

Article

# Effects of Iron Amendments on the Speciation of Arsenic in the Rice Rhizosphere after Drainage

Noriko Yamaguchi <sup>1,\*</sup>, Toshiaki Ohkura <sup>1</sup>, Atsuko Hikono <sup>1</sup>, Hiroshi Yamaguchi <sup>1</sup>,  
Yohey Hashimoto <sup>2</sup> and Tomoyuki Makino <sup>1</sup>

<sup>1</sup> Institute for Agro-Environmental Science, National Agriculture and Food Research Organization, 3-1-3, Tsukuba, Ibaraki 305-8604, Japan; tohkura@affrc.go.jp (T.O.); hikonoa996@affrc.go.jp (A.H.); yhiro@affrc.go.jp (H.Y.); T\_Makino@affrc.go.jp (T.M.)

<sup>2</sup> Department of Bioapplications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan; yhashim@cc.tuat.ac.jp

\* Correspondence: nyamag@affrc.go.jp; Tel./Fax: +81-29-838-8315

Received: 31 August 2017; Accepted: 29 November 2017; Published: 1 December 2017

**Abstract:** Applications of iron- (Fe-) bearing materials represent an effective countermeasure for decreasing the dissolution of arsenic (As) in soil under anaerobic conditions. In this study, we investigated the effects of Fe amendments (ferrihydrite-based and zero-valent iron- (ZVI-) based materials) on the speciation of As in rice cultivated soils and root-attached materials including Fe plaque when the soil shifts from anaerobic to aerobic conditions. Rice (*Oryza sativa* L.) was cultivated in pots filled with soil under continuous flooding conditions, and root distribution in the soil was restricted inside a cylinder made by nylon mesh. Soil and root samples were collected after drainage at different growth stages of the rice plants, which are represented by intermittent drainage and drainage at harvest. The speciation of As was determined by As K-edge X-ray absorption near edge structure (XANES) spectroscopy. The proportion of arsenite did not differ between the bulk soil and root-attached materials including Fe plaque, whereas a larger proportion of dimethylarsinic acid was found in the root-attached materials regardless of the application of Fe amendments. Observation of soil thin-sections showed that the application of Fe amendments caused an increase in Fe (hydr)oxide deposition around the roots as well as on the soil particles. In addition to Fe (hydr)oxide, sulfide was found to be associated with As under anaerobic conditions, notably for the ZVI-amended soil at the time of intermittent drainage. The concentration of As in the soil solution and As uptake by rice grains decreased, while As speciation near the roots was not influenced by the application of Fe amendments. In conclusion, Fe amendments mitigated As dissolution in the soil solution by providing a sorption site for As in bulk soil without altering As speciation near the roots.

**Keywords:** arsenic sulfide; dimethylarsinic acid; ferrihydrite; Fe plaque; paddy soil; zero-valent iron

## 1. Introduction

The chronic exposure to arsenic (As) is associated with an increased risk of carcinoma in humans. Increases in the As concentration in paddy rice are of great concern, especially in Asian countries where rice is consumed as a staple food [1]. Paddy rice is flooded during growing, which leads to the development of anaerobic conditions, leading to microbial reduction of arsenate (As-5) to arsenite (As-3). Since the adsorption affinity of As-3 to soil is weaker than that of As-5 [2], the As concentration in the soil solution is increased with the development of anaerobic conditions [3–5]. In addition, iron (Fe) (hydr)oxide, which adsorbs As, undergoes reductive dissolution, and then the As-3 associated with Fe (hydr)oxides is released into the solution as the adsorption phase diminishes [6]. In contrast, when rice is grown as an upland crop, soil conditions remain aerobic and the As concentration is significantly lower than in paddy rice [5,7,8] because the dissolution of As in the soil solution is

decreased. Therefore, growing rice without flooding is one of the strategies to reduce As concentrations in rice grains; however, soil flooding has many benefits for rice production, such as the control of weeds, the prevention of damage from continuous cultivation and cool weather [9]. It may also not be feasible to abandon the flooding practice for practical or economic reasons in areas that experience annual monsoon flooding. In addition, aerobic conditions cause an increase in the Cd concentration in rice grains [5,8]. To decrease the As uptake by rice while retaining the benefits of soil flooding, the As concentration in the soil solution must be reduced under anaerobic conditions. Several Fe-bearing materials have been shown to be effective in reducing the As concentration in soil solutions under anaerobic conditions and thereby reduce the As concentration in rice grains [10–15].

As well as Fe-bearing materials applied to soil, Fe plaque is known to influence As dissolution and uptake by rice [16–21]. The Fe plaque is deposited around the roots when  $\text{Fe}^{2+}$  is supplied from the soil solution due to the reductive dissolution of Fe minerals in soil under anaerobic conditions. Even when the soil matrix is predominantly under anaerobic conditions, the rice rhizosphere is partially aerobic due to the radial oxygen loss (ROL) through the root aerenchyma; thus,  $\text{Fe}^{2+}$  is oxidized and precipitated as ferric (hydr)oxide [20]. Fe plaque is dominantly composed of ferrihydrite, although crystalline minerals, such as goethite and lepidocrocite, are also present [20]. Since these Fe minerals strongly adsorb As, Fe plaque sequesters As around the roots [20]. Both As-3 and As-5 are present in Fe plaque [22]. Liu et al. [21] showed that As-5 is the dominant As species associated with the Fe plaque of roots in mature rice plants, whereas Yamaguchi et al. [23] showed that As-3 is the dominant species when the soil matrix is under anaerobic conditions and the proportion of As-5 increases at 1 month after harvest. The source of As sequestered in Fe plaque is the As-3 dissolved in the soil solution [4,7]. Therefore, the results of Yamaguchi et al. [23] implied that the As-3 associated with Fe plaque is not immediately oxidized to As-5. After the flooded water is drained, the oxidation of As-3 in the Fe plaque is faster than that in the bulk soil [23]. However, oxidation rate of As-3 associated with Fe plaque is not well understood so far.

The formation and distribution of Fe plaque is heterogeneous and influenced not only by the degree of ROL, which is associated with the growth stage [24] and genotype [25] of rice, but also by various soil-derived factors [23,26,27]. When larger amounts of  $\text{Fe}^{2+}$  are supplied from the soil by reductive dissolution, larger amounts of Fe plaque are formed around roots. Ultra et al. [28] showed that the addition of 0.1 wt % amorphous Fe (hydr)oxide results in an increase in Fe plaque formation and thereby a reduction in As uptake by rice. The addition of Fe-bearing materials to soil increases the source of  $\text{Fe}^{2+}$  dissolved in the soil solution, which possibly influences Fe plaque formation. Various types of Fe-bearing materials have been investigated to identify promising soil amendments for decreasing As uptake by rice [10–15]. However, the influence of these materials on Fe plaque formation and As sequestration in Fe plaque is not well understood.

We hypothesized that the application of Fe-bearing materials to paddy soil enhance the deposition of Fe plaque around rice roots, and thereby influences As speciation and its dissolution to soil solution. Whether the Fe plaque acts as a sink or as a source of As for rice roots is still controversial. When As is associated with Fe plaque in the rhizosphere as As-5, it is barely dissolved in the soil solution. However, if As-3 is a major species associated with Fe plaque, it can be dissociated from Fe plaque and become a source of As for root uptake by rice or any crop that is subsequently cultivated after the harvest of rice. Since As uptake by rice is different according to growth stage, the speciation of arsenic in the rhizosphere at different cultivation stages of rice must be clarified to understand the role of Fe plaque in the uptake of As from roots. The purpose of this study was to investigate the effects of Fe amendments on the speciation of As in the rice rhizosphere, including Fe plaque, when the soil shifts from anaerobic to aerobic conditions after drainage at different growth stages of rice. The deposition of Fe (hydr)oxide around roots and soil particles was observed by preparing resin-embedded thin-sections of soil. The speciation of As in the rhizosphere and bulk soil was determined by X-ray absorption near edge structure (XANES) spectroscopy. These results contribute to the understanding of how drainage

and aeration associated with rice root aerenchyma influence the oxidation of As-3 in the presence of Fe amendments.

## 2. Materials and Methods

### 2.1. Pot Experiment

The soil for the pot experiments was collected from a plowed layer of paddy field and passed through an 8-mm mesh sieve. The soil was classified as *Aeric Epiaquepts* according to US taxonomy, and the relevant soil properties, including the As concentration, are shown in Table S1. Two types of commercially available Fe-bearing materials, ferrihydrite-base material (FB, Ishihara Sangyo Kaisha, Ltd., Osaka, Japan) and zero-valent iron-based material (ZVI, Kobe Steel, Ltd., Tokyo, Japan), were used. Chemical fertilizer (0.2 g of nitrogen, 0.087 g of phosphorus and 0.17 g of potassium) and Fe-bearing material (17.9 g of FB or 10.1 g of ZVI; corresponding to 500 kg Fe ha<sup>-1</sup>) were mixed with 2.5 kg of oven-dried soil. Five sets of pots were prepared for each group: control soil (without Fe-bearing material), soil amended with FB and soil amended with ZVI, (15 pots in total).

A cylinder (80 mm in diameter and 200 mm in height) made of nylon mesh screen (NYTAL PA-25-63, Semitec, Osaka, Japan; mesh size: 63 µm) was filled with 0.6 kg of the soil and placed in the center of a pot (1/5000 a Wagner pot, 4000 cm<sup>3</sup>). The remaining soil was filled around the soil cylinder. The soil in each pot was mixed well with water to mimic the puddling of a paddy field before transplanting the rice seedlings. Three seedlings of *Oryza sativa* cv. Koshihikari were transplanted into the center of the soil cylinder to regulate the position of the rice root zone inside the compartment surrounded by the nylon mesh screen.

The soil surface was flooded continuously by tap water during rice cultivation. Two sets of pots for each of the three treatments (control, FB and ZVI) were used as samples of intermittent drainage. Fifty days after transplanting the rice seedlings, the flooding water was drained from the hole at the bottom of the pot, which mimics the intermittent drainage that occurs in paddy fields. The soil and root samples were collected 1 h and 7 days after the drainage as described in the following section. The remaining three sets of pots for each treatment were used as samples of drainage after harvest. Rice was grown until the grain maturing stage without intermittent drainage, and the flooding water was drained just before harvest (113 days after transplanting).

### 2.2. Eh, pH and Soil Solution Analyses

A platinum electrode (PRN-41, Fujiwara, Tokyo, Japan) for measuring the redox potential and a soil solution sampler were both buried at a depth of 5 cm from the soil surface inside the nylon mesh cylinder. The soil solution sampler was composed of a 10-cm porous part (OD 2.5 mm) and a connected polyethylene/polyvinyl chloride tube (5 Rhizon MOM 5 cm; Rhizosphere Research Products, Wageningen, The Netherlands); a polyvinyl chloride extension tube (PF; TOP Co., Ltd., Tokyo, Japan); and an evacuated plastic tube (Venoject II, TERUMO Co., Tokyo, Japan). The soil solution was collected six days before the intermittent drainage (44 days after transplanting) from five sets of pots for three treatments (control, FB and ZVI); 41 and 35 days before the drainage at harvest (9 and 15 days after heading; 72 and 78 days after transplanting, respectively) from three sets of pots for three treatments. The timing of soil solution sampling was within three weeks after heading when As uptake by rice is known to be high [5]. The soil solution was immediately acidified by HNO<sub>3</sub> to prevent precipitation of Fe (hydr)oxide. The soil redox potential and pH was recorded when the soil solution was collected. The As and Fe concentrations in the soil solution were determined with an inductively coupled plasma mass spectrometer (ICP-MS, Elan, PerkinElmer, Waltham, MA, USA, limit of quantification for As: 0.17 µg/L) and an inductively coupled plasma optical emission spectrometer (ICP-OES, VistaPro, Varian, Palo Alto, CA, USA, limit of quantification for Fe: 1 µg/L), respectively. Certified reference materials used were ERM-CA615 (Trace elements in groundwater: European Commission, JRC, IRMM, North-East Limburg, Geel, Belgium).

### 2.3. Soil and Root Sampling from Rice-Cultivation Pots

The nylon mesh limited root distribution and, hence, soil that was not directly influenced by the rice root was sampled from the compartment outside of the nylon mesh cylinder (MO) and soil that interacted with the rice roots (MI) and roots with root-attached materials including Fe plaque (RZ) was sampled from the area inside the nylon mesh cylinder. The soil and root samples were collected 1 h and 7 days after intermittent drainage and 1 h, 7 days and 27 days after drainage at harvest, as described in Section 2.2. Each sample was collected from one pot per treatment. In this study, replicated measurements of bulk soil and root samples were sacrificed to allow for time-resolved sampling points. However, the mixing of samples from depth-intervals within the separated zones (MI, MO, and RZ), serves as an averaging of information and provides confidence that the measurements are representative.

Wet soil samples (MO and MI) were collected from a depth of 3–6 cm from the surface from inside and outside of the nylon mesh cylinder, respectively. The well-mixed portion of wet soil paste was packed in a plastic bag (c.a. 15 mm × 15 mm × 1 mm), sealed, and immediately frozen in liquid N<sub>2</sub>. The samples were stored at −30 °C until XANES analysis was carried out.

The soil and roots inside the nylon mesh cylinder at depths of 0–3 and 6–9 cm were frozen in liquid N<sub>2</sub> and then freeze dried. Subsequently, the roots stained with the dark red color of Fe hydroxide (Fe plaque) were pulled out from the soil using tweezers. Visible soil particles attached to the roots were removed by hand or tweezers. The roots from depths of 0–3 cm and 6–9 cm obtained 1 h after intermittent drainage were mixed for the analyses because the quantity of roots obtained for the analyses was insufficient. The soil collected from outside of the cylinder was also freeze dried and then well mixed. These freeze-dried bulk soils (MO and MI) and RZ were used to determine the ammonium-oxalate extractable As and Fe concentrations (As<sub>o</sub> and Fe<sub>o</sub>) which is associated with poorly crystalline Fe hydroxide as described in the supporting information. The portions of freeze-dried roots were pulverized, and the powdered samples were pressed into pellets for the As speciation analysis by XANES spectroscopy.

### 2.4. Speciation of As by X-ray Absorption Near Edge Structure Spectroscopy

The arsenic K-edge XANES spectra were acquired using beamline BL01b1 at SPring-8 and beamline BL5S1 at the Aichi Synchrotron Radiation Center. A double-crystal Si(111) monochromator and two Rh-coated mirrors at a grazing incidence angle of 3.7 and 3.14 mrad were used at BL01b1 and BL5S1, respectively. The incoming beam was measured with an Ar 15% N<sub>2</sub> 85%-filled ion chamber. The XANES spectra of the soil paste (MO and MI) samples and RZ pellets were collected in the fluorescence detection mode using a 19-element Ge semiconductor detector, whereas those of the reference materials were collected in the transmission mode with an Ar 100%-filled ion chamber at ambient temperature. The frozen soil paste samples were thawed at an ambient temperature just before the analyses. During XANES analyses, the soil paste was kept sealed in a polyethylene bag because X-ray absorption by the polyethylene bag is negligible at the energy for As analyses. Our previous study showed that X-ray irradiation did not cause changes in As speciation for anaerobic soil paste during XANES analyses [4]. The composition of As species was evaluated by a linear combination fitting (LCF) of the XANES spectra with reference compounds. The reference compounds included Na<sub>2</sub>AsO<sub>3</sub> (rsenite, As-3 Wako pure chemical, Osaka, Japan), NaHAsO<sub>4</sub> (arsenate, As-5 Wako pure chemical, Osaka, Japan), dimethylarsinic acid (DMA, Hayashi pure chemical, Osaka, Japan), orpiment (As<sub>2</sub>S<sub>3</sub> Wako pure chemical, Osaka, Japan), and arsenopyrite (FeAsS, Francisco I. Madero Mine, Mexico, N's Mineral, Niigata, Japan). The fitting range was ±10 eV from E<sub>0</sub>. The Athena in Demeter 0.9.25 program package was used to subtract the pre-edge background and normalize the spectra and LCFs. In the LCFs, the fraction of each reference compound was set as an adjustable parameter, and optimization was achieved by minimizing the residual of the fit, which was defined as the normalized root-square difference between the data and the fit (R-factor).

### 2.5. Analyses of Rice Grain

Rice grains were finely ground using a shaking mill (Shake Master; Bio Medical Science Inc., Tokyo, Japan) and 0.2 g of the powdered grain was digested at 105 °C using a hot block acid digestion system (DigiPREP MS; SCP Sciences, Inc., Quebec, QC, Canada). After cooling, 1 ml of 30% hydrogen peroxide solution was added, and the mixture was heated again for 1 h at 105 °C. The concentrations of As in the acid-digested solution were determined by ICPMS. Certified reference material used was NMIJ CRM7502-a (White rice flour: National Metrology Institute, AIST, Tsukuba, Japan).

### 2.6. Preparation of Soil Thin-Sections

The soil cylinder covered by the nylon mesh was pulled from the pot, and then the nylon mesh was removed. The soil cylinder was cut to a height of 3 cm. The soil inside the nylon mesh cylinder at a depth of 3–6 cm from the surface was immediately frozen using liquid nitrogen and then freeze dried. The freeze-dried soil block was embedded in unsaturated polyester resin (Sundhoma, DIC material, Tokyo, Japan) under vacuum. The resin-embedded soil block was kept in a draft chamber at ambient temperature for two weeks until the resin solidified. The solidified resin-embedded soil block was cut by bandsaw (Andosaw, TA300L, Aichi, Japan) and then polished using a grinder (PSG-52, Okamoto, Gunma, Japan) to a thickness of 30–50 µm. The method of soil thin-section preparation is described by Yamaguchi et al. [23]. The thin-section image was obtained using an image scanner under a white color background (CanonScan LiDE 700F, Canon, Tokyo, Japan) and observed under a digital microscope (Keyence VHX-100, Osaka, Japan).

### 2.7. Statistic Analysis

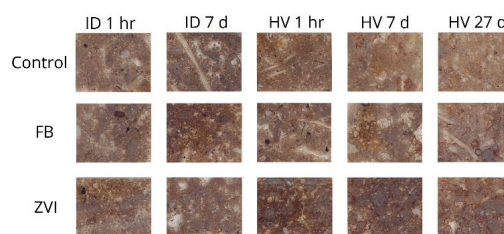
Statistical analysis was conducted with an add-in program for MS Excel (BellCurve for Excel; Social Survey Research Information Co., Ltd., Tokyo, Japan). Before analysis of variance, normality of sample variances was assessed by Levene's test for pH, As and Fe concentration of soil solutions, and Eh of soils during rice-pot cultivation. Two-way analysis of variance (two-way ANOVA) with sampling time and treatments as factors was used to analyze pH and Eh followed by the Tukey–Kramer multiple comparison test, whereas the Kruskal–Wallis test (nonparametric test) was applied for As and Fe followed by Steel–Dwass multiple comparison test.

## 3. Results

### 3.1. Observation of the Soil Thin-Sections

Reddish Fe hydroxide was heterogeneously distributed around the soil aggregates and roots (Figure 1). In the thin-section of soil collected 1 h after drainage, roots entirely coated by Fe plaque and those with scattered Fe plaque were observed together (Figure S1). Without the Fe amendments, Fe hydroxide deposits were observed only in a limited area around the roots in the thin-section of soil at 1 h after drainage. Seven days after intermittent drainage, the deposited Fe hydroxide around the soil aggregate and roots was increased. However, part of the root surface still did not have obvious Fe plaque deposits. Seven days after intermittent drainage, a larger area around the roots and soil aggregate had Fe hydroxide deposits in the thin-section of soil amended with FB compared with the soil amended with ZVI and without the Fe amendments.





**Figure 1.** Scanned images of the soil thin-section. ID: intermittent drainage, HV: drainage at harvest. Area of reddish color shows signs of Fe (hydr)oxide deposition. Black bar indicates 10 mm.

Similar to the intermittent drainage results, roots entirely coated by Fe plaque and those with scattered Fe plaque were observed together at 1 h after drainage at harvest. The deposition of Fe (hydr)oxide around the roots and soil aggregates increased with time after drainage for the FB-amended soil. In contrast, a larger area of soil was stained by the color of the Fe (hydr)oxide in the thin-section of soil amended with ZVI after drainage at harvest. In addition, most of the roots were coated with Fe plaque in the soil amended with ZVI after drainage at harvest.

### 3.2. Soil Redox Potential, pH and As and Fe Dissolution in the Soil Solution

The results of ANOVA with days of flooding and materials as factors, and multiple comparison tests, are summarized in Table 1. Before analysis of variance, normality of sample variance was rejected for As and Fe by Levene's test. Accordingly, two-way analysis of variance (ANOVA) was conducted for Eh and pH, whereas the Kruskal–Wallis test (nonparametric test) was applied for As and Fe. There was no significance in interaction between the days of flooding and materials for pH and Eh. Regardless of the Fe amendment application, the Eh dropped below  $-220$  mV (Table S2). The Eh values were not significantly influenced by the application of the Fe amendments or days of flooding (Table 1). The soil pH was not significantly affected by the Fe-amendments, but it was slightly higher at sampling after 72 days of flooding than at the other sampling occasions (Table 1). The concentration of As in the soil solution was significantly decreased ( $p < 0.05$ ) by the application of FB and ZVI. The As concentration increased with time and was higher at 78 than at 50 days of flooding though it was not significant (Table 1). The concentrations of Fe in the soil solutions of the FB and ZVI were lower than that in the control, and significant decrease in Fe concentration was observed after 72 days of flooding. The arsenic concentration in brown rice was decreased by 17% and 38% due to the application of FB and ZVI, respectively (Table S2).

**Table 1.** Results of statistical analyses investigating the effects of days of flooding and Fe-bearing materials on Eh, pH, As and Fe concentrations in soil solution.

	Eh	pH	As	Fe
	mV		$\mu\text{mol L}^{-1}$	$\text{mmol L}^{-1}$
<b>Days of flooding</b>				
50 d (6 d before intermittent drainage)	$-234 \pm 6a$	$6.70 \pm 0.04a$	$3.05 \pm 0.42a$	$4.85 \pm 0.20a$
72 d (41 d before drainage at harvest)	$-229 \pm 8a$	$6.81 \pm 0.02b$	$3.51 \pm 0.52a$	$3.82 \pm 0.20b$
78 d (35 days before drainage at harvest)	$-226 \pm 3a$	$6.75 \pm 0.02ab$	$3.96 \pm 0.62a$	$4.32 \pm 0.44ab$
<b>Materials</b>				
Control	$-236 \pm 3a$	$6.71 \pm 0.03a$	$5.46 \pm 0.18a$	$5.08 \pm 0.33a$
FB	$-235 \pm 4a$	$6.80 \pm 0.04a$	$3.00 \pm 0.21b$	$3.80 \pm 0.18b$
ZVI	$-221 \pm 10a$	$6.72 \pm 0.03a$	$1.81 \pm 0.21c$	$4.39 \pm 0.24ab$
<b>Analysis of Variance</b>				
Days of flooding	n.s.	*	n.s.	**
Materials	n.s.	n.s.	**	*
Days of flooding x Materials	n.s.	n.s.	-	-

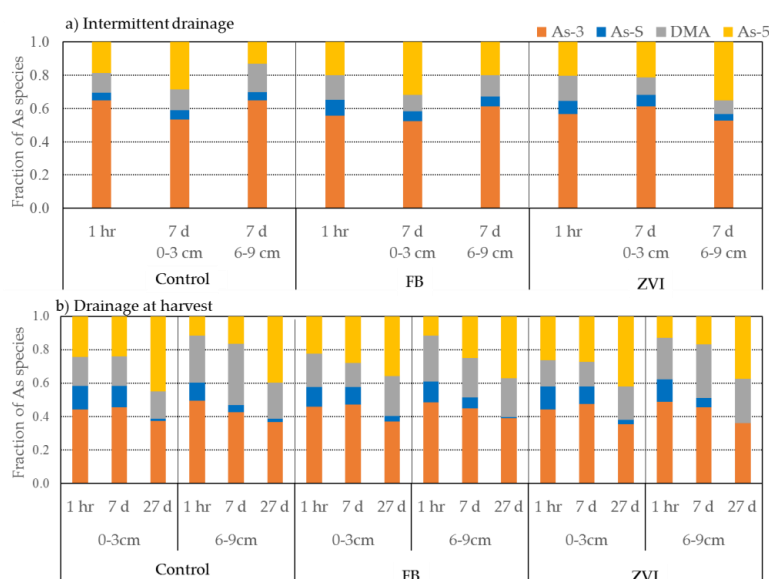
Data represents mean values and standard error (SE). Two-way analysis of variance (ANOVA) was used for Eh and pH, whereas the Kruskal–Wallis test (nonparametric test) was used for As and Fe. Values with different letters are significantly different as judged by the Tukey–Kramer multiple comparison test for Eh and pH, and Steel–Dwass test for As and Fe at  $p < 0.05$ . \* and \*\*: Significant at 0.05 and 0.01 probability on analysis of variance. n.s.: No significant difference.

### 3.3. As Speciation

#### 3.3.1. Root-Attached Materials (RZ)

Within seven days after intermittent drainage, more than 50% of the As in the RZ was in the form of As-3 (Figure 2 and Table S3). The fraction of As-5 was higher at a depth of 0–3 cm than at 6–9 cm for RZ collected from the control and the FB-amended pot. In contrast, the fraction of As-5 was lower at a depth of 0–3 cm than at 6–9 cm for the RZ from the ZVI-amended pot (Figure 2a). The XANES spectra of the RZ could not be fit by the combination of the As-3 and As-5 spectra (Figure S2), and the fitting results showed that dimethylarsinic acid (DMA) was prerequisite to fit the XANES spectra for RZ (Table S5). A clear difference was not found among the fractions of DMA and As-S in the RZ, regardless of the application of Fe amendment, depth from the surface or elapsed time after intermittent drainage.

After drainage at harvest, the fraction of As-5 was higher at a depth of 0–3 cm than at 6–9 cm for RZ at 1 h and 7 days after drainage at harvest regardless of the application of Fe amendments (Figure 2b). The fraction of As-3 tended to decrease with time after drainage and, conversely, those of As-5 were increased. More than 30% of the As remained as As-3 in the RZ within 27 days after drainage at harvest despite the aerobic conditions of the soil. The fraction of DMA in RZ after drainage at harvest was higher than those after intermittent drainage. In addition, the fraction of DMA did not materially change over time after drainage. The fraction of As-S decreased with time regardless of the application of Fe amendments and the depth from the surface. After 27 days, As speciation in RZ was analogous regardless of the application of Fe amendment or the depth from the surface.



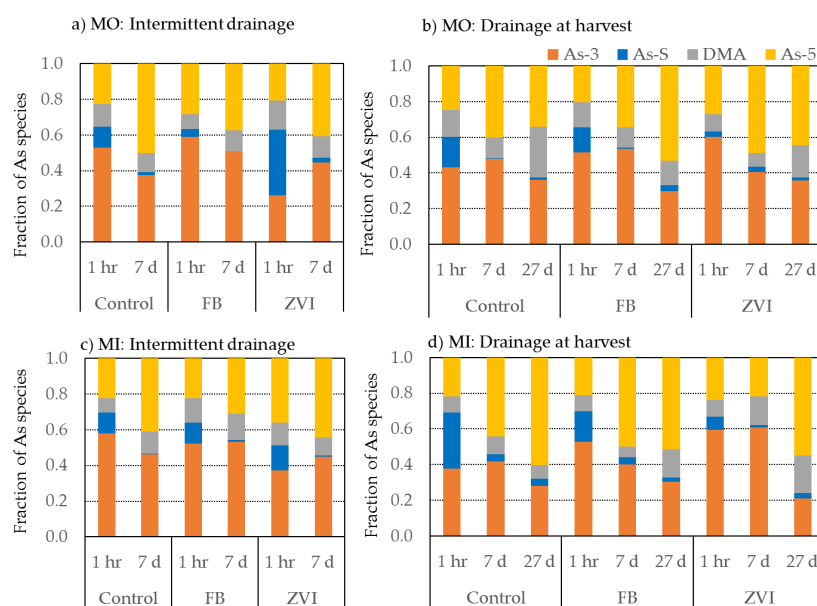
**Figure 2.** Speciation of As in the Fe plaque and root-attached materials (RZ) collected after intermittent drainage and drainage at harvest.

#### 3.3.2. Bulk Soil (MO and MI)

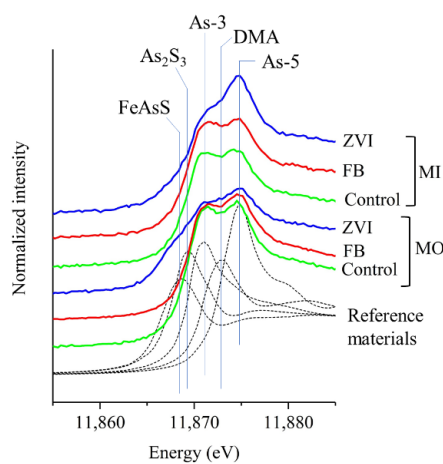
The fraction of As-5 exhibited a time-dependent increase after drainage (Figure 3 and Table S4). There was not a large difference among the speciation of As in the MO and MI after intermittent drainage, except for the ZVI-treated soil that was sampled 1 h after drainage (Figure 3a,c). The XANES spectra of the MO collected from the ZVI-amended pot had a shoulder of approximately 11,865 to 11,870 eV (Figure 4). One hour after intermittent drainage, 37% and 17% of As species in MO and MI taken from the ZVI-amended soil was assigned to As associated with sulfur ( $\text{As}_2\text{S}_3$  and arsenopyrite), respectively, whereas 5–12% of As was assigned to As-S for the control and FB-amended soil. We did not evaluate the fraction of  $\text{As}_2\text{S}_3$  and arsenopyrite separately, but the sum of their fraction was

shown as As-S. Seven days after intermittent drainage, the fraction of As-S decreased regardless of the application of the Fe amendment.

After drainage at harvest, the fractions of As-S in the MO and MI decreased with increasing time (Figure 3b,d). This decreasing trend was similar to that observed for the RZ. Although the fraction of As-S was highest for the MO from the ZVI-amended pot 1 h after intermittent drainage, it was lower than that of the control and the FB-amended pot 1 h after drainage at harvest. The As-3 fraction in the MO and MI from the control pot did not show considerable changes with time, whereas it decreased for the FB- and ZVI-amended pots. After 27 days, the As speciation in the MO and MI was similar in the FB-amended pots, whereas the proportion of As-5 was higher in the MI than in the MO for the control and the ZVI-amended pots. Nearly 10% of the As in soil was assigned to DMA, and the fraction of DMA tended to be higher 27 days after drainage at harvest.



**Figure 3.** Arsenic speciation of soils collected from outside (MO) and inside (MI) the nylon mesh compartment after intermittent drainage and drainage at harvest. FB denotes ferrihydrite-base; ZVI denotes zero-valent iron.



**Figure 4.** Arsenic K-edge XANES results for bulk soils collected from inside (MI) and outside (MO) the nylon mesh compartment 1 h after intermittent drainage.



## 4. Discussion

### 4.1. Deposition of Fe Hydroxide after Drainage

#### 4.1.1. Intermittent Drainage

Part of the ferric (hydr)oxide in submerged paddy soil is subject to reductive dissolution. When  $O_2$  is supplied, the dissolved  $Fe^{2+}$  is oxidized to  $Fe^{3+}$  and then deposited as ferric (hydr)oxide. Because  $O_2$  is delivered through the root aerenchyma, the rice rhizosphere is partially aerobic even when the bulk soil is in an anaerobic condition; therefore, Fe plaque appears on the root surface. The acid-ammonium extraction method, which is commonly used to dissolve poorly crystalline Fe minerals in soil, cannot distinguish the Fe minerals that were originally present in soil and secondary precipitated Fe (hydr)oxide from  $Fe^{2+}$  in soil solution. Because of the heterogeneous deposition of Fe (hydr)oxide, secondary precipitated Fe (hydr)oxide is visually observed as a reddish color area in the soil thin-section. The area of reddish color, which is identified as deposited Fe (hydr)oxide, was limited to an area around part of the roots in the thin-section from the soil without the Fe amendment at one hour after intermittent drainage. However, Fe (hydr)oxide was deposited around both the roots and the soil particles when the soil was amended with FB and ZVI (Figure 1), which was likely because the addition of the Fe amendment caused an increased source of Fe hydroxide to be deposited after intermittent drainage. This result is corroborated by the increased concentration of  $Fe_o$  observed after the addition of FB and ZVI (Figure S3c). The application of FB and ZVI did not cause an increase of Fe dissolution under anaerobic conditions but increased deposition of Fe (hydr)oxide, which acts as a sorbent for As. This was likely one of the mechanisms where application of FB and ZVI caused decreased As concentration in the soil solution (Table 1). Regardless of the addition of Fe amendments, Fe plaque deposition was heterogeneous around the roots, with certain roots entirely coated by Fe plaque and others presenting only fragments of Fe plaque, which is consistent with the results of a previous study [23] (Figure S1). This finding may have been caused by an insufficient supply of  $O_2$  through the aerenchyma because the rice is not yet well grown at the time of intermittent drainage.

Seven days after intermittent drainage, reddish Fe (hydr)oxide deposits appeared around the roots and soil matrix regardless of the Fe amendment application. The Fe hydroxide deposits were more distinct for the FB-amended soil than for the control or the ZVI-amended soil. Since FB is mainly composed of ferrihydrite and supplied dissolved  $Fe^{2+}$  under anaerobic conditions, a greater amount of Fe (hydr)oxide was deposited than that under the control condition. Hashimoto et al. [29] showed that the corrosion of ZVI causes Fe hydroxide deposits on the surface of ZVI particles, whereas the core of the ZVI remained after anaerobic incubation. The dissolution of amended FB was likely more rapid than that of ZVI; therefore, the Fe hydroxide deposits were more clearly observed for the FB-amended soil than for the ZVI-amended soil after intermittent drainage.

#### 4.1.2. Drainage at Harvest

Deposition of Fe hydroxide at the roots and soil particles was more distinct after drainage at harvest than that after 7 days of intermittent drainage, except in the FB-amended soil. This was likely because a longer period under anaerobic soil conditions supplied more dissolved  $Fe^{2+}$  as a source of deposited Fe (hydr)oxide. The concentrations of  $Fe_o$  in MI (Figure S3) and root attached materials RZ (Figure S4) tended to be higher 1 h after drainage at harvest than 1 h after intermittent drainage, in line with the results of thin-section analyses. Li et al. [30] showed that the formation of Fe plaque increases with the stage of rice growth, which is consistent with our results. In contrast, Schmidt et al. [31] suggested that Fe plaque formation decreases during the late stage of rice growth. These controversial results imply that many factors, including the soil type, nutrient condition and rice cultivar, influence the formation of Fe plaque [20]. One hour after drainage at harvest, deposition of Fe (hydr)oxide was observed both on the roots and soil particles. The deposition of Fe (hydr)oxide was more distinct for the ZVI-amended soil than for control or the FB-amended soil, which was probably

because more  $\text{Fe}^{2+}$  was supplied from the ZVI amendment and its corrosion products due to the continuous anaerobic conditions. After intermittent drainage, the deposition of Fe (hydr)oxide was more distinct for the FB-amended soil than for the ZVI amended soil as discussed in the previous section. The different timing of the increased Fe (hydr)oxide deposition for the FB- and ZVI-amended soil can likely be attributed to the different rates of reductive dissolution of ferrihydrite and zero-valent iron. The deposition of Fe (hydr)oxide did not show a clear difference between the control and the FB-amended soil (Figure 1). Due to the prolonged flooding conditions, the dissolution of  $\text{Fe}^{2+}$  from the control soil is increased, and the difference between the  $\text{Fe}^{2+}$  supplied from FB-amended soil and the control may be reduced.

#### 4.1.3. Deposition of Fe Plaques Around Roots

Regardless of the application of the Fe amendments and the time after drainage, roots entirely coated by Fe plaque were observed with those with a dispersed Fe plaque (Figure S1) as also shown by Seyfferth et al. [22]. This heterogeneous distribution of Fe plaque was likely caused by the heterogeneous supply of  $\text{Fe}^{2+}$  and  $\text{O}_2$  around the roots. The reddish color of Fe (hydr)oxide was observed in areas where newly formed lateral roots were emerging (yellow arrows in Figure S1), even around roots with less Fe (hydr)oxide deposition. The leakage of  $\text{O}_2$  may be limited around old roots, whereas it was active at the root tip or the point of lateral emergence. The lateral root junction was shown to be hotspots of As-3 uptake [32]. Ultra et al. [28] showed that the addition of 0.1 wt % amorphous Fe (hydr)oxide resulted in the increased formation of Fe plaque, which reduced the As uptake by rice. Our results showed that the application of the Fe amendment enhanced the deposition of Fe (hydr)oxide; however, deposition was not preferentially increased around the roots.

#### 4.2. Oxidation of As after Drainage

Drainage of the flooding water promotes the supply of  $\text{O}_2$  from the atmosphere to the soil and results in a shift to aerobic conditions in the surface soil. The proportion of As-5 in the RZ at a depth of 0–3 cm was higher than that at 6–9 cm at 7 days after intermittent drainage for the control and FB-amended pots. However, even in the RZ at a depth of 0–3 cm, more than 50% of the As was in the form of As-3 (Figure 2a). Oxidation of As-3 was not prominent after intermittent drainage in the RZ. After drainage at harvest, part of the As-3 was oxidized over time, the proportion of As-S was decreased and the proportion of As-5 was increased. Dong et al. [33] showed that the rate of oxidation of As-3 was 28–38% per day based on a microcosm experiment. In their experiment,  $\text{O}_2$  was supplied to a soil slurry via bubbling air. The oxidation speed of As-3 in our pot experiment was much slower than the speed estimated by Dong et al. [33]. This difference was attributed to the slow and heterogeneous  $\text{O}_2$  diffusion in the soil and Fe plaque of the rhizosphere in the rice-cultivated pot. Compared with the MO, a larger proportion of As-3 was oxidized to As-5 in the MI than the control and the ZVI amended pots after drainage at harvest. The presence of rice roots may influence the oxidation of As-3 in soil.

On the root attached materials, which include Fe plaque (RZ, Figure 2), the proportion of As-3 was not materially different from that in the bulk soil (MI and MO, Figure 3). Liu et al. [21] indicated that oxidative reactions near the roots (that is, derived from  $\text{O}_2$  and/or oxidants supplied from the rice root) caused a predominance of As-5 over As-3 for mature rice roots after harvest. However, our results showed that As-3 was still present on the root-attached material (RZ) after harvest (Figure 2) as previously reported [22,23]. Since the dominant source of As on the root-associated materials in RZ was As-3 supplied from the soil solution [4,7], the presence of As-5 in RZ implied an oxidative reaction of As-3 to As-5 in the rhizosphere. However, our results indicated that oxidation of As-3 was not sufficiently pronounced to generate higher proportions of As-5 in the rhizosphere than that in the bulk soil under our experimental conditions. Intermittent drainage is effective to decrease As concentration in soil solution [34]. The decrease of As concentration was not caused by the oxidation

of As-3 to As-5. Oxidation of  $\text{Fe}^{2+}$  followed by precipitation of the Fe (hydr)oxide provided sorption sites for As-3 thereby decreasing As dissolution after drainage.

The linear combination fitting (LCF) of the XANES spectra indicated that DMA was present both in the RZ and the bulk soil (Figures 3 and 4). Arao et al. [35] detected DMA in the soil solution collected from rice-cultivated soil under flooded conditions. However, DMA was not detected when the same soil was incubated under flooded conditions without rice cultivation. Arao et al. [35] suggested that bacteria associated with the rice rhizosphere may be responsible for the methylation of arsenite. A bacterium responsible for As methylation was isolated from the roots of rice plants grown in paddy soil under anaerobic conditions [36]. The higher proportion of DMA in RZ than that in bulk soil (Figures 3 and 4) likely resulted from the contribution of the rhizosphere bacteria to the formation of DMA. The proportion of DMA was higher in the RZ after drainage at harvest than after intermittent drainage (Figure 2). Since DMA is strongly adsorbed on Fe (hydr)oxide [37,38], it can be sequestered in Fe (hydr)oxide in the rhizosphere, including in Fe plaque [22].

#### 4.3. Influence of Fe Amendments

The major components in the FB and ZVI used in this study were ferrihydrite and zero-valent iron, respectively. When zero-valent iron is incubated under anaerobic conditions, Fe (hydr)oxide is formed on the surface of the ZVI [29,39]. Ferrihydrite and the corrosion products of the ZVI undergo reductive dissolution and provide  $\text{Fe}^{2+}$  to the soil solution. However, the application of 1 wt % of FB and ZVI to the soil did not increase the concentration of  $\text{Fe}^{2+}$  in the soil solution when the Eh was  $-243 \sim -226$  mV (Table 1). In contrast, the concentration of As in the soil solution was significantly decreased by the application of FB and ZVI under anaerobic conditions (Table 1), which is consistent with the results of previous studies [10] because of the increase in sorption sites for As derived from FB and ZVI. In addition, sulfide precipitation due to sulfate reduction scavenged Fe and As from solution, thereby cause decreased concentration of Fe and As in soil solution (Table 1).

The application of the Fe amendments had little influence on the As speciation in the RZ. The amounts of Fe and As deposition in the RZ were controlled by their concentrations in the soil solution, which is controlled by the soil conditions, such as Eh and pH. The application of the Fe amendments caused a decrease in the As concentration in the soil solution (Table 1), which resulted in the decreased concentration of As in RZ (Figure S4), whereas the speciation of As in the RZ was not influenced by the application of the Fe amendments (Figure 2).

The speciation of As in the bulk soil (MO and MI) showed only a slight difference under the application of FB and ZVI compared with the control, except for the MO collected from the ZVI amended pot at 1 h after intermittent drainage (Figure 3). A larger proportion of As in the MO collected from the ZVI amended pot was present as As-S ( $\text{As}_2\text{S}_3$  and arsenopyrite) at 1 h after intermittent drainage compared with the proportion in the control and the FB amended pot (Figures 3 and 4). The increased As-S contents under anaerobic soil incubation with 1 wt % of ZVI was consistent with the results observed by Suda et al. [15]. Jia et al. [40] showed that the application of sulfate to paddy soil resulted in the decreased concentration of As in the soil solution, and they concluded that microbial sulfate reduction had a role in immobilizing the As in anaerobic soil by facilitating the precipitation of immobile arsenic sulfide. Sulfate reduction followed by the formation of arsenic sulfide can be further enhanced by the addition of ZVI, which was used in this study. The removal of As from contaminated groundwater by ZVI in the presence of sulfate was caused by the formation of arsenic sulfide [41,42]. The ZVI used in this study contained 1 wt % of sulfur [29]. Hashimoto et al. [29] compared the Cd speciation in soil amended with ZVI of different S contents under anaerobic conditions and found that CdS was formed around the ZVI with high S contents while Cd-O was the major species around the ZVI with low S contents. The sulfide supplied from the S in ZVI may have contributed to the increased proportion of arsenic sulfide in the ZVI-amended soil at the timing of intermittent drainage. However, the proportion of As-S in the ZVI-amended soil was lower than that in the FB-amended soil and in the control after drainage at harvest. Despite the decreased fraction of As-S in MO, concentration

of As in soil solution was lower for ZVI-amended soil than that in control and FB-amended soil (Table 1). It was likely that the sink of As was partly shifted from sulfide to Fe hydr(oxide) in the ZVI-amended soil after prolonged anaerobic condition. Due to the prolonged anaerobic condition, amounts of  $\text{Fe}^{2+}$  from soil and/or ZVI were increased and the  $\text{Fe}^{2+}$  scavenge sulfide to form precipitation of FeS. In addition, deposition of Fe (hydr)oxide was increased as indicated from the thin-section analyses (Figure 1). Since deposition of FeS was not distinguishable from Fe (hydr)oxide in the soil thin-section, analyses of Fe speciation are needed to explain this mechanism though we did not determine XANES spectra of Fe in this study.

At the surface of ferrihydrite, the oxidation of As-3 to As-5 is catalyzed by Fe(II) in the presence of dissolved  $\text{O}_2$  [43]. Micro scale observations of As speciation around rice roots indicated that the oxidation of As associated with Fe plaque was faster than that in bulk soil [23]. However, the enhanced oxidation of As-3 by the application of Fe amendments was not observed in this study. Although the application of FB and ZVI caused an increased concentration of  $\text{Fe}_o$  (Figure S3), the fluctuations related to heterogeneous oxidation were more pronounced than the influence of Fe amendments on As speciation both in the bulk soil and in the rhizosphere.

## 5. Conclusions

The application of FB and ZVI to paddy soil resulted in a decrease in the dissolution of As into the soil solution under anaerobic conditions. At the time of intermittent drainage, formation of As sulfide was an important mechanism to restrict As dissolution in soil solution when soil was amended with ZVI. However, the fraction of As-S decreased in ZVI-amended soil after prolonged anaerobic conditions, possibly because sulfides were scavenged by increased  $\text{Fe}^{2+}$  dissolution. Despite the lower fraction of As-S than control, As concentration in soil solution of FB and ZVI amended soil was lower than the control. In addition to the sorption ability of As when FB and ZVI were applied, increased precipitation of Fe (hydr)oxide from dissolved  $\text{Fe}^{2+}$  provided sorption sites with As under anaerobic conditions. The decreased concentration of As in the soil solution limited supply of As to the rhizosphere, and therefore the sequestration of As with Fe (hydr)oxide in the rice rhizosphere was decreased by the application of FB and ZVI. Due to the ROL from the roots, the rice rhizosphere is known to be more oxic than bulk soil. Therefore,  $\text{Fe}^{2+}$  dissolved in the soil solution is deposited around roots as Fe plaque. The application of FB and ZVI caused increased deposition of Fe plaque as well as Fe (hydr)oxide deposition around soil particles while their appearances were heterogeneous. Despite the oxidation of  $\text{Fe}^{2+}$  followed by the deposition of Fe (hydr)oxide, our results showed that oxidation of As-3 was not sufficiently pronounced to generate higher proportions of As-5 in the rhizosphere than in the bulk soil, regardless of the application of Fe-bearing materials. It is likely that the partial aeration caused by ROL from roots was not enough to accelerate oxidation of As-3 to As-5. Therefore, intermittent drainage had little influence on oxidation of As-3 to As-5 both in rhizosphere and bulk soil. The fraction of DMA was higher in the rhizosphere than in bulk soil due to the possible influence from root-associated micro-organisms. After drainage, the oxidation process followed by Fe (hydr)oxide deposition occurred heterogeneously around soil particles and rice roots. Although heterogeneous, the application of FB and ZVI resulted in greater Fe (hydr)oxide deposition around the roots and soil particles. The deposited Fe (hydr)oxide-sorbed As-3 therefore contributed to the decreased concentration of As in the soil solution and rice grain. However, the influence of Fe amendments on the oxidation of As after drainage was not as pronounced as the heterogeneous oxidation process in the rice cultivated soil and rhizosphere with Fe plaque.

**Supplementary Materials:** The followings are available online at <http://www.mdpi.com/2411-5126/1/1/6/s1>, Table S1. Soil properties and As concentrations. Table S2. Eh, pH, Fe and As concentrations in the soil solution before drainage. Table S3. Results of linear combination fitting of As-K edge XANES for bulk soil collected from inside (MI) and outside (MO) of nylon mesh cylinder. Table S4. Results of linear combination fitting of As-K edge XANES for root-attached materials (RZ). Table S5. Fractions of As species obtained from a linear combination fitting of the XANES spectra with and without the inclusion of DMA as a reference compound for fitting. Figure S1. Microscopic images of selected areas of the soil thin-sections shown in Figure 1. White and yellow

arrows indicate roots with scattered Fe plaque coating and the position of lateral root emergence, respectively. Figure S2. Linear combination fitting of XANES spectra with and without the inclusion of DMA as a reference compound. Normalized spectra of RZ collected at a depth of 6–9 cm from the control pot 1 h after drainage at harvest (a) and normalized spectra of MI collected from the control pot 27 days after intermittent drainage (b). Figure S3. Concentrations of As and Fe extracted by acid ammonium oxalate solution ( $As_o$  and  $Fe_o$ ) from the bulk soil from the inner (MI) and outer compartments (MO) of the nylon mesh containing the soil cylinder with rice roots. Figure S4. Concentrations of As and Fe extracted by acid ammonium oxalate solution ( $As_o$  and  $Fe_o$ ) from root-attached materials (RZ).

**Acknowledgments:** This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Research project for improving food safety and animal health, As-210) and a grant-in-aid for Scientific Research (Proposal No. 22380046). The XANES measurements were performed with the approval of the Aichi Synchrotron Radiation Center (Proposal No. 201606072) and Japan Synchrotron Radiation Research Institute (Proposal No. 2016A1255).

**Author Contributions:** N.Y. and T.M. designed the experiments, H.Y. and T.M. performed the rice cultivation and soil sampling, A.H. and T.O. made the soil thin-sections, T.M. performed the extraction experiments and the statistical analyses, N.Y. and Y.H. performed the XANES analyses, N.Y. wrote the paper, and T.M., T.O. and Y.H. edited the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## References

1. Stone, R. Food safety—Arsenic and paddy rice: A neglected cancer risk? *Science* **2008**, *321*, 184–185. [[CrossRef](#)] [[PubMed](#)]
2. Manning, B.A.; Goldberg, S. Arsenic(III) and arsenic(V) absorption on three California soils. *Soil Sci.* **1997**, *162*, 886–895. [[CrossRef](#)]
3. Takahashi, Y.; Minamikawa, R.; Hattori, K.H.; Kurishima, K.; Kihou, N. Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. *Environ. Sci. Technol.* **2004**, *38*, 1038–1044. [[CrossRef](#)] [[PubMed](#)]
4. Yamaguchi, N.; Nakamura, T.; Dong, D.; Takahashi, Y.; Amachi, S.; Makino, T. Arsenic release from flooded paddy soils is influenced by speciation, Eh, pH, and iron dissolution. *Chemosphere* **2011**, *83*, 925–932. [[CrossRef](#)] [[PubMed](#)]
5. 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](#)] [[PubMed](#)]
6. Nickson, R.T.; McArthur, J.M.; Ravenscroft, P.; Burgess, W.G.; Ahmed, K.M. Mechanism of arsenic release to groundwater, Bangladesh and west Bengal. *Appl. Geochem.* **2000**, *15*, 403–413. [[CrossRef](#)]
7. 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](#)] [[PubMed](#)]
8. Moreno-Jimenez, E.; Meharg, A.A.; Smolders, E.; Manzano, R.; Becerra, D.; Sanchez-Llerena, J.; Albarran, A.; Lopez-Pinero, A. Sprinkler irrigation of rice fields reduces grain arsenic but enhances cadmium. *Sci. Total Environ.* **2014**, *485*, 468–473. [[CrossRef](#)] [[PubMed](#)]
9. Kyuma, K. *Paddy Soil Science*; Kyoto University Press: Kyoto, Japan; Trans Pacific Press: Melbourne, Australia, 2004.
10. Makino, T.; Nakamura, K.; Katou, H.; Ishikawa, S.; Ito, M.; Honma, T.; Miyazaki, N.; Takehisa, K.; Sano, S.; Matsumoto, S.; et al. Simultaneous decrease of arsenic and cadmium in rice (*Oryza sativa* L.) plants cultivated under submerged field conditions by the application of iron-bearing materials. *Soil Sci. Plant Nutr.* **2016**, *62*, 340–348. [[CrossRef](#)]
11. 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](#)]
12. 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](#)]
13. Suda, A.; Baba, K.; Akahane, I.; Makino, T. Use of water-treatment residue containing polysilicate-iron to stabilize arsenic in flooded soils and attenuate arsenic uptake by rice (*Oryza sativa* L.) plants. *Soil Sci. Plant Nutr.* **2016**, *62*, 111–116. [[CrossRef](#)]



14. Suda, A.; Baba, K.; Yamaguchi, N.; Akahane, I.; Makino, T. The effects of soil amendments on arsenic concentrations in soil solutions after long-term flooded incubation. *Soil Sci. Plant Nutr.* **2015**, *61*, 592–602. [[CrossRef](#)]
15. Suda, A.; Makino, T.; Yamaguchi, N.; Taniguchi, H. Arsenic immobilization in anaerobic soils by application of by-product iron materials obtained from the casting industry. *Soil Sci. Plant Nutr.* **2017**. [[CrossRef](#)]
16. Liu, W.J.; Zhu, Y.G.; Smith, F.A. Effects of iron and manganese plaques on arsenic uptake by rice seedlings (*Oryza sativa* L.) grown in solution culture supplied with arsenate and arsenite. *Plant Soil* **2005**, *277*, 127–138. [[CrossRef](#)]
17. Garnier, J.M.; Travassac, F.; Lenoble, V.; Rose, J.; Zheng, Y.; Hossain, M.S.; Chowdhury, S.H.; Biswas, A.K.; Ahmed, K.M.; Cheng, Z. Temporal variations in arsenic uptake by rice plants in Bangladesh: The role of iron plaque in paddy fields irrigated with groundwater. *Sci. Total Environ.* **2010**, *408*, 4185–4193. [[CrossRef](#)] [[PubMed](#)]
18. Syu, C.H.; Jiang, P.Y.; Huang, H.H.; Chen, W.T.; Lin, T.H.; Lee, D.Y. Arsenic sequestration in iron plaque and its effect on as uptake by rice plants grown in paddy soils with high contents of as, iron oxides, and organic matter. *Soil Sci. Plant Nutr.* **2013**, *59*, 463–471. [[CrossRef](#)]
19. Li, J.; Dong, F.; Lu, Y.; Yan, Q.; Shim, H. Mechanisms controlling arsenic uptake in rice grown in mining impacted regions in south China. *PLoS ONE* **2014**, *9*. [[CrossRef](#)] [[PubMed](#)]
20. Khan, N.; Seshadri, B.; Bolan, N.; Saint, C.P.; Kirkham, M.B.; Chowdhury, S.; Yamaguchi, N.; Lee, D.Y.; Li, G.; Kunhikrishnan, A.; et al. Root iron plaque on wetland plants as a dynamic pool of nutrients and contaminants. In *Advances in Agronomy*; Sparks, D.L., Ed.; Elsevier: Amsterdam, The Netherlands, 2016; Volume 138, pp. 1–96.
21. Liu, W.J.; Zhu, Y.G.; Hu, Y.; Williams, P.N.; Gault, A.G.; Meharg, A.A.; Charnock, J.M.; Smith, F.A. Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (*Oryza sativa* L.). *Environ. Sci. Technol.* **2006**, *40*, 5730–5736. [[CrossRef](#)] [[PubMed](#)]
22. Seyfferth, A.L.; Webb, S.M.; Andrews, J.C.; Fendorf, S. Arsenic localization, speciation, and co-occurrence with iron on rice (*Oryza sativa* L.) roots having variable Fe coatings. *Environ. Sci. Technol.* **2010**, *44*, 8108–8113. [[CrossRef](#)] [[PubMed](#)]
23. 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](#)]
24. Yu, H.Y.; Wang, X.Q.; Li, F.B.; Li, B.; Liu, C.P.; Wang, Q.; Lei, J. Arsenic mobility and bioavailability in paddy soil under iron compound amendments at different growth stages of rice. *Environ. Pollut.* **2017**, *224*, 136–147. [[CrossRef](#)] [[PubMed](#)]
25. Syu, C.-H.; Lee, C.-H.; Jiang, P.-Y.; Chen, M.-K.; Lee, D.-Y. Comparison of as sequestration in iron plaque and uptake by different genotypes of rice plants grown in as-contaminated paddy soils. *Plant Soil* **2014**, *374*, 411–422. [[CrossRef](#)]
26. Violante, A.; Barberis, E.; Pigna, M.; Boero, V. Factors affecting the formation, nature, and properties of iron precipitation products at the soil-root interface. *J. Plant Nutr.* **2003**, *26*, 1889–1908. [[CrossRef](#)]
27. Frommer, J.; Voegelin, A.; Dittmar, J.; Marcus, M.A.; Kretzschmar, R. Biogeochemical processes and arsenic enrichment around rice roots in paddy soil: Results from micro-focused X-ray spectroscopy. *Eur. J. Soil Sci.* **2011**, *62*, 305–317. [[CrossRef](#)]
28. Ultra, V.U.; Nakayama, A.; Tanaka, S.; Kang, Y.M.; Sakurai, K.; Iwasaki, K. Potential for the alleviation of arsenic toxicity in paddy rice using amorphous iron-(hydr)oxide amendments. *Soil Sci. Plant Nutr.* **2009**, *55*, 160–169. [[CrossRef](#)]
29. Hashimoto, Y.; Furuya, M.; Yamaguchi, N.; Makino, T. Zerovalent iron with high sulfur content enhances the formation of cadmium sulfide in reduced paddy soils. *Soil Sci. Soc. Am. J.* **2016**, *80*, 55–63. [[CrossRef](#)]
30. Li, R.; Zhou, Z.; Zhang, Y.; Xie, X.; Li, Y.; Shen, X. Uptake and accumulation characteristics of arsenic and iron plaque in rice at different growth stages. *Commun. Soil Sci. Plant Anal.* **2015**, *46*, 2509–2522. [[CrossRef](#)]
31. Schmidt, H.; Eickhorst, T.; Tippkötter, R. Monitoring of root growth and redox conditions in paddy soil rhizotrons by redox electrodes and image analysis. *Plant Soil* **2010**, *341*, 221–232. [[CrossRef](#)]
32. Seyfferth, A.L.; Ross, J.; Webb, S.M. Evidence for the root-uptake of arsenite at lateral root junctions and root apices in rice (*Oryza sativa* L.). *Soils* **2017**, *1*, 3. [[CrossRef](#)]

33. Dong, D.T.; Yamaguchi, N.; Makino, T.; Amachi, S. Effect of soil microorganisms on arsenite oxidation in paddy soils under oxic conditions. *Soil Sci. Plant Nutr.* **2014**, *60*, 377–383. [[CrossRef](#)]
34. Nakamura, K.; Katou, H. Arsenic and cadmium solubilization and immobilization in paddy soils in response to alternate submergence and drainage. In *Competitive Sorption and Transport of Heavy Metals in Soils and Geological Media*; Selim, H.M., Ed.; CRC Press: Boca Raton, FL, USA, 2012; pp. 379–404.
35. Arao, T.; Kawasaki, A.; Baba, K.; Matsumoto, S. Effects of arsenic compound amendment on arsenic speciation in rice grain. *Environ. Sci. Technol.* **2011**, *45*, 1291–1297. [[CrossRef](#)] [[PubMed](#)]
36. Kuramata, M.; Sakakibara, F.; Kataoka, R.; Abe, T.; Asano, M.; Baba, K.; Takagi, K.; Ishikawa, S. Arsenic biotransformation by *Streptomyces* sp isolated from rice rhizosphere. *Environ. Microbiol.* **2015**, *17*, 1897–1909. [[CrossRef](#)] [[PubMed](#)]
37. Shimizu, M.; Arai, Y.; Sparks, D.L. Multiscale assessment of methylarsenic reactivity in soil. 2. Distribution and speciation in soil. *Environ. Sci. Technol.* **2011**, *45*, 4300–4306. [[CrossRef](#)] [[PubMed](#)]
38. Shimizu, M.; Arai, Y.; Sparks, D.L. Multiscale assessment of methylarsenic reactivity in soil. 1. Sorption and desorption on soils. *Environ. Sci. Technol.* **2011**, *45*, 4293–4299. [[CrossRef](#)] [[PubMed](#)]
39. Zhu, L.; Gao, K.T.; Jin, J.; Lin, H.Z.; Xu, X.Y. Analysis of ZVI corrosion products and their functions in the combined ZVI and anaerobic sludge system. *Environ. Sci. Pollut. Res.* **2014**, *21*, 12747–12756. [[CrossRef](#)] [[PubMed](#)]
40. Jia, Y.; Bao, P.; Zhu, Y.G. Arsenic bioavailability to rice plant in paddy soil: Influence of microbial sulfate reduction. *J. Soils Sediments* **2015**, *15*, 1960–1967. [[CrossRef](#)]
41. Ludwig, R.D.; Smyth, D.J.A.; Blowes, D.W.; Spink, L.E.; Wilkin, R.T.; Jewett, D.G.; Weisener, C.J. Treatment of arsenic, heavy metals, and acidity using a mixed PVI-compost PRB. *Environ. Sci. Technol.* **2009**, *43*, 1970–1976. [[CrossRef](#)] [[PubMed](#)]
42. Kober, R.; Welter, E.; Ebert, M.; Dahmke, A. Removal of arsenic from groundwater by zerovalent iron and the role of sulfide. *Environ. Sci. Technol.* **2005**, *39*, 8038–8044. [[CrossRef](#)] [[PubMed](#)]
43. Ona-Nguema, G.; Morin, G.; Wang, Y.H.; Foster, A.L.; Juillot, F.; Calas, G.; Brown, G.E. XANES evidence for rapid arsenic(III) oxidation at magnetite and ferrihydrite surfaces by dissolved O<sub>2</sub> via Fe<sup>2+</sup>-mediated reactions. *Environ. Sci. Technol.* **2010**, *44*, 5416–5422. [[CrossRef](#)] [[PubMed](#)]



© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).