pots. Tap water was added on a daily basis to maintain flooding of the soils, and pots were rearranged randomly every week until two weeks before harvest. After harvested (about 120 days of growth), the rice plants were separated into grain (brown rice), husk, straw, and root fractions. Rice straw and roots were washed thoroughly in tap water, and then rinsed with deionized water. Iron plaque on fresh root surfaces was then extracted using a dithionite-citrate-bicarbonate (DCB) solution as described previously (Otte et al., 1989; Taylor and Crowder, 1983). After iron plaque extraction, all plant materials were dried at 70 °C for 72 h and ground for further analysis. The roots sampled were hand-shaken and the soil adhering to the roots was considered as rhizosphere material (Rollwagen and Zasoski, 1988). All samples were stored in plastic bags. The soil samples were further air-dried, ground and passed through a 100-mesh sieve before subsequent analysis.

2.3. Analysis of As and Cd in rice plants

Approximately 1 g of each plant sample was digested with 1.0 mL of HClO₄, 1.5 mL of H₂SO₄ and 4.0 mL of HNO₃ at 110–130 °C until a clear solution was obtained. The digested samples were filtered through a 0.45- μ m filter and diluted to 25 mL with ultra-pure deionized water. Then, the As concentration was analyzed with a HG-AFS (SA-10) and the total Cd was analyzed using a graphite furnace atomic absorption spectrometer. Quality assurance and quality control were performed for blank and standard for every ten samples using duplicates, and certified standard reference materials (GBW(E)070008 for soil As and GBW(E)10014 for plant As, and GBW(E)07429 for soil Cd and GBW(E)10023 for plant Cd) were obtained from the National Research Center for Standards in China. The plant sample recoveries of the spiked standards ranged from 97% to 102% for As, and 95%–107% for Cd, respectively.

2.4. Analysis of As, Cd, and Fe fractions in the soil

A five-step sequential extraction scheme was employed to determine the operationally defined Cd and As fractions according to the procedures described by Tessier et al. (1979) (Table S13) and Wenzel et al. (2001) (Table S14), respectively. In brief, 1 g of dried soil was weighed into a 50 mL centrifuge tube in a N₂ atmosphere. For each step, 20 mL of extractant was added, and the mixtures were shaken in a rotary shaker at 160 rpm at room temperature.

Table 1
The pot trials designed in this study.

Treatments		Soil/kg	Biochar/kg	ZVI/kg	ZVI-biochar/kg
Ctr	unamended paddy soil	10	0	0	0
0.5 Fe–C	1% w/w ZVI-biochar (0.5% Fe)	9.9	0	0	0.1
1.0 Fe–C	1% w/w ZVI-biochar (1% Fe)	9.9	0	0	0.1
2.5 Fe–C	1% w/w ZVI-biochar (2.5% Fe)	9.9	0	0	0.1
5.0 Fe–C	1% w/w ZVI-biochar (5% Fe)	9.9	0	0	0.1
C	1% w/w biochar	9.9	0.1	0	0
Fe	0.05% zero-valent iron (ZVI)	9.995	0	0.005	0

Then, the suspension was centrifuged at 3000 rpm for 15 min, and each step was repeated three times, all the supernatants were collected, and the final volume was adjusted to 50 mL with deionized water. The supernatants were further passed through a 0.22- μ m filter and determined for the Cd and As concentrations, as described above. Among all the treatments, the rate of recovery of Cd and As in each extraction step to total Cd and As content in rhizosphere soil ranged from $91 \pm 3\%$ to $97 \pm 2\%$ for Cd, and from $94 \pm 1\%$ to $94 \pm 5\%$ for As, respectively, suggesting that the extract methods used have a high accuracy and precision in recovering Cd and As from 1 g rhizosphere soil. Free Fe oxides, including crystalline and amorphous Fe oxides in the rhizosphere soils were extracted with a DCB solution (DCB-Fe) (Mehra and Jackson, 1960) and a 0.2 M NH_4^+ -oxalate buffer (pH 3.0) (Oxalate-Fe) (Mckeague and Day, 1966), respectively. The extracts were passed through a 0.45- μ m filter and further analyzed for the iron concentration by ICP-AES (PerkinElmer, Inc. America). In addition, HCl-extractable Fe(II) (HCl-Fe(II)) was also determined based on the described method (Lovley and Phillips, 1988). Further details regarding the extraction procedure are provided in our previous study (Liu et al., 2015).

2.5. Analysis of Fe, Cd and As contents in Fe plaque

After DCB extraction (Hu et al., 2014), the iron plaque extracts were passed through a 0.22- μ m filter and analyzed for Cd and As contents as described above. Further details can be found in our previous studies (Liu et al., 2015; Yu et al., 2016b). The Fe content in iron plaque was determined by ICP-AES.

2.6. Statistical analysis

Statistical and correlation analyses were performed using the SPSS 18.0 (SPSS, Inc. Chicago, USA) software. Statistically significant differences were determined by Duncan's multiple range test and $P < .05$ was considered significant. The correlation analysis was conducted using a Pearson's correlation test with a significance level of $P < .05$ (two-tailed).

3. Results

3.1. Cd and As accumulation in rice

The Cd and As contents in the roots, straws, husks and rice grains in different treatments at the mature stage are given in Fig. 1. In untreated soil (Ctr), the accumulation of Cd and As in different rice tissues were clearly decreased in the order of root > straw > grain. The grain Cd content in the Ctr ($2.0 \pm 0.06 \text{ mg kg}^{-1}$) was approximately 10-fold higher than the Cd limit in rice (0.2 mg kg^{-1}) recommended by the WHO (Liu et al., 2005) (Fig. 1a), and the grain As ($0.43 \pm 0.04 \text{ mg kg}^{-1}$) was twice as high as the allowable As limit (0.2 mg kg^{-1}) specified by the Chinese food security standards (Fig. 1e). Compared with the

control, in the single biochar amendment, the Cd in roots, straw, husks, and grains were significantly decreased (Fig. 1b–d), while the As in different rice tissues showed no significant change (Fig. 1g–h). Conversely, in the ZVI alone treatment, the Cd and As in roots, straw, husks, and grains showed a reverse behavior. In comparison with the untreated soil, the decrease of Cd and As in rice grains in the amendments with ZVI or biochar alone was less than 30% (Fig. 1a, e). Interestingly, after the simultaneous application of equivalent biochar and ZVI (5.0 Fe–C), a dramatic decrease in both As and Cd was observed in all rice tissues. In particular, the concentrations of Cd ($0.15 \pm 0.01 \text{ mg kg}^{-1}$) and As ($0.17 \pm 0.01 \text{ mg kg}^{-1}$) in rice grains were decreased by 93% and 61% compared with the untreated soil, respectively, which were both below the allowable Cd (0.2 mg kg^{-1}) and As (0.2 mg kg^{-1}) limits (Fig. 1a, e). The substantial decrease in the 5.0 Fe–C treatment (>60%) was much higher than the total decrease ratio (<30%) of the single Fe and biochar treatments, indicating that the occurrence of interaction between Fe and biochar may play a key role in alleviating both As and Cd. The effects of ZVI dosage (0.5%, 1%, 2.5%, and 5%) in Fe–C composites were also examined, and the results showed that the Cd and As contents in different rice tissues were clearly decreased in the order of 0.5 Fe–C > 1.0 Fe–C > 2.5 Fe–C > 5.0 Fe–C, suggesting that a ZVI-biochar mixture with a high Fe content can dramatically reduce Cd and As accumulation in rice.

The grain yields of the treatment with Fe–C and the control had no significant differences (Table S15). To illustrate the differences in grain As and Cd concentrations due to the dilution effects of grain yields or the decrease in arsenic bioavailability, the As and Cd in rice grains were normalized by the dry weight (g pot^{-1}) of rice grains. Compared with the control, the normalized As and Cd in brown rice decreased significantly in all treatments with Fe–C, suggesting that the differences in grain As and Cd concentrations were not caused by the dilution effects of grain yields but by the decrease in As and Cd bioavailability (Fig. S12). Therefore, it is reasonable to correlate the As and Cd concentrations in different parts of the rice plant with the As, Cd and Fe species in soils.

3.2. Cd, As, and Fe in rhizosphere soils

It was reported that MgCl_2 -Cd and OAc-Cd normally represented the bioavailable Cd for rice plants, while NH_2OH -HCl-Cd implied the immobilized Cd in the soil (Fan et al., 2014; Niazi et al., 2011). Similarly, $(\text{NH}_4)_2\text{SO}_4$ -As and $\text{NH}_4\text{H}_2\text{PO}_4$ -As suggested the bioavailable As in the soil, and Oxalate-As represented the less bioavailable As (Baumann and Fisher, 2011; Fan et al., 2002; Tang et al., 2007). In untreated soil (Ctr), the MgCl_2 -Cd, OAc-Cd, and NH_2OH -HCl-Cd occupied 1.1%, 4.4% and 5.6% of the total Cd content, respectively (Fig. 2a–c). Meanwhile, the concentrations of $(\text{NH}_4)_2\text{SO}_4$ -As, $\text{NH}_4\text{H}_2\text{PO}_4$ -As, and Oxalate-As accounted for 0.3%, 7.4%, and 19.0% of the total As, respectively (Fig. 2f–h). These results implied that most of the Cd in the paddy soil existed as the residual and organic-bound forms (Fig. 2d and e), and that of As existed as residual and Fe oxides forms (Fig. 2i and j). Comparison with the Ctr,

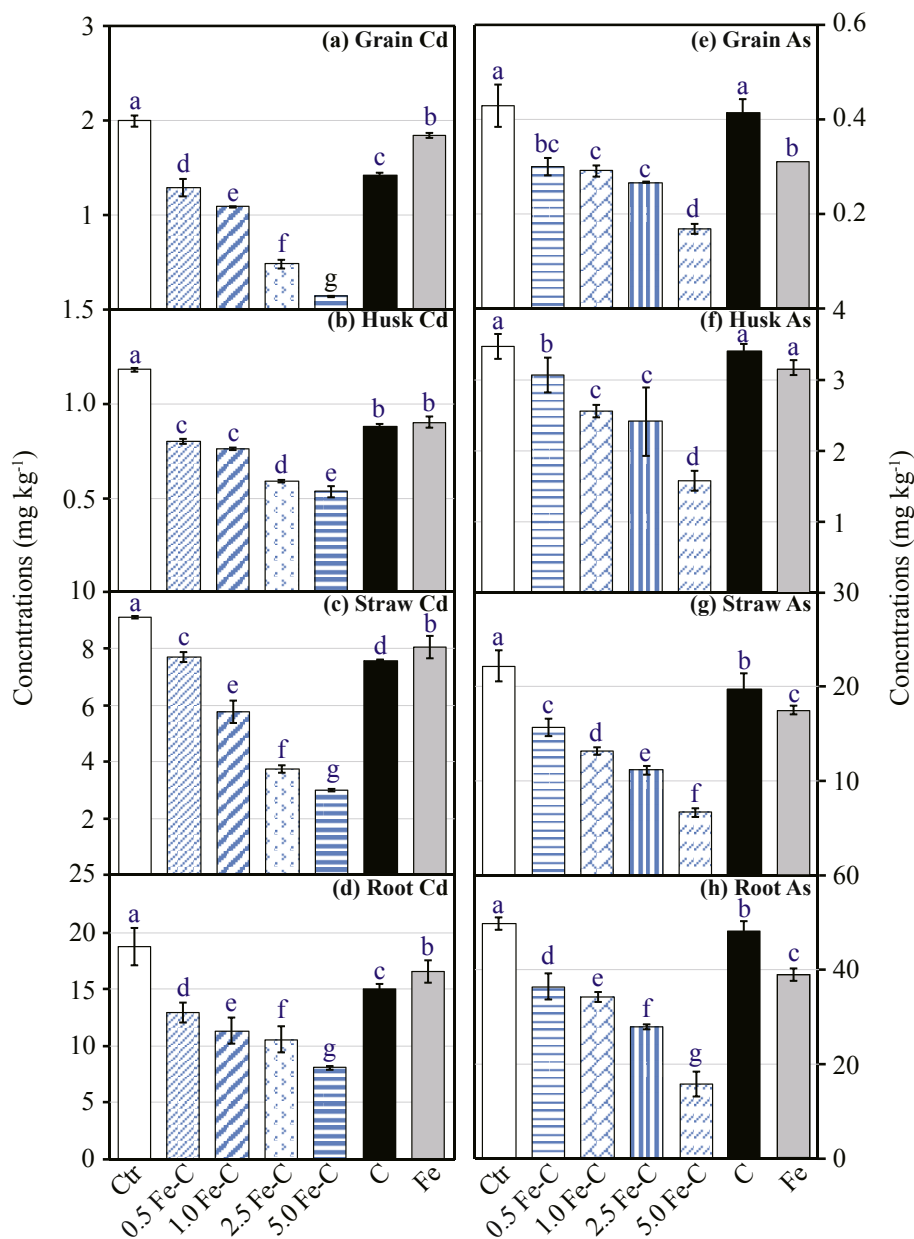


Fig. 1. Concentrations of Cd and As in different rice plant tissues under different treatments. (a), grain Cd; (b) husk Cd; (c) straw Cd; (d) root Cd; (e) grain As; (f) husk As; (g) straw As; (h) root As. Ctr: untreated paddy soil; C: biochar; Fe: zero-valent iron; 0.5 Fe–C: ZVI-biochar with 0.5% Fe; 1.0 Fe–C: ZVI-biochar with 1% Fe; 2.5 Fe–C: ZVI-biochar with 2.5% Fe; 5.0 Fe–C: ZVI-biochar with 5% Fe. Significant differences are indicated by different letters ($P < .05$). Bars represent standard errors ($n = 3$).

$MgCl_2$ –Cd and OAc–Cd decreased significantly in all the biochar and/or ZVI treatments in the order of Ctr > C > Fe > 0.5 Fe–C > 1.0 Fe–C > 2.5 Fe–C > 5.0 Fe–C, which was similar to the trend observed for $(NH_4)_2SO_4$ –As and $NH_4H_2PO_4$ –As. Conversely, NH_2OH –HCl–Cd and Oxalate–As in all the amendments showed a significant increasing trend, except for the amendment with biochar. A further correlation analysis showed that the contents of $MgCl_2$ –Cd and OAc–Cd or $(NH_4)_2SO_4$ –As and $NH_4H_2PO_4$ –As were significantly and positively correlated with Cd or As in grains, husks and straw (Figs. S13 and S14), respectively. Nevertheless, NH_2OH –HCl–Cd and Oxalate–As showed the opposite trend (Figs. S13 and S14). From the above results, we can determine that the bioavailable Cd and As were much lower in the combined amendment than those in the single biochar or single ZVI amendments, indicating that ZVI-biochar composite can

substantially enhance the transformation of Cd and As fractions from bioavailable Cd and As to the immobilized fractions (residual fractions). In addition, the ZVI-biochar mixture with a high Fe dosage had a positive effect on the immobilization of Cd and As.

It was well known that the DCB-Fe represented free Fe oxides (crystalline and amorphous forms), and Oxalate-Fe and HCl-Fe(II) usually represented the Fe forms that are available for microbial reduction in soils (Lovley and Phillips, 1988; Munch and Ottow, 1980). As shown in Fig. 3, compared with the untreated soil, the DCB-Fe and Oxalate-Fe in all the amendments increased significantly in the order of Ctr < C < Fe < 0.5 Fe–C < 1.0 Fe–C < 2.5 Fe–C < 5.0 Fe–C. In contrast, HCl-Fe(II) showed the completely opposite trend, basically decreasing in the above order. Obviously, higher DCB-Fe and Oxalate-Fe, and lower HCl-Fe(II) were observed in the Fe–C combined amendments than those in the single biochar or

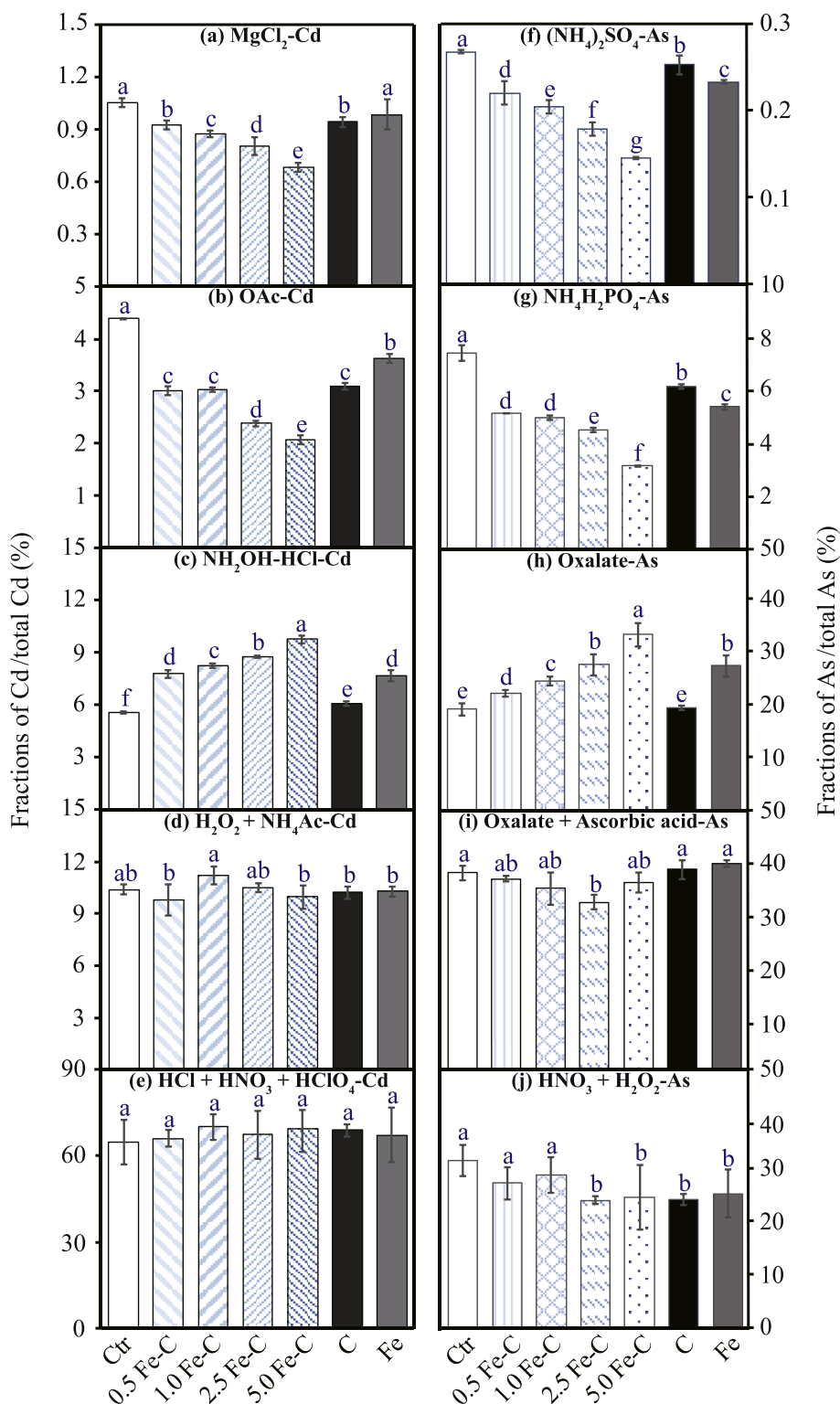


Fig. 2. The percentage content of Cd and As fractions in the total Cd and As contents in each treatment. Sequential extraction procedures were used to determine the Cd and As fractions in soil, respectively. Significant differences are indicated by different letters ($P < .05$). Bars represent standard errors ($n = 3$).

single ZVI amendments, suggesting that microbial Fe(III) reduction was inhibited but the amount of amorphous Fe(III) oxyhydroxide was greatly increased by Fe-biochar composite. A further correlation analysis also indicated that oxalate-Fe was significantly and negatively correlated with the contents of bioavailable Cd and As forms (Fig. 4). In contrast, HCl-Fe(II) showed a different trend.

Collectively, increasing the content of Fe in the amorphous Fe oxides may reduce the amount of available Cd and As fractions in soil (Fig. 5). However, enhancing Fe(III) reduction can elevate the contents of bioavailable Cd and As.

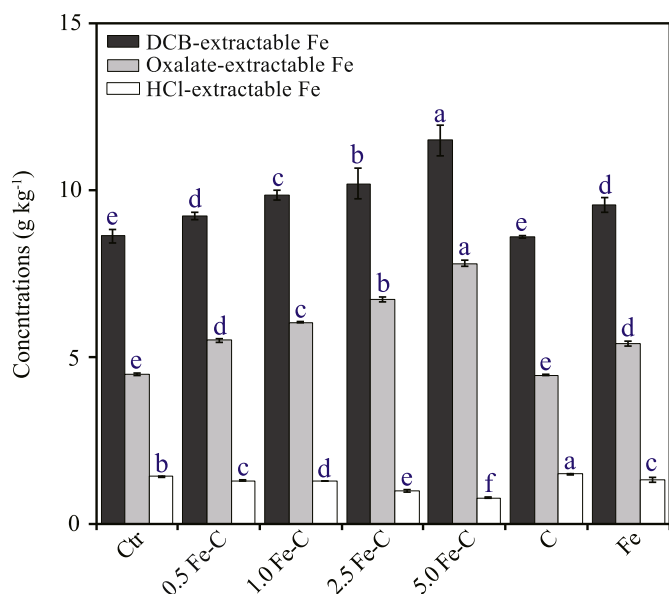


Fig. 3. Concentrations of Fe species in soils, including DCB-extractable Fe (DCB-Fe), Oxalate-extractable Fe (Oxalate-Fe), and HCl-extractable Fe(II) (HCl-Fe(II)). Significant differences are indicated by different letters ($P < .05$). Bars represent standard errors ($n = 3$).

3.3. Cd, As, and Fe in Fe plaque

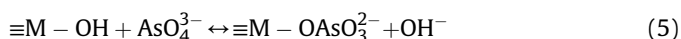
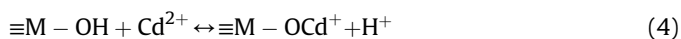
Both the Fe and As contents in Fe plaque in the biochar amendment were significantly higher than those in the Ctr (Fig. 6), while the plaque-Cd in the biochar amendment decreased slightly. The concentrations of Fe, Cd and As in Fe plaque all increased significantly in the ZVI amendment (Fig. 6). Among the four combined amendments, the contents of Fe, Cd and As in Fe plaque were all significantly ($P < .05$) increased in the order of 0.5 Fe-C < 1.0 Fe-C < 2.5 Fe-C < 5.0 Fe-C compared with Ctr (Fig. 6). In particular, in 5.0 Fe-C (ZVI-biochar with 5% Fe), the contents of Fe ($45.33 \pm 1.53 \text{ mg g}^{-1}$), Cd ($8.54 \pm 0.05 \text{ mg kg}^{-1}$) and As ($418.85 \pm 26.07 \text{ mg kg}^{-1}$) in Fe plaque were approximately 4, 6 and 14-fold higher than those in the Ctr, respectively (Fig. 6). Overall, in the equivalent biochar and ZVI (5.0 Fe-C) amendments, the contents of Fe, Cd, and As in Fe plaque were much higher than those in the single biochar or single ZVI amendment, demonstrating that the combined amendment of biochar and ZVI can greatly enhance Fe plaque formation, thus increasing Cd and As accumulation in Fe plaque.

4. Discussion

Since the biogeochemical behavior of Cd and As in paddy fields is generally opposite, their remediation usually encounters the inconsistent transformation of Cd and As, i.e., the immobilization of one heavy metal will cause the mobilization of the other one or have nearly no impact on the other heavy metal. Based on this study, the single applications of biochar or ZVI had limited impacts on the immobilization of As and Cd (<30%), but the combined amendments of biochar and ZVI substantially enhanced the immobilization of As and Cd (93% and 61%). Hence, in addition to the roles of single biochar and single ZVI amendments, some interaction between biochar and ZVI may play a key role in immobilizing Cd and As simultaneously in the Fe-C combined treatments. The roles of biochar, ZVI, and their interaction will be discussed in detail as follows.

4.1. Role of biochar

The results in Fig. 1 suggested that biochar did have positive impacts on Cd and As alleviation, but biochar could reduce the bioavailability of Cd more effectively than that of As, as seen in Fig. 2. Biochar has been widely reported as a soil amendment to alleviate the bioavailability of heavy metals due to its huge surface area, alkaline pH, strong sorption capacity and high cation exchange capacity (Beesley and Marmiroli, 2011; Beesley et al., 2011; Bian et al., 2014; Glaser et al., 2002; Uchimiya et al., 2010; Zhang et al., 2013). Biochar's liming effect can lead to the raise in soil pH, which can greatly reduce the mobility and bioavailability of Cd (Houben et al., 2013), mainly by increasing the net negative charge of variably charged soil constituents (Lindsay, 1979; McBride et al., 2010; Bradl, 2004) and the density of cation exchange sites (Harvey et al., 2011). This can further favor metal precipitation, decrease metal solubility and promote metal adsorption on the biochar particles. In this study, the rhizosphere pH substantially increased from 4.7 to 5.6 after biochar amendment (Fig. S15), which can also greatly affect Cd and As bioavailability (Masscheleyn et al., 1991; Yu et al., 2016b; Houben and Sonnet, 2015). Therefore, the adsorption of cations (e.g., Cd²⁺) can be enhanced after the amendment with biochar. As exists predominantly in anionic forms, so biochar may not show strong affinity of As for functional groups (carboxylic and phenolic) situated on the surface of oxidized biochar particles. However, As has a smaller ionic radius (0.58 Å), which may favor its diffusion into micropores of biochar when amended with biochar with time compared with that of Cd (0.97 Å) (Nguyen et al., 2008). This can in part contribute to As sorption in the presence of biochar. In addition, some studies also indicate that biochar addition can enhance the bioreduction of As(V) to more soluble As(III) (Chen et al., 2016), probably resulting in the occurrence of As mobilization. FTIR analysis showed the presence of aromatic C=C stretch and C-OH stretch in the biochar (Fig. S1). Due to the possible proton release or uptake of hydroxyl group situated on the surface of biochar ($\equiv\text{MOH}$), the acid-base reactions on the surface of biochar might occur as Rxns. (1) and (2). The surface complexation of Cd/As onto biochar was also likely to occur as Rxns. (3)–(5). Cd may be adsorbed on the deprotonated functional group (i.e. $\equiv\text{MO}^-$) as Rxn (3), which was not a pH-dependent reaction as no proton was involved in this reaction. Besides the direct Cd adsorption on $\equiv\text{MO}^-$, we think the Cd²⁺ could displace protons on the $\equiv\text{MOH}$ functional group via surface complexation processes according to the similar literature (Lu et al., 2012; Zhang et al., 2013). Since the protons were involved in the reaction of surface complexation between Cd²⁺ and $\equiv\text{MOH}$, the soil pH could obviously influence the Cd²⁺ surface complexation on $\equiv\text{MOH}$.



According to a previous pot experiment, the root-induced acidification of the rhizosphere can also affect the rhizospheric pH possibly by counteracting the liming effect of biochar to influence fractions of available Cd; also, the pH has a dominant role in controlling the carbonate-bound Cd solubility (Houben and Sonnet,

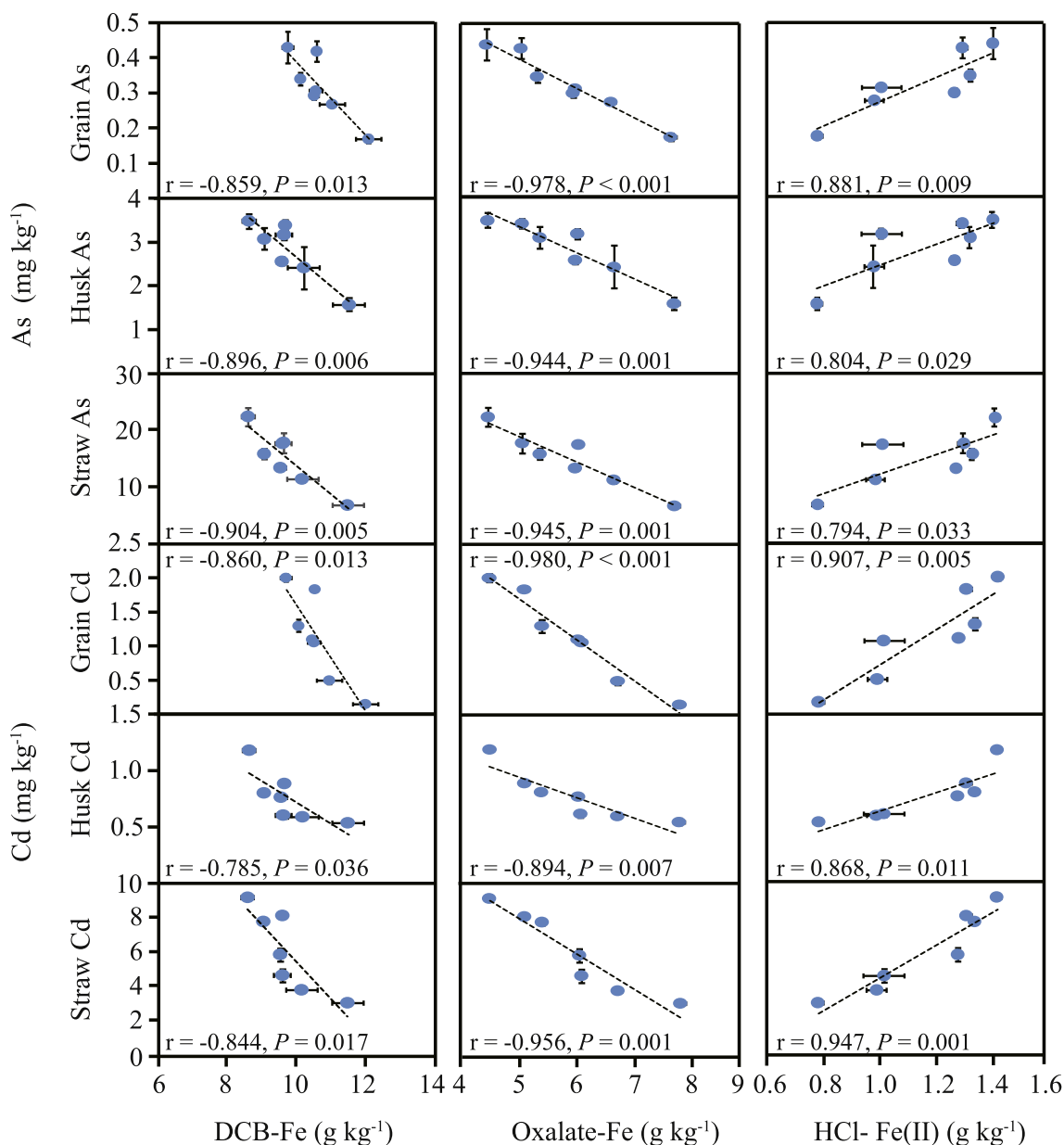


Fig. 4. The correlations between the concentrations of Fe species in soil (DCB-Fe, Oxalate-Fe and HCl-Fe(II)) and Cd or As contents in different tissues of rice (grains, husks and straw).

2015). In this study, negative correlations between the pH and fractions of bioavailable Cd were observed (Fig. S16a), but no statistically significant differences were found between pH and bioavailable As (Fig. S16b), indicating that the Cd species might be more sensitive to pH than As species. Collectively, the influence of the biochar amendment on Cd bioavailability is primarily due to its high cation binding capacity and strong adsorption capacity and the increase in pH, and for As, biochar may mainly affect the Fe transformation to further influence the mobility of As, which is discussed in next section.

4.2. Role of ZVI

With the ZVI amendment, the bioavailability of As decreased significantly, while that of Cd just decreased slightly (Fig. 2). Meanwhile, the amorphous Fe oxide-bound As increased

significantly, and the immobilized Cd increased as well (Fig. 2). ZVI is a very reactive reducing reagent that can easily be oxidized by the O₂ in the paddy soils and transformed into amorphous iron oxyhydroxides (Rxn. (6)). The reactions between ZVI and H⁺/H₂O will generate Fe²⁺ (Rxn. (7) and (8)), which can be further oxidized via biotic (iron oxidizing microbes, FeOBs) or abiotic processes (Rxn. (9) and (10)), followed by the formation of amorphous iron oxyhydroxides, such as ferrihydrite (*am*-FeOOH). The newly formed iron minerals from the above corrosion processes of ZVI can provide many new surface adsorption sites for As and Cd adsorption, and the As and Cd may also be incorporated into the new minerals, resulting in an increase of As and Cd immobilization.



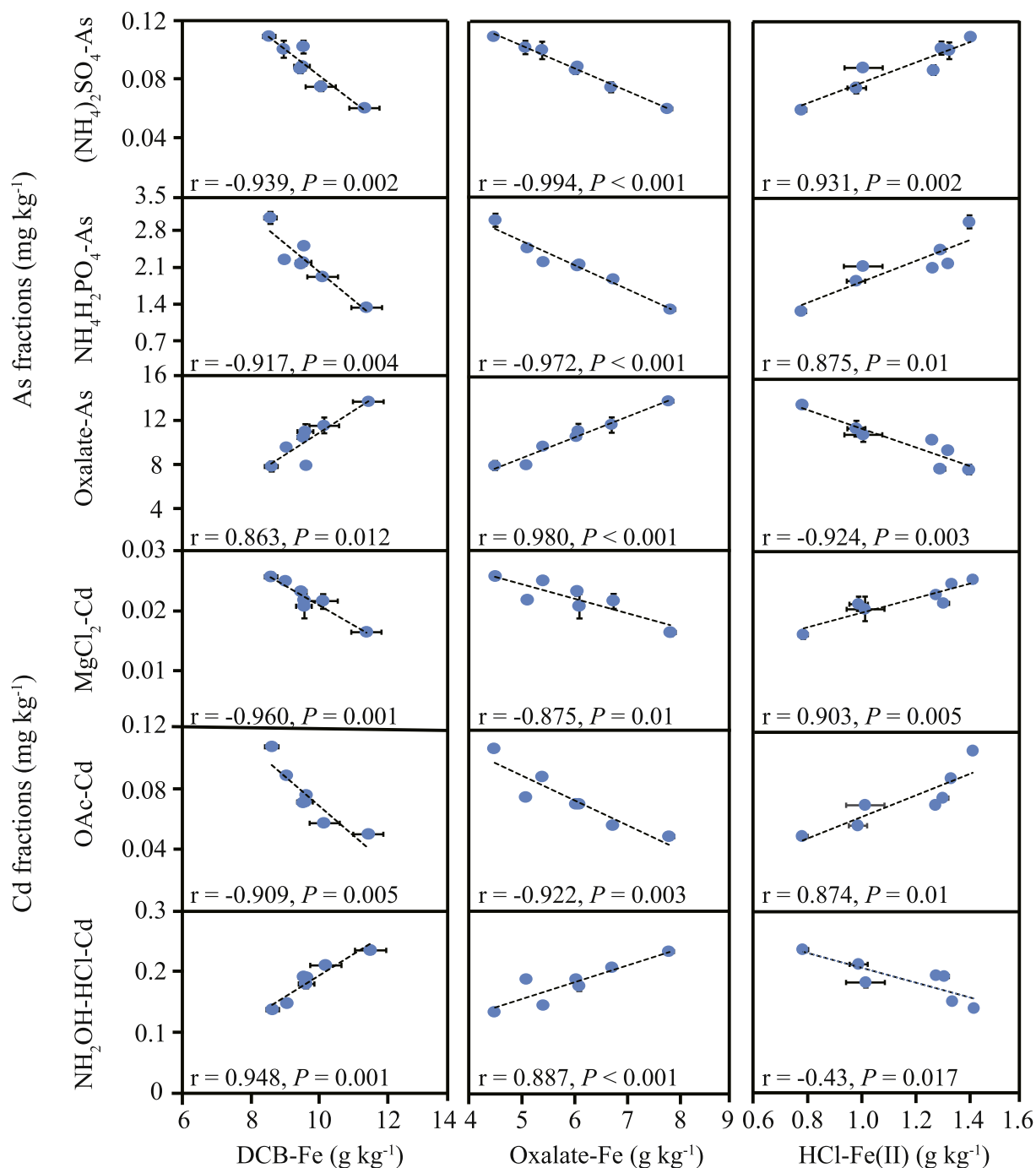
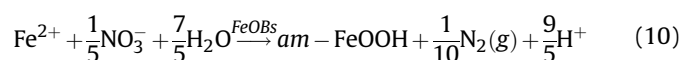
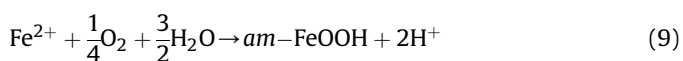
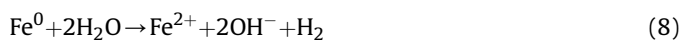


Fig. 5. The correlations between the concentrations of Fe species in soil (DCB-Fe, Oxalate-Fe and HCl-Fe(II)) and the Cd fractions (MgCl₂-Cd, OAc-Cd, and NH₂OH-HCl-Cd) or As fractions ((NH₄)₂SO₄-As, NH₄H₂PO₄-As, and Oxalate-As) in soil.



With the amendment of ZVI, the pH would increase by consuming proton during the corrosion of Fe⁰ (Rxns. (7) and (8)), though some new protons may be released by the partial oxidation of Fe²⁺ (Rxns. (9)–(10)). The increase of bulk pH will be favorable for the Cd²⁺ immobilization due to the low solubility of Cd²⁺ at a high pH. The oxidation of Fe²⁺ resulted in the generation of the iron oxyhydroxides, which can be an important sink for both As and Cd. The results in Fig. 3 confirmed the increase of the contents of crystalline and amorphous Fe oxides, and the concomitant decrease of bioavailable As and Cd. In addition, the increase of the Fe plaque with the ZVI amendment can also enhance the immobilization of

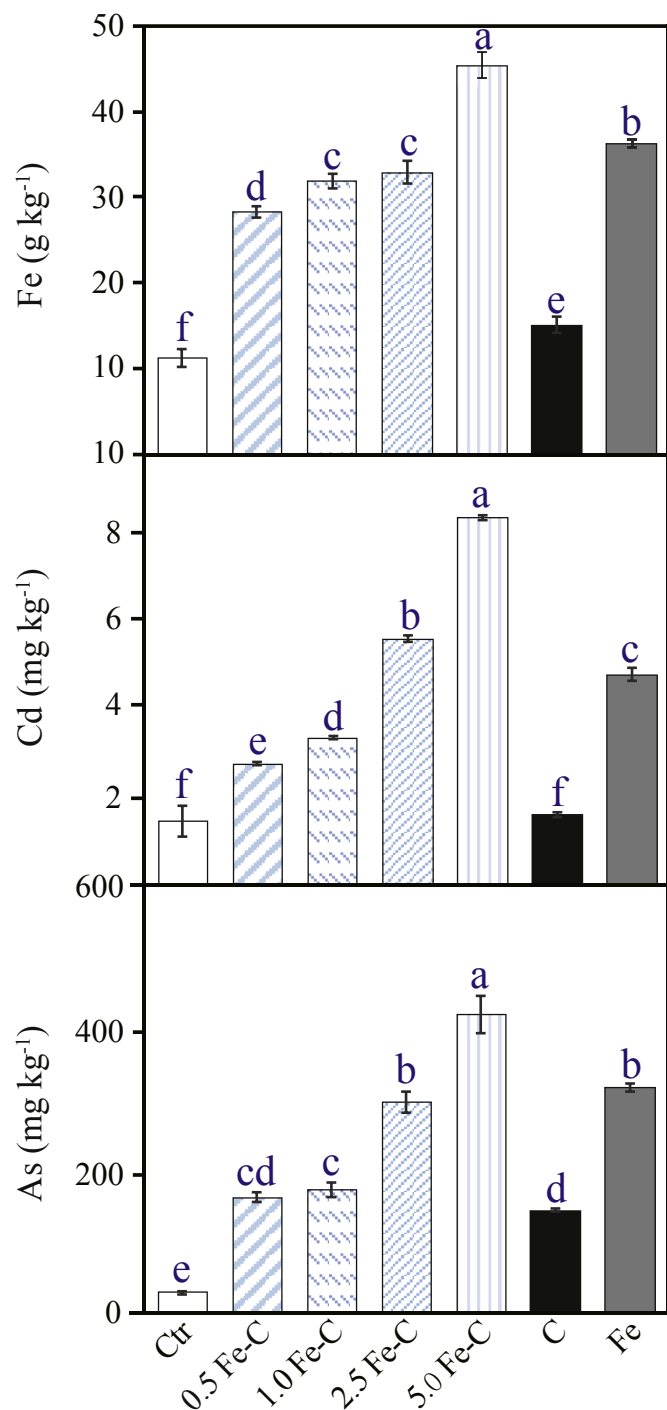
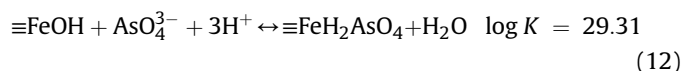
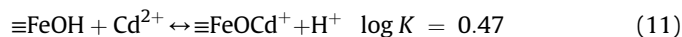


Fig. 6. Concentrations of Fe, Cd, and As in the root Fe plaque of paddy soil under different treatments. Significant differences are indicated by different letters ($P < .05$). Bars represent standard errors ($n = 3$).

Cd and As (Fig. 6), which was further confirmed by the positive correlations between the Fe contents in the amorphous iron oxides and those in Fe plaque (Tables S16 and S17).

Thus, increasing the amorphous Fe oxides in rhizosphere soil can enhance the formation of Fe plaque and Cd and As accumulation in Fe plaque. In fact, the affinity of Cd to Fe oxides may not be very strong in comparison with As (Chen et al., 1997). The surface complexation constant of AsO_4^{3-} on iron oxides ($\log K = 29.31$) was much higher than that of Cd^{2+} iron oxides (0.47) (Rxns. (11) and

(12)), suggesting that the affinity of As to Fe oxides was much stronger than that of Cd. Furthermore, $\equiv\text{FeOcd}^+$ and $\equiv\text{FeH}_2\text{AsO}_4$ in Rxns. (11) and (12) stand for the surface complexes of Cd^{2+} and AsO_4^{3-} on the surface hydroxyl group of iron oxyhydroxides ($\equiv\text{FeOH}$), which were directly obtained from the database of Visual Minteq. $\equiv\text{FeOH}$ in Rxns. (11) and (12) stands for the surface hydroxyl group of iron oxyhydroxides.



The Fe oxides have unique roles in immobilizing As and Cd, particularly for As. Using ZVI as a soil amendment to stabilize As contamination has been widely reported in previous studies (Cundy et al., 2008; Kumpiene et al., 2006; McBride and Martínez, 2000). However, in multi-contaminated sites, although the ZVI amendment showed unique roles in reducing As bioavailability, it may have a different impact on other elements, such as increasing Cd mobility (Hartley et al., 2004). Compared with the untreated soil (pH 4.7), the ZVI amendment also increased the soil pH (pH 5.4), which also contributed to Cd immobilization as described above. In summary, the increased amounts of amorphous Fe oxides induced by ZVI addition may be the key factor in immobilizing As using the ZVI amendment.

4.3. The interaction between biochar and ZVI

In the combined biochar and ZVI amendments, both the Cd and As contents in different rice tissues decreased significantly (Fig. 1). With increasing Fe contents in the Fe-biochar mixture, the bioavailability of Cd and As decreased (Fig. 2); however, the immobilized Cd and As in soils and Fe, Cd and As in Fe plaque increased significantly (Fig. 6). Similar to single biochar or single ZVI amendments, the combined ZVI-biochar amendment also increased the soil pH (pH 5.0–5.6) in comparison with the untreated soil (pH 4.7) (Fig. S15), which can further affect Cd and As bioavailability. Based on the above discussions, biochar can highly adsorb Cd but weakly immobilize As, and ZVI can greatly immobilize As but slightly adsorb Cd; therefore, for Cd and As co-contaminated paddy soil, using the single biochar or single ZVI did not effectively reduce the bioavailability of both Cd and As. The combined amendment of ZVI and biochar might have the combined effect of biochar and ZVI that simultaneously immobilizes Cd and As. However, the results suggested that the extents of Cd alleviation with Fe-biochar mixtures (90%) were much higher than the total extent (30%) of single Fe (10%) and single biochar (20%) amendment (Fig. 1), implying that, in addition the single effects of Fe and biochar, the interaction between Fe and biochar may play an important role and contribute heavily to the overall Cd alleviation with Fe-biochar mixtures. It was reported that the presence of carbon might accelerate the corrosion of ZVI and result in the rapid formation of iron oxides (Dou et al., 2010). The possible mechanism of the acceleration in corrosion of ZVI in the presence of biochar/carbon can be reasonably assigned to electron acceleration between ZVI and biochar due to biochar's electrical conductivity (Klöpffel et al., 2014). Due to its condensed aromatic structures and quinone/hydroquinone moieties, biochar can facilitate electron transfer (Chen et al., 2014; Kappler et al., 2014; Xu et al., 2014). The FTIR spectrum of the biochar suggested the presence of aryl carbonyl carbon group ($\text{C}=\text{O}$) at 1653 cm^{-1} and methyl group ($\text{C}-\text{H}$) at 1385 cm^{-1} (O'Reilly and Mosher, 1983; Tatzber et al., 2010) (Fig. S11), again demonstrating that it has the potential in

facilitating electron transfer. This suggests that ZVI corrosion in the biochar/ZVI mixture can be effectively enhanced. Thus, it can be proposed that after contacting the surface of biochar, the ZVI might be corroded quickly due to the possible catalytic effect of the biochar surface on the corrosion reactions (Rxns. (6) and (7)), resulting in the formation of iron oxides, which can enhance the immobilization of Cd and As.

In this study, the Fe ratio in the ZVI-biochar mixture, which is applied on a dry weight basis of biochar to paddy soil, is very low. The highest Fe ratio (5%, w/w, based on a dry weight basis of biochar, which is equivalent to 0.05% w/w based on a dry weight of soil) is much lower than the reported application rates of Fe (higher than 5% w/w, based on a dry weight basis of soil), which can cause soil aggregate cementation and reduced soil porosity (Kumpiene et al., 2008). Our results demonstrate that the pre-mixed ZVI-biochar mixture has great potential for remediating Cd and As co-contaminated paddy soil.

5. Conclusion

In this study, a combination of biochar and ZVI was applied to paddy soil contaminated with both Cd and As to investigate its potential impact on Cd and As bioavailability. The pot trials showed that the ZVI-biochar treatments simultaneously reduced the mobility and bioavailability of Cd and As in co-contaminated paddy soil. In comparison with the single biochar or single ZVI amendments, the ZVI-biochar mixtures have synergistic effects of biochar and ZVI. Briefly, the mixtures greatly enhanced the transformation of Cd and As fractions from bioavailable Cd and As forms to less bioavailable fractions. In addition, the mixtures also increased the amorphous Fe oxides concentration and enhanced the formation of iron plaque. Interestingly, the Fe ratio in the ZVI-biochar mixture was positively related with the combined ZVI-biochar effect. Taken together, for treating Cd and As co-contaminated sites, the combined ZVI-biochar treatment can be a good choice in the future.

Notes

We have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.chemosphere.2017.12.081>.

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