Article

Combined Effect of Ferrous Ion and Biochar on Cadmium and Arsenic Accumulation in Rice

Qun Rong [1,2], Kai Zhong [2], Fangyuan Li [2], He Huang [1,2], Chuanzhang Li [1,3], Xinyu Nong [2] and Chaolan Zhang [1,2,*]

[1] College of Life Science and Technology GuangXi University, Nanning 530004, China;
[2] College of Resources, Environment and Materials GuangXi University, Nanning 530004, China;
[3] Guangxi Zhuang Autonomous Region Environmental Monitoring Central Station, Nanning 530004, China

Abstract: Excessive accumulation of cadmium (Cd) and arsenic (As) in rice (*Oryza sativa* L.) poses a potential health risk to populations. Cd and As exhibit opposite geochemical behavior in paddy soil, using appropriate remediation materials to reduce their migration and inhibit their uptake by rice is a great challenge. A pot culture experiment was conducted to investigate the effects of application of silkworm excrement biochar (BC) and ferrous sulfate (Fe(II)) on available Cd and As in paddy soils and their uptake by rice. Results showed that the application of BC + Fe significantly accelerated the tillering of rice plants, and the addition of BC alone to soil did not have a significant effect on the pH of soil, while applied 1% (w/w) BC and 1% (w/w) Fe(II) (1BC-1 Fe(II)) treatment could markedly reduce the soil pH. BC+Fe(II) could reduce the content of available Cd (reduced by 10%–23%) and As (reduced by 6%–33%) in soil. BC+Fe(II) has a distinct decreasing effect on the available As, thus inhibiting As uptake in rice tissues, and the effect was more obvious with an increasing mass ratio of Fe in BC+Fe(II) treatment. BC+Fe(II) decreased bioaccumulation factors (BF) of As compared to control and BC alone treatments. Compared with As, Cd was more readily transferred from the root to the shoot and accumulated in rice eventually. These findings provide a safe and reliable remediation strategy though application of BC+Fe(II) in Cd and As co-contaminated soil. However, the improvement effect of amendments should be paid a special attention on soil pH.

Keywords: silkworm excrement biochar; heavy metal; paddy soil; phytoavailability

1. Introduction

Anthropogenic activities and mining are major sources of metals accumulation in soils, especially in agricultural soils [1,2]. The pollution of Cd in paddy soils is a widespread problem due to its potential harm to plants and human health [3]. Rice (*Oryza sativa* L.) is a widely cultivated crop in China [1], and also a common dietary source for Cd and As exposure. A number of studies have reported that metal contents exceed safe values [4]. Cultivated in flooded soil, with the enhanced bioavailability of As in soil due to the anaerobic conditions, the rice take in more [5]. In addition, As is more easily taken in rice than other cereals [6]. Owing to high redox potential, aerobic conditions in paddy soil can obviously inhibit the uptake of As in grains, whereas rice growing tends to increase uptake of Cd in rice under the aerobic conditions [7,8]. For ensuring the grain safety, how to simultaneously reduce the uptake of Cd and As in rice has become a vital issue and drawn much public attention.

Biochar is a carbon-rich material produced by the thermochemical conversion of biomass in a anoxic-high temperature condition, commonly using biomass materials including agricultural waste, animal and plant residues, etc. [9]. In recent years, many researchers have highlighted that the biochar as a novel soil remediation or amendment was effective in remediating inorganic contaminants [10,11]. Agricultural solid waste has been widely used as a source of biochar materials [12,13]. The treatments with biochar reduce Cd transferring from soil to rice owing to its surface absorption and functional groups (e.g., −OH) complexation in order to immobilize metal cations in soils [14–16]. However, biochar did not show a consistent effect in As contaminated soil, nor did it effectively reduce the content of As in rice. The chemical properties of Cd and As in soil are usually opposite, so it is a challenge to find a composite soil amendment for eliminating the content in rice as well as ecological risk for both contaminants. There are numerous iron-based materials that have been proved to be effective in the immobilization of As in soil. Zero valent iron, iron (hydr) oxides have been extensively reported on immobilizing As in contaminated soils [17,18]. In addition, the effect of soluble Fe on reducing the bioavailability of As is better than that of metallic Fe [19]. The possible reason is that amorphous iron oxides with As can form Fe-As compounds on its surfaces with low solubility [20]. These reports suggest that decreasing As bioavailability and bioaccessibility in soil by applying Fe-material may be an effective method to immobilize As. In addition, Fe(II) and Fe(III) salts are considered to be a more effective amendments for As contaminated soil because of their chemical reactions in the soil that are more effective than iron oxides [18,21,22]. Therefore, Fe(II) as the main remediation material was proved to be effective in reducing the water-soluble As in soil [12,23].

Combining Fe-base materials and biochar to amend Cd and As co-contaminated soil have been reported, such as Fe(II) combination with compost, as a promising approach to deal with metals co-existing in high concentrations in soil [24,25]. However, in the paddy soils system, soil functions and services should be improved while considering the mitigation of metal toxicity. Therefore, based on these characteristics and our previous studies [12,26], biochar and ferrous ion were chosen to be amendments settling the Cd-As contaminated paddy soil, in order to figure out the disposal procedure of the materials and the translocation efficiency reduction of the two heavy metals mentioned above in contaminated rice plant soil. The objects of this study were (1) to investigate the effect of the biochar and ferrous ion on the bioavailable of Cd and As in contaminated soils and metals uptake by rice and (2) to explore the possible mechanism using biochar and ferrous ion immobilization As and Cd in soils.

2. Materials and Methods

2.1. Materials

The raw material of silkworm excrement biochar (BC) was collected from the Guangxi Silk-Worm Technology Promotion Centre. The procedure of preparing biochar (BC) has been reported in our previous study [12]. Briefly, after dried naturally in a plastic tray, the silkworm excrement was transferred into a crucible. Then, the crucible was then heated in a muffle furnace (Nabertherm LT40, BRE, GER; heating rate: 20 °C/min; ventilatory rate: 2 mL N_2/min) at 500 °C for 3 h, and then cooled to room temperature. BC was pulverized and stored in a glass container prior to characterization and pot experiment. The basic physicochemical properties of BC were determined, and the results were shown in Table 1.

Table 1. Soil and biochar characteristics.

Item	pH	Total Organic Carbon (TOC) %	Cation Exchange Capacity (CEC) cmol/kg	Available N mg/kg	Available P mg/kg	Available K mg/kg	Total As mg/kg	Total Cd mg/kg	Available As mg/kg	Available Cd mg/kg
Soil	7.6	14.6	9.25	131.5	26.5	122.0	27.3	7.7	1.8	3.0
Biochar	9.4	227.8	71.59	ND	ND	ND	ND	ND	ND	ND

Ferrous sulfate (Fe(II)) was purchased from Shanghai Sinopharm Chemical Reagent Co. Ltd., China, which is analytical grade.

The contaminated soil was classified as a typical calcareous soil and collected in a paddy field adjacent to a mining in Daxin, Guangxi Zhuang Autonomous Region, China. Soil samples were dried naturally in shade. The pH, total organic carbon (TOC), cation exchange capacity (CEC), available N, available P, available K, As, and Cd contents of soil were determined, and the results were shown in Table 1.

The rice variety (*Oryza sativa* L.) is Baiyou1191, which purchased from the agricultural science and technology market of Nanning, Guangxi Zhuang Autonomous Region, China. The entire growing period of rice is about three months.

The concentrations of Cd and as in the solutions were determined by inductively coupled plasma mass spectrometry (ICP-MS, iCAP Q, Thermo Scientific, MA, USA). ND indicates lower than the detection limit, Cd is 0.0125 mg/kg, and As is 0.025 mg/kg.

2.2. Pot Experiments

The amendments were consisted of BC and/or Fe(II). The treatments were as follows: (1) Control, untreated soil (9 kg dry weight); (2) BC, alone applied 1% (w/w, dry weight soil) biochar per pot, (3)1BC-1Fe(II), applied 0.5% biochar and 0.5% ferrous sulfate per pot; (4) 3BC-1Fe(II), applied 0.75% biochar and 0.25% ferrous sulfate per pot; (5) 5BC-1Fe(II), applied 0.83% biochar and 0.17% ferrous sulfate per pot. The amendments were mixed with soil and placed in each pot. Basic fertilizer was applied at 54.0 g N($CO(NH_2)_2$), 3.6 g P($Ca(H_2PO_4)_2 \cdot H_2O$), and 2.9 g K(KCl) of each pot, and each treatment was repeated three times.

The treated soil was submerged (2–3 cm above the soil surface) with deionized water for a week and randomly arranged in a greenhouse. The rice seeds were sterilized with 30% H_2O_2 for 10 min, then rinsed with deionized water and soaked for 24 h. Rice seeds were germinated in pots filled with vermiculite in a greenhouse until five leaves seeding out, and then three rice seedlings, with uniform sizes were transplanted into each pot on 30 August 2015. Effective tiller numbers of each treatment contained 3 sub-samples on the tillering stage (16 October 2015), and each sub-sample effective tiller numbers were measured for three tested plants in each pot. Plants were reaped on 19 November 2015.

The procedure of water management are as follows: maintain a 2 cm layer of water until the tillering stage, 3 cm layer of water at tillering stage, and then dried at the jointing stage, 3 cm layer of water at the blooming stage, 1 cm layer of water was maintained at the maturity stage, dried out for cleavage until 7 days before the harvest. A fixed vertical ruler was used for measuring the depth of layer water in the whole growth stage.

Rhizosphere soil was collected during rice harvest, and each soil sample contained 3 sub-samples. The collected soil samples were air-dried and screened to <2 mm for pH, available Cd and As measurements. The plant tissues including root, straw, husk, and grain were collected. Each sample consisted of 3 subsamples. The rice tissues were thoroughly washed with ultrapure water (18.2 MΩ·cm) eventually. The root and straw were dried at 105 °C for 1 h, and dried at 60 °C for 48 h in a dryer. Grain were dried at 60 °C for 48 h before being decorticated. The samples were powdered with a stainless steel grinder and passed through a 0.25 mm sieve before chemical analysis. All the plant tissues were crushed using a stainless steel mill and the powder stored in a silica gel dryer before chemical analysis.

2.3. Chemical Analyses

Soil pH was measured with a glass electrode using a soil to carbon dioxide-free water ratio of 1:2.5 (w/v, UB-10, Denver Instrument, Arvada, CA, USA). For determination of soil total organic carbon (TOC), 0.3 g soil was added to 50 mL glass tubes, and the tubes were placed in a paraffin oil bath for 5 min at 170 °C with 5 mL of $K_2Cr_2O_7$-H_2SO_4 solution, and the mixture was titrated with ferrous sulfate solution after cooling. The soil cation exchange capacity (CEC), available N, available P, and available K were measured using the $BaCl_2$-$MgSO_4$ exchange, NaOH hydrolyzed-H_3BO_3 absorption, sodium bicarbonate extraction Mo-Sb anti-spectrophotometry, and ammonium acetate extraction flame photometry method, respectively [27]. Soil samples of 0.2000 g were digested in a ternary mixture of HF, HNO_3, and $HClO_4$ (4/5/2, v/v) acid in microwave digester (Mars 6, CEM, BSN, USA) at 220 °C for 30 min until a clear solution about 1–2 mL was obtained. Then, the solution was transferred into a volumetric flask, and adjusted to 50 mL with 3% (v/v) HNO_3 for later analysis. Plant samples of 0.5000 g were digested with $HNO_3/HClO_4$ (8/2, v/v) in a microwave digester. Available Cd was extracted by diethylene triamine pentaacetic acid-triethanolamine (DTPA) according to ISO 14870 [28], and available As were extracted by 0.5 M $NaHCO_3$ [29]. All of the extracts were diluted with Milli-Q water and filtered using 0.45 μm nylon filter membranes and subsequently analyzed for Cd and As concentrations using inductively coupled plasma mass spectrometry (ICP-MS, iCAP Q, Thermo Scientific, Waltham, MA, USA). The analysis methods for As and Cd in rice tissues have been described in detail in previous studies [30,31]. The resulting solutions were diluted to 50 mL in 3% HNO_3 in volumetric flasks, filtered, and stored at 4 °C prior to analysis.

The glassware used for Cd and As analyses in the experiment was soaked with 10% HNO_3 before. To ensure the quality, 20 samples including blank samples, duplicate samples, and reference material were digested. For the whole procedure, soil and plants were digested using documented standard materials for quality control (GBW10044 (grain) and GBW07454 (soil)).

2.4. Statistical Analysis

The data were analyzed using an analysis of variance. Duncan's multiple range tests were used to detect differences between means for the fixed effects at the probability levels of $p < 0.05$ and $p < 0.01$ (IBM SPSS Statistics 19.0, Armonk, NY, USA). The figures were drawn with Origin Pro 8.0 (OriginLab, Northampton, MA, USA).

The bioaccumulation factor (BAF) and translocation factor (TF) were calculated based on previous studies [22], and presented as follows:

$$\mathrm{BAF_{root}} = \mathrm{C_{root}}/\mathrm{C_{soil}},$$

$$\mathrm{BAF_{straw}} = \mathrm{C_{straw}}/\mathrm{C_{soil}},$$

$$\mathrm{BAF_{husk}} = \mathrm{C_{husk}}/\mathrm{C_{soil}},$$

$$\mathrm{BAF_{rice}} = \mathrm{C_{rice}}/\mathrm{C_{soil}},$$

$$\mathrm{TF_{rs}} = \mathrm{C_{straw}}/\mathrm{C_{root}},$$

$$\mathrm{TF_{sg}} = \mathrm{C_{grain}}/\mathrm{C_{straw}},$$

where C_{root}, C_{straw}, C_{husk}, C_{grain}, and C_{soil} are the contents ($mg \cdot kg^{-1}$) of Cd or As in root, straws, husk, grain, and soil, respectively.

3. Results

3.1. Effective Tiller Number

Metal pollution inhibited the growth of rice effective tillers, which could be alleviated by BC+Fe(II) treatment (Figure 1, Table 2). The treatments of BC+Fe(II) increased effective tiller number, especially the 1BC-1Fe(II) treatment, whose tiller number was >2-fold greater than that of the control. The effective tiller number was increased with increasing Fe(II) ratio in BC+Fe(II) treatments. Nevertheless, the BC treatment was minor reduction than control, but there was no significant difference. These results showed that the increase of effective tiller number should be attributed to the addition of Fe(II).

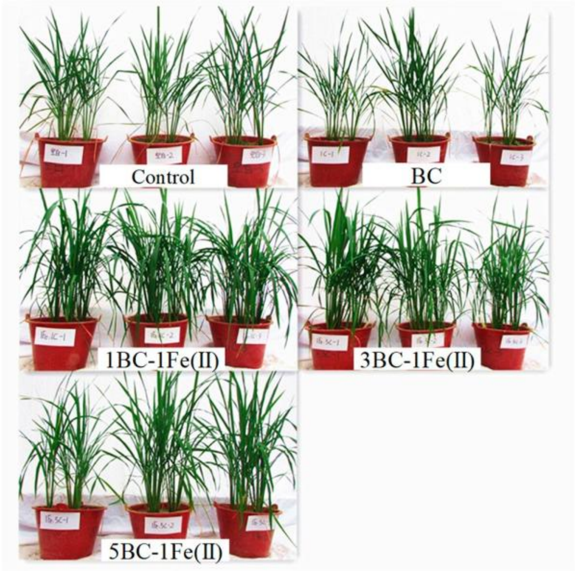

Figure 1. Growth of rice at the tillering stage.

Table 2. Effective tiller number of per rice plant.

Treatments	Effective Tiller Number (per Plant)
Control	4.1 ± 0.2 c
BC	3.6 ± 0.7 c
1BC-1Fe(II)	9.2 ± 0.2 a
3BC-1Fe(II)	7.1 ± 1.1 b
5BC-1Fe(II)	4.7 ± 0.6 c

Data are means ± standard error ($n = 3$). Lowercase letters indicate significant differences ($p < 0.05$). Control, untreated soil; BC, applied 1% (w/w, dry weight soil) biochar; 1BC-1Fe(II), applied 0.5% biochar and 0.5% ferrous sulfate; 3BC-1Fe(II), applied 0.75% biochar and 0.25% ferrous sulfate; 5BC-1Fe(II), applied 0.83% biochar and 0.17% ferrous sulfate.

3.2. *Soil pH*

As shown in Figure 2, the soil pH was decreased 0.7 units under 1BC-1Fe(II) treatment compared to control (pH 7.8). The soil pH presents a gradual increase trend with adding of BC mass in BC+Fe(II) treatments. However, it is worth noting that the pH of the BC treatment (7.9) was slightly increased compared with control; there is no significant difference ($p < 0.05$). Generally, changes of soil pH are the result of a combination of BC and Fe(II), and these are mainly contributed by BC application.

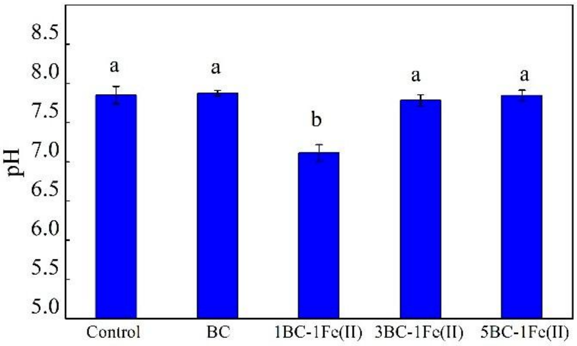

Figure 2. The pH in different treatments. Lowercase letters indicate significant differences ($p < 0.05$). The data are mean ± standard error ($n = 3$), bars represent standard errors ($n = 3$). Control, untreated soil; BC, applied 1% biochar; 1BC-1Fe(II), applied 0.5% biochar and 0.5% ferrous sulfate; 3BC-1Fe(II), applied 0.75% biochar and 0.25% ferrous sulfate; 5BC-1Fe(II), applied 0.83% biochar and 0.17% ferrous sulfate.

3.3. *Available Cd and As*

To analyze the bioavailability of Cd in soil, DTPA extraction method was used to obtain available Cd content under different treatments (Figure 3). Compared with control, the available Cd were reduced in all treated soil, which were significantly decreased by 23% in 5BC-1Fe(II) ($p < 0.05$). It is worth noting that, although BC treatment reduced the available Cd, it had no significant difference with control ($p < 0.05$), as well as 1BC-1Fe(II) and 3BC-1Fe(II) treatments. Combination of BC and Fe(II) could effectively retain the available Cd in soil, and this effect was mainly caused by application of BC.

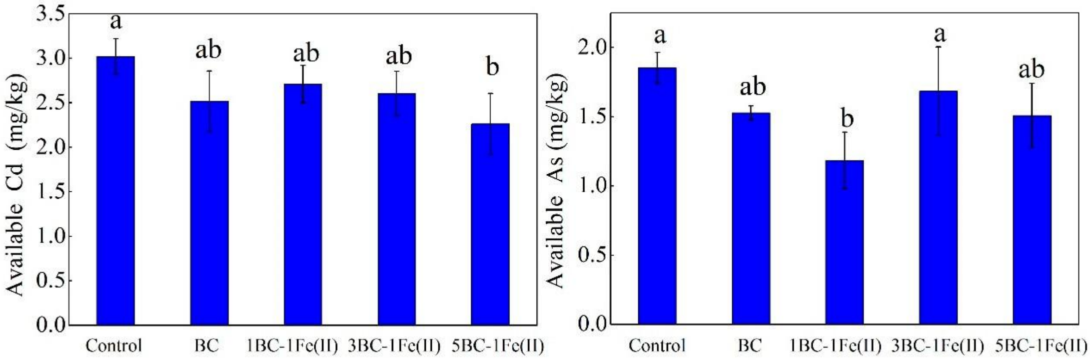

Figure 3. The effect of different treatments on available Cd and As in soil. Different letters indicate that the differences are statistically significant ($p < 0.05$). The data are mean ± standard error ($n = 3$), bars represent standard errors ($n = 3$). Control, untreated soil; BC, applied 1% biochar; 1BC-1Fe(II), applied 0.5% biochar and 0.5% ferrous sulfate; 3BC-1Fe(II), applied 0.75% biochar and 0.25% ferrous sulfate; 5BC-1Fe(II), applied 0.83% biochar, and 0.17% ferrous sulfate.

We further analyzed the changes of available As concentrations after application of amendments (Figure 3). The available As concentration is lowest in 1BC-1Fe(II) treatment, which reduced 33% compared to control. In addition, the BC, 3BC-1Fe(II), and 5BC-1Fe(II) also reduced the content of available As, but there was no significant difference compared to control.

3.4. *Cd and As Contents in Rice Tissues*

The Cd contents and distribution in the root, straw, husk, and grain were affected by applied amendments (Figure 4). The distribution of Cd in rice tissues completely followed as order root > straw > husk > grain in this study. In all amendments, 1BC-1Fe(II) treatment could promote the uptake of Cd in rice tissues. We noted that all treatments promoted the root absorption of Cd, but only 1BC-Fe(II) showed significantly difference, which was increased by 262% compared to control. In addition, the highest content of Cd in straw, husk, and grain under 1BC-1Fe(II) treatment, while the lower Cd content was presented in BC, 3BC-1Fe(II) and 5BC-1Fe(II) treatments. Figure 4 shows that the grain Cd content was highest in 1BC-1Fe(II) treatment, which is 1.3 ± 0.3 mg·kg^{-1}, while the other three treatments are lower. What needs to be illustrated is that the yield of rice in control treatment was significantly affected due to special growing conditions of excessive heavy metal in soil without BC and Fe(II) amendment. Therefore, the plants growth was inhibited, resulting in low yields and inability to digest analysis of grain. Based on the distribution of Cd in plant tissue after treatments, we suppose that the trend of Cd uptake in grain is consistent with root, husk, and straw under the four treatments, Cd content in grain will be lower than 1BC-1Fe(II) treatment while higher than control, 3BC-1Fe(II), and 5BC-1Fe(II) treatments.

The sequence of As accumulation in plant is root > straw > husk > grain; this is consistent with the distribution of Cd. The contents of As in root, straw, husk and grain are 22.00–78.08 mg·kg^{-1}, 1.01–3.02 mg·kg^{-1}, 0.10–0.19 mg·kg^{-1} and 0.01–0.23 mg·kg^{-1}, respectively, which indicates that there is a significant difference in the uptake of As in different tissues. Compared to control, there was no marked difference of As contents in plant tissues under BC treatment, indicating that BC had no significant effect on inhibiting tissues uptaking As. Conversely, the As uptake in tissues increasing with the mass decreased of Fe(II) in BC+Fe(II) treatments, and 1BC-1Fe(II) treatment was more prominent. Similarly, the growth of rice has been inhibited in untreated soil (control), resulting in low yields and inability for digest analysis of grain, which analyzed the distribution of As under different treatments and speculated that the As content in grain was the same as that in BC treatment.

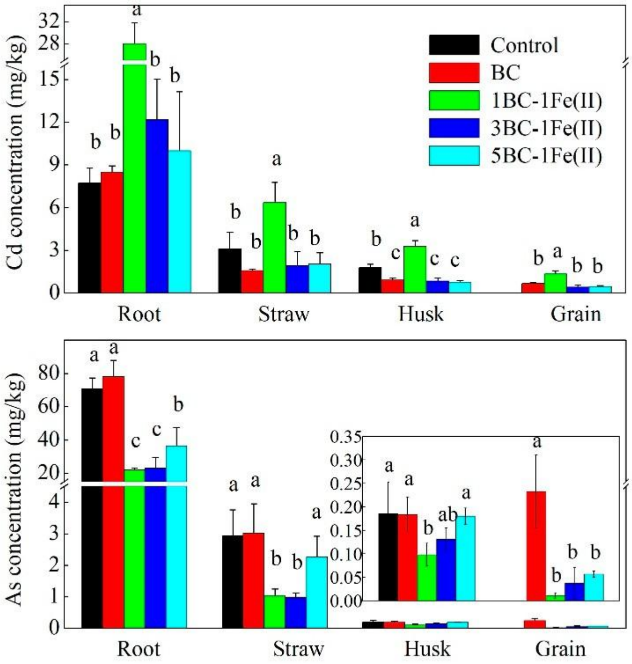

Figure 4. The Cd and As contents in rice tissues. The un-amendment soil (Control) inhibited the ear formation of rice (grain is null in control treatment). Different letters indicate that the differences are statistically significant ($p < 0.05$). The data are mean ± standard error ($n = 3$), bars represent standard errors ($n = 3$). Control, untreated soil; BC, applied 1% (w/w) biochar; 1BC-1Fe(II), applied 0.5% biochar and 0.5% ferrous sulfate; 3BC-1Fe(II), applied 0.75% biochar and 0.25% ferrous sulfate; 5BC-1Fe(II), applied 0.83% biochar, and 0.17% ferrous sulfate.

3.5. Comparison of Cd and As Translocation

In this study, total As (27.3 mg·kg^{-1}) was much higher than Cd content (7.7 mg·kg^{-1}) in soil, but Cd accumulation in grain was much higher than As (Figure 4). BAF were calculated based on the ratio of the heavy metal uptake by different tissues to the total metal content in soil (Tables 3 and 4). In general, the amendment treatment which has poor BAF value could be suitable for immobilization of Cd or As to decrease their availability to the rice in the soil. The BAF value of Cd was the highest in 1BC-1Fe(II) treatment, which was the same as the pH reduction effect. This indicates that soil pH is the main affecting factor of BAF. In addition, the BAF value of Cd gradually decreased from root to grain migration. Combined with the analysis of TF, we found that it is easier to transfer Cd from root to stem than from stem to grain. The BAF of As have significantly higher of root and straw uptake ability than husk and grain, it ranged from 0.04 to 2.86 in root and straw, whereas, for husk and rice, it only ranged from 0.04×10^{-2} to 0.90×10^{-2}, indicating that As in root and straw was much easier to be accumulated than in husk and grain. When Fe(II) was added, the accumulation of As in plants was lower than that in control and BC treatment, indicating that Fe(II) inhibited the migration of As to plants.

Furthermore, the TF, representing the transfer capacity of metals in different tissues were calculated (Tables 3 and 4). The TF from root to straw (TFrs) were in the range between 0.17 to 0.37 and 0.04 to 0.06 for Cd and As, respectively, and Cd and As from straw to grain (TFsg) ranged from 0.21 to 0.47 and from 0.02 to 0.13, respectively, indicating that the translocation of As from root to straw and from straw to grain is more difficult than that of Cd. However, the application of BC did not alter (increased or decreased) the As BAF and TF.

Table 3. Bioaccumulation factors (BF) and translocation factors (TF) of Cd.

Treatment	BAFroot	BAFstraw	BAFhusk	BAFgrain	TFrs	TFsg
Control	1.05 ± 0.24 c	0.41 ± 0.17 b	0.23 ± 0.04 b	–	0.37 ± 0.09 a	–
BC	1.09 ± 0.09 bc	0.20 ± 0.11 c	0.12 ± 0.03 c	0.09 ± 0.01 b	0.19 ± 0.02 b	0.47 ± 0.05 a
1BC-1Fe(II)	3.62 ± 0.65 a	0.84 ± 0.20 a	0.43 ± 0.07 a	0.17 ± 0.03 a	0.24 ± 0.07 b	0.25 ± 0.06 b
3BC-1Fe(II)	1.64 ± 0.46 b	0.29 ± 0.14 bc	0.11 ± 0.03 c	0.06 ± 0.02 c	0.17 ± 0.04 b	0.21 ± 0.02 b
5BC-1Fe(II)	1.54 ± 0.30 bc	0.26 ± 0.12 bc	0.11 ± 0.04 c	0.06 ± 0.01 c	0.21 ± 0.03 b	0.29 ± 0.08 b

– BAF and TF value could not be calculated due to the lack of data on the control treatments. The data are mean ± SD ($n = 3$). Different lowercase letters indicate a significant difference at $p < 0.05$.

Table 4. Bioaccumulation factors (BF) and translocation factors (TF) of As.

Treatment	BAFroot	BAFstraw	BAFhusk ($\times 10^{-2}$)	BAFgrain ($\times 10^{-2}$)	TFrs	TFsg
Control	2.69 ± 0.29 a	0.12 ± 0.03 a	0.64 ± 0.21 a	–	0.04 ± 0.01 b	–
BC	2.86 ± 0.44 a	0.11 ± 0.04 a	0.66 ± 0.15 ab	0.90 ± 0.30 a	0.04 ± 0.01 b	0.13 ± 0.04 a
1BC-1Fe(II)	0.83 ± 0.10 b	0.04 ± 0.01 b	0.38 ± 0.10 b	0.04 ± 0.02 c	0.04 ± 0.01 b	0.02 ± 0.01 b
3BC-1Fe(II)	0.86 ± 0.29 b	0.04 ± 0.01 b	0.52 ± 0.17 ab	0.20 ± 0.10 b	0.04 ± 0.01 b	0.03 ± 0.01 b
5BC-1Fe(II)	1.46 ± 0.44 c	0.08 ± 0.03 c	0.60 ± 0.14 ab	0.20 ± 0.01 b	0.06 ± 0.01 a	0.03 ± 0.02 b

– BAF and TF value could not be calculated due to the lack of data on the control treatments. The data are mean ± SD ($n = 3$). Different lowercase letters indicate a significant difference at $p < 0.05$.

4. Discussion

The yield of rice is associated with the tiller number of plants. More rice plant tillers mean a higher yield of rice [32]. As is well known, Fe as an essential mineral elements for the chloroplast synthesis and enzymes involved in the biosynthesis express has a very important role in graminaceous plants [33], and thus promotes plant growth [24,34]. The iron may generate the formation of iron plaque on the surface of root, promoting the growth and bifurcation of root [23]. Iron plaque both increases the uptake of nutrients and promotes the growth of rice; these results can be found in field applications [35]. In addition, extra addition of SO_4^{2+} into the heavy metal contaminated soil could increase the plant height and the soil biomass [36].

In soil, pH is a key factor which influence the availability of heavy metals [37]. As is easily transferred in soils with a pH between 3 and 8 [38]. On the contrary, in a low pH condition, the availability and mobility of Cd are increasing. In previous study, the limed biochar had been proved to be effective in eliminating soil contamination by increasing the soil pH [39–41]. In our study, BC has a high pH value (pH 9.4) which could induce a liming effect on the mobility of Cd. Furthermore, with the pH increasing, the number of positively charged sites on soil minerals decreases, lowering the sorption capacity of negatively charged oxy-anions of As [42]. However, in our study, the pH slightly increased with biochar addition, and the changes in soil pH were brought about by the Fe(II) application. The pH value was significantly decreased in 1BC-1Fe(II) treatment owing that Fe(II) accounted for a significant portion of amendment. A similar result was reported by Cui [43], who found that addition of Fe(II) could obviously decrease the soil pH value. It is well known that the Fe-hydroxides may result in a lower pH. It can be ascribed to the hydrolysis and precipitation of Fe(III)-hydroxides, after the oxidation of Fe(II) to Fe(III) in the air. With a relatively rapid kinetics, Fe(II) can be oxidized to the trivalent state [44]; this process produces protons and thus may lower the pH [45].

Heavy metal's toxicity in the soil and its potential risk of entering human body was evaluated by its bioavailability. The mobility and bioavailability of metals can be eliminated dramatically by applying soil amendments in the multiple contaminated soils [11,46]. The mechanisms of stabilizing the As in soil can be concluded in two aspects: the generation of amorphous or crystalline Fe(III) arsenate compounds and the other one is absorption on the layer of iron (oxy)hydroxides, including zero talent iron, iron hydroxides, and oxides, which have the ability to remove both As(III) and As(v) from soil [22,30]. Fe(II) could also be oxidized to Fe(III) when exposed to an aerobic environment, and, with oxidized forms, it was possible to subsequently remove As from contaminated soil by adsorption and co-precipitation [47]. The Fe(III) ions were precipitated as Fe(III) hydroxide when BC was amended. The iron-bearing mineral was formed and bonded arsenate and arsenite, which may play a significant role in As immobilization of Fe(II) in our study [24,25].

In addition to the liming effect, biochar may provide other benefits including adsorption of both cations and anions which may reduce nutrients' leaching owing to high surface charge density, high surface area, internal porosity, and the presence of both polar and non-polar surface sites [12,48]. In general, the liming procedure can enhance the heavy metal barrier effect. The effect of the amendments on Cd differed from that of As, owing to the application of Fe(II), soil pH decreased by 0.7 units in 1BC-1Fe(II) treatment. The immobilization of heavy metals can be achieved by adsorption on the surface of biochar. It can be divided into two categories: specific and non-specific adsorption. Specific adsorption indicates that the metals were forming coordination bonds to surface in the inner layer, while nonspecific adsorption related to those metals was adsorbed by simple coulombic interaction in the diffuse electric double layer [49]. Harvey [50] reported that Cd^{2+} was adsorbed by biochar occurring predominantly via two distinct cation-π bonding mechanisms involving Cd^{2+}–π bonding with soft ligands (e.g., C = O) or electron-rich domains on aromatic structures. Such adsorption processes are probably responsible for our study. The results of this study are particularly pleasing regarding the application of this biochar to Cd and As contaminated soils, since it demonstrated that available Cd and As concentrations were decreased. However, soil pH was not distinctly changed in BC alone treatments compared to control. Thus, it can be deduced that the decrease of Cd mobility may be attributed to absorption by BC rather than pH increase. In the present study, the available As can be eliminated by Fe(II), but the DTPA-extractable Cd increased. These antilogous results may owe to the variance of soil pH after addition of Fe(II) [51].

Related to the free iron oxide, the iron plaques on the outside layer of roots can accelerate the inhibition of As [22,52]. Due to iron plaque being ubiquitous on rice roots and a barrier against As uptake, it should not be ignored while detecting the As inhibition mechanism of rice plants. The As content and BAF in root, husks, and grain increased proportionally to decreasing amounts of external Fe(II) (Figure 4, Table 4). The application of Fe(II) showed significant reduction of the As content in rice root, indicating that Fe(II) application brings about the decrease mobility of As by raising the level of free Fe oxide in the soil. The high affinity of root iron plaques to arsenate and altered the arsenate uptake of rice seedlings [53,54]. It can be presumed that the root iron plaque has the ability to obstruct As uptake, thus reduces the migration of As from soil to rice plant. Iron-bearing mineral and iron plaque play a key role in immobilization of As contaminated soil. Cd has a higher TFrs value than As (Tables 3 and 4), indicating that the translocation of As from root to straw and from straw to rice is more difficult than that of Cd. The study has shown that aquaporin channels can take As(III) and Cd [55]. In addition, Cd can also enter the rice by an absorption channel of mineral elements [56], transported from roots to the stem through the phloem, then directionally distributed through the microtubules. Therefore, the differences between metal contents and distribution in rice tissues may be the reason for different transport mechanisms when the rice plant uptakes the Cd and As (V). As (V) is taken up via phosphate transporters of plants [57] because both arsenate and phosphate have a tetrahedral structure [58,59]. Matsumoto reported that the application of Fe materials had a significant decreasing effect on the contents and proportions of inorganic-As (iAs) in rice grain, indicating that, compared to the translocation of organic As from shoots/roots to grains [60], the same process of iAs is much more difficult. This made the decrease in the proportion of iAs in the rice grain. The TF of As depends not only on total As, but its morphology (III, V) is also an important factor. Therefore, the treatments with addition of BC, BC+Fe(II) made a difference in the soil characteristics like pH, Fe fractions, As species, and Cd migration, resulting in different Cd and As uptake from soil to rice plants.

5. Conclusions

This study illustrates the addition of BC and Fe(II) under an appropriate mass ratio could guarantee the growth of rice in As and Cd co-contaminated paddy soil, and the toxicity of Cd and As to rice growth was significantly reduced. The BC+Fe(II) treatment can reduce the available As and Cd in soil. The Fe(II) significantly inhibited As uptake by rice and the translocation from roots to shoots, and BC could decrease the accumulation and migration of Cd. The 1BC-1Fe(II) treatment markedly reduced As uptake and soil pH significantly, while more Cd accumulates in the rice plant and one should be catious about the risk of causing damage to human health. Furthermore, in this study, when Fe(II) and BC were used together, the inhibitory effect of Cd and As uptake by rice in straw, husk, and grain could be significantly observed with increasing the mass ratio of biochar. The combined use of BC and Fe(II) as amendments in the Cd-As co-contaminated soil should be taken into consideration due to its pH decreasing effect on soil.

Author Contributions: Conceptualization, Q.R. and C.Z.; Methodology, H.H. and F.L.; Project administration, C.L.; Software, C.L.; Writing—original draft, Q.R. and K.Z.; Writing—review and editing, C.L. and X.N. All authors have read and agreed to the published version of the manuscript.

Funding: The study was supported by the National Key Research and Development Program of China (2018YFD0800700, 2017YFD0801300) and the National Natural Science Foundation of China (No. 41461091).

Conflicts of Interest: No potential conflict of interest was reported by the authors.

References

1. Liu, X.; Tian, G.; Jiang, D.; Zhang, C.; Kong, L. Cadmium (Cd) distribution and contamination in chinese paddy soils on national scale. *Environ. Sci. Pollut. Res.* **2016**, *23*, 17941–17952. [CrossRef] [PubMed]
2. Zhao, F.J.; Ma, Y.; Zhu, Y.G.; Tang, Z.; Mcgrath, S.P. Soil contamination in china: Current status and mitigation strategies. *Environ. Sci. Technol.* **2015**, *49*, 750–759. [CrossRef] [PubMed]
3. Chen, H.P.; Tang, Z.; Wang, P.; Zhao, F.J. Geographical variations of cadmium and arsenic concentrations and arsenic speciation in chinese rice. *Environ. Pollut.* **2018**, *238*, 482–490. [CrossRef] [PubMed]
4. Chen, H.P.; Yang, X.P.; Wang, P.; Wang, Z.X.; Li, M.; Zhao, F.J. Dietary cadmium intake from rice and vegetables and potential health risk: A case study in xiangtan, southern china. *Sci. Total Environ.* **2018**, *639*, 271–277. [CrossRef]
5. Xu, X.Y.; McGrath, S.P.; Meharg, A.A.; Zhao, F.J. Growing rice aerobically markedly decreases arsenic accumulation. *Environ. Sci. Technol.* **2008**, *42*, 5574–5579. [CrossRef]
6. Rinklebe, J.; Shaheen, S.M.; Frohne, T. Amendment of biochar reduces the release of toxic elements under dynamic redox conditions in a contaminated floodplain soil. *Chemosphere* **2016**, *142*, 41–47. [CrossRef]
7. Hu, P.; Ouyang, Y.; Wu, L.; Shen, L.; Luo, Y.; Christie, P. Effects of water management on arsenic and cadmium speciation and accumulation in an upland rice cultivar. *J. Environ. Sci.* **2015**, *27*, 225–231. [CrossRef]
8. Arao, T.; Kawasaki, A.; Baba, K.; Mori, S.; Matsumoto, S. Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in japanese rice. *Environ. Sci. Technol.* **2009**, *43*, 9361–9367. [CrossRef]
9. Ahmad, M.; Rajapaksha, A.U.; Lim, J.E.; Zhang, M.; Bolan, N.; Mohan, D.; Vithanage, M.; Lee, S.S.; Ok, Y.S. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* **2014**, *99*, 19–33. [CrossRef]
10. Peng, X.; Deng, Y.E.; Peng, Y.; Yue, K. Effects of biochar addition on toxic element concentrations in plants: A meta-analysis. *Sci. Total Environ.* **2018**, *616*, 970–977. [CrossRef]
11. He, E.K.; Yang, Y.X.; Xu, Z.B.; Qiu, H.; Yang, F.; Peijnenburg, W.J.G.M.; Zhang, W.H.; Qiu, R.L.; Wang, S.Z. Two years of aging influences the distribution and lability of metal(loid)s in a contaminated soil amended with different biochars. *Sci. Total Environ.* **2019**, *673*, 245–253. [CrossRef] [PubMed]
12. Bian, P.Y.; Zhang, J.J.; Zhang, C.L.; Huang, H.; Rong, Q.; Wu, H.X.; Li, X.; Xu, M.M.; Liu, Y.; Ren, S.W. Effects of silk-worm excrement biochar combined with different iron-based materials on the speciation of cadmium and lead in soil. *Appl. Sci.* **2018**, *8*, 1999. [CrossRef]

13. Huang, H.; Zhang, C.L.; Zhang, P.; Cao, M.Z.; Xu, G.P.; Wu, H.X.; Zhang, J.J.; Li, C.Z.; Rong, Q. Effects of biochar amendment on the sorption and degradation of atrazine in different soils. *Soil Sediment Contam.* **2018**, *27*, 643–657. [CrossRef]
14. Beesley, L.; Moreno-Jimenez, E.; Gomez-Eyles, J.L.; Harris, E.; Robinson, B.; Sizmur, T. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.* **2011**, *159*, 3269–3282. [CrossRef] [PubMed]
15. Fellet, G.; Marchiol, L.; Delle Vedove, G.; Peressotti, A. Application of biochar on mine tailings: Effects and perspectives for land reclamation. *Chemosphere* **2011**, *83*, 1262–1267. [CrossRef]
16. Yue, L.; Lian, F.; Han, Y.; Bao, Q.L.; Wang, Z.Y.; Xing, B.S. The effect of biochar nanoparticles on rice plant growth and the uptake of heavy metals: Implications for agronomic benefits and potential risk. *Sci. Total Environ.* **2019**, *656*, 9–18. [CrossRef]
17. Zhang, M.Y.; Wang, Y.; Zhao, D.Y.; Pan, G. Immobilization of arsenic in soils by stabilized nanoscale zero-valent iron, iron sulfide (FeS), and magnetite (Fe_3O_4) particles. *Chin. Sci. Bull.* **2010**, *55*, 365–372. (In Chinese) [CrossRef]
18. Wang, X.Q.; Yu, H.Y.; Li, F.B.; Liu, T.X.; Wu, W.J.; Liu, C.P.; Liu, C.S.; Zhang, X.Q. Enhanced immobilization of arsenic and cadmium in a paddy soil by combined applications of woody peat and $Fe(NO_3)_3$: Possible mechanisms and environmental implications. *Sci. Total Environ.* **2019**, *649*, 535–543. [CrossRef] [PubMed]
19. Subacz, J.L.; Barnett, M.O.; Jardine, P.M.; Stewart, M.A. Decreasing arsenic bioaccessibility/bioavailability in soils with iron amendments. *J. Environ. Sci. Eng. A* **2007**, *42*, 1317–1329. [CrossRef] [PubMed]
20. Wang, X.; Zhang, H.; Wang, L.L.; Chen, J.; Xu, S.Q.; Hou, H.J.; Shi, Y.; Zhang, J.D.; Ma, M.; Tsang, D.C.W.; et al. Transformation of arsenic during realgar tailings stabilization using ferrous sulfate in a pilot-scale treatment. *Sci. Total Environ.* **2019**, *668*, 32–39. [CrossRef] [PubMed]
21. Komarek, M.; Vanek, A.; Ettler, V. Chemical stabilization of metals and arsenic in contaminated soils using oxides—A review. *Environ. Pollut.* **2013**, *172*, 9–22. [CrossRef] [PubMed]
22. Wang, X.Q.; Li, F.B.; Yuan, C.L.; Li, B.; Liu, T.X.; Liu, C.S.; Du, Y.H.; Liu, C.P. The translocation of antimony in soil-rice system with comparisons to arsenic: Alleviation of their accumulation in rice by simultaneous use of fe(ii) and no3. *Sci. Total Environ.* **2019**, *650*, 633–641. [CrossRef] [PubMed]
23. Zou, L.N.; Zhang, S.; Duan, D.C.; Liang, X.Q.; Shi, J.Y.; Xu, J.M.; Tang, X.J. Effects of ferrous sulfate amendment and water management on rice growth and metal(loid) accumulation in arsenic and lead co-contaminated soil. *Environ. Sci. Pollut. Res.* **2018**, *25*, 8888–8902. [CrossRef] [PubMed]
24. Fresno, T.; Moreno, J.E.; Peñalosa, J.M. Assessing the combination of iron sulfate and organic materials as amendment for an arsenic and copper contaminated soil. A chemical and ecotoxicological approach. *Chemosphere* **2016**, *165*, 539–546. [CrossRef]
25. Fresno, T.; Moreno, J.E.; Zornoza, P.; Peñalosa, J.M.; Fresno, T.; Moreno, J.E.; Zornoza, P.; Peñalosa, J.M.; Fresno, T.; Moreno, J.E. Aided phytostabilisation of As- and Cu-contaminated soils using white lupin and combined iron and organic amendments. *J. Environ. Manag.* **2017**, *205*, 142–150. [CrossRef]
26. Lu, M.X.; Li, F.Y.; Zhng, C.L.; Li, C.Z.; Yang, W.W.; Zhang, J.J.; Huang, H.; Wu, H.H. Effects of different soil amendments on the immobilization and remediation of cadmium and arsenic in soil. *J. Guangxi Univ.* **2016**, *41*, 1667–1675. (In Chinese)
27. Bao, S.D. *Soil Agro-Chemistrical Analysis (Third Edition)*; China Agriculture Press: Beijing, China, 2018; pp. 39–83. (In Chinese)
28. DIN ISO. *ISO-14870, 2011: Soil Quality—Extraction of Trace Elements by Buffered Dtpa Solution, Standardization*; Din ISO: Berlin, Germany, 2001.
29. Woolson, E.A.; Axley, J.H.; Kearney, P.C. Correlation between available soil arsenic, estimated by six methods, and response of corn (*Zea mays* l.)1. *Soil Sci. Soc. Am. J.* **1971**, *35*, 101–105. [CrossRef]
30. Huang, G.X.; Ding, C.F.; Hu, Z.Y.; Cui, C.H.; Zhang, T.L.; Wang, X.X. Topdressing iron fertilizer coupled with pre-immobilization in acidic paddy fields reduced cadmium uptake by rice (*Oryza sativa* l.). *Sci. Total Environ.* **2018**, *636*, 1040–1047. [CrossRef]
31. Liu, C.P.; Luo, C.L.; Gao, Y.; Li, F.B.; Lin, L.W.; Wu, C.A.; Li, X.D. Arsenic contamination and potential health risk implications at an abandoned tungsten mine, southern china. *Environ. Pollut.* **2010**, *158*, 820–826. [CrossRef]
32. Wang, B.; Li, J.Y. Understanding the molecular bases of agronomic trait improvement in rice. *Plant Cell* **2019**, *31*, 1416–1417. [CrossRef]

33. White, P.J.; Broadley, M.R. Biofortification of crops with seven mineral elements often lacking in human diets – Iron, Zinc, Copper, Calcium, Magnesium, Selenium and Iodine. *New Phytol.* **2009**, *182*, 49–84. [CrossRef] [PubMed]
34. Liu, H.J.; Zhang, J.L.; Christie, P.; Zhang, F.S. Influence of iron plaque on uptake and accumulation of cd by rice (*Oryza sativa* L.) seedlings grown in soil. *Sci. Total Environ.* **2008**, *394*, 361–368. [CrossRef] [PubMed]
35. Vromman, D.; Lutts, S.; Lefevre, I.; Somer, L.; De Vreese, O.; Slejkovec, Z.; Quinet, M. Effects of simultaneous arsenic and iron toxicities on rice (*Oryza sativa* L.) development, yield-related parameters and as and fe accumulation in relation to as speciation in the grains. *Plant. Soil.* **2013**, *371*, 199–217. [CrossRef]
36. Yi, H.Z.; Zhu, Y.G.; Li, M.; Zhang, L.G.; Cao, Z.H.; Smith, E.A. Sulfur (S)-induced enhancement of iron plaque formation in the rhizosphere reduces arsenic accumulation in rice (*Oryza sativa* L.) seedlings. *Environ. Pollut.* **2007**, *147*, 387–393.
37. Hooda, P.S.; Alloway, B.J. Cadmium and lead sorption behaviour of selected english and indian soils. *Geoderma* **1998**, *84*, 121–134. [CrossRef]
38. Fitz, W.J.; Wenzel, W.W. Arsenic transformations in the soil-rhizosphere-plant system: Fundamentals and potential application to phytoremediation. *J. Biotechnol.* **2002**, *99*, 259–278. [CrossRef]
39. Liang, B.; Lehmann, J.; Solomon, D.; Kinyangi, J.; Grossman, J.; O'Neill, B.; Skjemstad, J.O.; Thies, J.; Luizão, F.J.; Petersen, J.; et al. Black carbon increases cation exchange capacity in soils. *Soil Sci. Soc. Am. J.* **2006**, *70*, 1719. [CrossRef]
40. Chen, L.J.; Jiang, Y.J.; Liang, C.; Luo, Y.; Xu, Q.S.; Han, C.; Zhao, Q.G.; Sun, B. Competitive interaction with keystone taxa induced negative priming under biochar amendments. *Microbiome* **2019**, *7*, 77. [CrossRef]
41. Kumpiene, J.; Lagerkvist, A.; Maurice, C. Stabilization of as, cr, cu, pb and zn in soil using amendments—A review. *Waste Manag.* **2008**, *28*, 215–225. [CrossRef]
42. Wilson, S.C.; Lockwood, P.V.; Ashley, P.M.; Tighe, M. The chemistry and behaviour of antimony in the soil environment with comparisons to arsenic: A critical review. *Environ. Pollut.* **2010**, *158*, 1169–1181. [CrossRef]
43. Cui, Y.S.; Xin, D.; Weng, L.P.; Riemsdijk, W.H.V. Assessment of in situ immobilization of lead (pPb) and arsenic (As) in contaminated soils with phosphate and iron: Solubility and bioaccessibility. *Water Air Soil Pollut.* **2010**, *213*, 95–104. [CrossRef]
44. Olowe, A.A.; Génin, J.M.R. The mechanism of oxidation of ferrous hydroxide in sulphated aqueous media: Importance of the initial ratio of the reactants. *Corros Sci.* **1991**, *32*, 965–984. [CrossRef]
45. Lin, R.G.; Spicer, R.L.; Tungate, F.L.; Davis, B.H. A study of the oxidation of ferrous hydroxide in slightly basic solution to produce γ-FeOOH. *Colloids Surf. A* **1996**, *113*, 79–96. [CrossRef]
46. Wang, R.Z.; Wei, S.; Jia, P.H.; Liu, T.; Hou, D.D.; Xie, R.H.; Lin, Z.; Ge, J.; Qiao, Y.B.; Chang, X.Y.; et al. Biochar significantly alters rhizobacterial communities and reduces cd concentration in rice grains grown on cd-contaminated soils. *Sci. Total Environ.* **2019**, *676*, 627–638. [CrossRef] [PubMed]
47. Katsoyiannis, I.A.; Zouboulis, A.I. Application of biological processes for the removal of arsenic from groundwaters. *Water Res.* **2004**, *38*, 17–26. [CrossRef] [PubMed]
48. Laird, D.; Fleming, P.; Wang, B.Q.; Horton, R.; Karlen, D. Biochar impact on nutrient leaching from a midwestern agricultural soil. *Geoderma* **2010**, *158*, 436–442. [CrossRef]
49. Bolan, N.S.; Naidu, R.; Syers, J.K.; Tillman, R.W. Surface charge and solute interactions in soils. *Adv. Agron.* **1999**, *67*, 87–140.
50. Harvey, O.R.; Herbert, B.E.; Rhue, R.D.; Kuo, L.J. Metal interactions at the biochar-water interface: Energetics and structure-sorption relationships elucidated by flow adsorption microcalorimetry. *Environ. Sci. Technol.* **2011**, *45*, 5550–5556. [CrossRef]
51. Fresno, T.; Penalosa, J.M.; Flagmeier, M.; Moreno-Jimenez, E. Aided phytostabilisation over two years using iron sulphate and organic amendments: Effects on soil quality and rye production. *Chemosphere* **2020**, *240*, 124827. [CrossRef]
52. Chen, Z.; Zhu, Y.G.; Liu, W.J.; Meharg, A.A. Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. *New Phytol.* **2005**, *165*, 91–97. [CrossRef]
53. Matsumoto, S.; Kasuga, J.; Taiki, N.; Makino, T.; Arao, T. Inhibition of arsenic accumulation in japanese rice by the application of iron and silicate materials. *Catena* **2015**, *135*, 328–335. [CrossRef]
54. Yamaguchi, N.; Ohkura, T.; Takahashi, Y.; Maejima, Y.; Arao, T. Arsenic distribution and speciation near rice roots influenced by iron plaques and redox conditions of the soil matrix. *Environ. Sci. Technol.* **2014**, *48*, 1549–1556. [CrossRef] [PubMed]

55. Kamiya, T.; Fujiwara, T. Arabidopsis nip1;1 transports antimonite and determines antimonite sensitivity. *Plant Cell Physiol.* **2009**, *50*, 1977–1981. [CrossRef]
56. Ishikawa, S.; Ishimaru, Y.; Igura, M.; Kuramata, M.; Abe, T.; Senoura, T.; Hase, Y.; Arao, T.; Nishizawa, N.K.; Nakanishi, H. Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 4950–4951. [CrossRef]
57. Zhao, F.J.; McGrath, S.P.; Meharg, A.A. Arsenic as a food chain contaminant: Mechanisms of plant uptake and metabolism and mitigation strategies. In *Annual Review of Plant Biology*; Annual Reviews: Palo Alto, CA, USA, 2010; Volume 61, pp. 535–559.
58. Tanaka, K.; Fujimaki, S.; Fujiwara, T.; Yoneyama, T.; Hayashi, H. Quantitative estimation of the contribution of the phloem in cadmium transport to grains in rice plants (*oryza sativa* l.). *Soil Sci. Plant Nutr.* **2007**, *53*, 72–77. [CrossRef]
59. Kato, M.; Ishikawa, S.; Inagaki, K.; Chiba, K.; Hayashi, H.; Yanagisawa, S.; Yoneyama, T. Possible chemical forms of cadmium and varietal differences in cadmium concentrations in the phloem sap of rice plants (*oryza sativa* L.). *Soil Sci. Plant Nutr.* **2010**, *56*, 839–847. [CrossRef]
60. Matsumoto, S.; Kasuga, J.; Makino, T.; Arao, T. Evaluation of the effects of application of iron materials on the accumulation and speciation of arsenic in rice grain grown on uncontaminated soil with relatively high levels of arsenic. *Environ. Exp. Bot.* **2016**, *125*, 42–51. [CrossRef]