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Effects of iron and manganese plaques on arsenic uptake by rice seedlings (*Oryza sativa* L.) grown in solution culture supplied with arsenate and arsenite

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Abstract

We have shown previously that phosphorus nutrition and iron plaque on the surface of rice roots influence arsenate uptake and translocation by rice in hydroponic culture. We have now investigated the role of iron (Fe) and manganese (Mn) plaque on arsenate and arsenite uptake and translocation in rice seedlings grown hydroponically. Fe and Mn plaques were clearly visible as reddish or brown coatings on the root surface after 12 h induction, and Fe plaque was much more apparent than Mn plaque. Arsenite or arsenate supply did not decrease plant dry weights significantly. There were significant differences in shoot dry weights but little difference in root dry weights between some plaque treatments. Arsenic (As) concentrations in Fe plaque when arsenate was supplied were significantly higher than those in no plaque (control) and Mn plaque treatments, and much higher than those supplied with arsenite. This showed that Fe plaque on the rice root had higher affinity to arsenate than to arsenite. In Fe plaque treatment, the results indicated that most As was sequestered in roots when arsenite was supplied and most As concentrated in Fe plaque when arsenate was supplied. Most As was accumulated in rice roots in Mn plaque and no plaque treatments for both As species.

Introduction

Arsenic (As) is a ubiquitous and potentially toxic element in the environment, and is observed in living tissues, water and soil derived from both natural and anthropogenic sources. Chronic As poisoning by drinking tube well water and consuming As-contaminated food has become a chemical disaster in Southeast Asia (Meharg, 2004). Rice (*Oryza sativa*) is the staple food for people in many parts of the world, particularly Southeast Asia. Apart from the health risks directly associated with the consumption of As-contaminated drinking water, Meharg and Rahman (2003) have demonstrated that As passes into rice through irrigation water pumped from contaminated tube wells in Bangladesh. Under irrigation with As-contaminated groundwater, rice straw, which is fed to cattle, can accumulate high levels (up to 92 mg kg^{-1}) of arsenic (Abedin et al., 2002a). In addition, rice grown on As-contaminated soils can accumulate high levels of arsenic in grains (Xie and Huang, 1998). Consequently, there are health risks to cattle and humans through the food chain.

The toxicity and bioavailability of As in the environment depends not only on the total concentration, but also on its chemical species (Chen

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and Wang, 1990; Cullen and Reimer, 1989). Arsenic released into the environment has both inorganic and organic forms. Generally inorganic As species are thought to be more mobile and more toxic than organic forms (Byrne et al., 1995; Philips, 1990). Arsenate is the predominant species in aerobic soils, whereas arsenite dominates under anaerobic conditions such as paddy soil (Marin et al., 1993; Masscheleyn et al., 1991; Onken and Hossner, 1995, 1996; Smith et al., 1998). Abedin et al. (2002a) found that arsenite was the most predominant species, followed by dimethylarsinic acid (DMAA), arsenate, and monomethylarsonic (MMAA), in the rice rhizosphere under flooded paddy conditions irrigated with arsenate-contaminated water.

The bioavailability of As in paddy soil is affected by some physico-chemical properties of bulk soil, such as presence of iron (Fe) (hydro-) oxides. The functional groups of Fe hydroxides may sequester some cations and anions in sediment and soil (Kuo, 1986). Iron (hydro-) oxide has a high affinity for arsenate in soil or solution (Belzile and Tessier, 1990; Jain et al., 1999; Meng et al., 2002). In addition, a coating of Fe hydroxides/oxides known as 'iron plaque' is commonly formed on the roots of aquatic plant species, such as *Oryza sativa* L. Iron plaque is the consequence of the oxidation of roots by release of oxygen and oxidants into the rhizosphere (Armstrong, 1967; Chen et al., 1980). It has been reported to consist of a mixture of amorphous or crystalline Fe (Bacha and Hossner, 1977; Chen et al., 1980). Otte et al. (1991) showed that Fe plaque plays an important role in mediating As accumulation in the salt marsh plant *Aster tripolium* growing in flooded soil. Liu et al. (2004a, b) suggested that Fe plaque on the root surface of rice has a high affinity to arsenate.

Iron plaque can also accumulate a variety of heavy metals and metalloids, such as Cu, Ni, Mn, Cd and As (Greipsson, 1994; Liu et al., 2004a, b; Otte et al., 1989; Taylor and Crowder, 1983a; Ye et al., 1998). In particular, Fe and Mn usually co-exist (adsorption and co-precipitation) in root plaques of aquatic plants. Because Fe oxides and hydroxides precipitate at lower redox potentials than Mn oxides, Fe is the main element and Mn is secondary in the plaques (Christensen and Sand-Jensen, 1998; Crowder & St-Cyr, 1991). A field study showed that the concentrations of Fe and Mn were positively correlated in the coating on the root surface of *Phragmites australis* (St-Cyr and Crowder, 1990).

In rice paddy soil, arsenate and arsenite co-exist in the soil solution (Abedin et al., 2002b; Smith et al., 1998). It is possible that there are two pathways for arsenite to enter into rice roots. In the rhizosphere, part of arsenite may be oxidized to arsenate, which has high affinity for iron plaque, co-precipitate with Fe(III) and adsorb on to the plaque (Otte et al., 1991). At the root–plaque interface, siderophores or phytosiderophores exuded by microbes or rice roots may complex with Fe(III) and mobilize Fe-bound arsenate, taken up through phosphate co-transporters. This may lead to simultaneous uptake of Fe and arsenate. In the second possible pathway, arsenite may be accumulated on Fe plaque in the form of $H_3AsO_3^0$ and then transported into rice roots via aquaporins (Meharg and Jardine, 2003).

In this study, the effects of Fe and Mn plaques on arsenite or arsenate uptake by rice seedlings were investigated under hydroponic culture. A previous experiment demonstrated that iron plaque had a high affinity to arsenate and thus affected the uptake of arsenate by rice seedlings (Liu et al., 2004a). However, the effect of iron plaque on adsorption and uptake of arsenite by rice plant remains unclear. Compared with Fe plaque, studies on Mn plaque are few and accumulation of metals/metalloids in Mn plaque is unclear, and need further investigation. Therefore, the objectives of this study were to (1) investigate the effects of Fe and Mn plaques on As uptake and translocation by rice seedlings when arsenate or arsenite was supplied to the growth solution, respectively; (2) investigate the differences between Fe and Mn plaques in affecting As uptake and translocation.

Materials and methods

Preparation of rice seedlings

Seeds of rice (*Oryza sativa* L.) (Cultivar: Yuanyou-1) were obtained from Professor Li Damo, Institute of Subtropical Regional Agriculture, Chinese Academy of Sciences. Seeds were

sterilized in 30% H_2O_2 (wt:wt) solution for 15 min followed by thorough washing with deionized water. The seeds were germinated in moist perlite. After 3 weeks, uniform seedlings were selected and transplanted to PVC pots (75 mm diameter and 140 mm height, two seedlings per pot) containing 500 mL 1/3 strength nutrient solution. The composition of nutrient solution was modified from that of Hewitt (1966) and contained 5 m*M* NH_4NO_3, 2 m*M* K_2SO_4, 4 m*M* $CaCl_2$, 1.5 m*M* $MgSO_4 \cdot 7H_2O$, 1.3 m*M* KH_2PO_4, 50 μM Fe(II)-ethylenediaminetetraacetic acid (EDTA), 10 μM H_3BO_4, 1.0 μM $ZnSO_4 \cdot 7H_2O$, 1.0 μM $CuSO_4 \cdot 5H_2O$, 5.0 μM $MnSO_4 \cdot H_2O$, 0.5 μM $Na_2MoO_4 \cdot 2H_2O$, and 0.2 μM $CoSO_4 \cdot 7H_2O$. The solution was changed twice a week, and solution pH value was adjusted to 5.5 using 0.1 *M* KOH or HCl.

Growth conditions

All experiments were carried out in a controlled environment greenhouse with ambient light (roughly 13 h day/11 h night, 500–1100 μmol m^{-1} s^{-1}). Temperature fluctuation in the greenhouse was between 20 and 35 °C at day and night. The relative humidity was 70%.

Experimental treatments

Experiment I: Effects of iron and manganese plaques on arsenate and arsenite uptake and As translocation

After 3 weeks, Fe plaque and Mn plaques were induced on roots as follows. All seedlings were first put into deionized water for 12 h to minimize interference from other elements with Fe and Mn. They were then transferred into 500 mL solution with 0.36 mmol Fe^{2+} L^{-1} for 24 h (Fe^{2+} as $FeSO_4 \cdot 7H_2O$ and Mn^{2+} as $MnSO_4 \cdot H_2O$), respectively. Solution pH was adjusted to 5.5 for Fe plaque treatment and 7.0 for Mn plaque treatment using 0.1 *M* KOH or HCl. Control seedlings were also put into distilled water (pH 5.5).

After Fe and Mn plaques were induced, all seedlings were rinsed with deionized water for three times, then grown in 1/3 strength normal nutrient solution for 3 days before exposure to As treatments. After 3 days, plants were transplanted in 1/3 strength nutrient solutions with 6.67 μmol As L^{-1} or 13.3 μmol As L^{-1} as $Na_3AsO_4 \cdot 12H_2O$ or $NaAsO_2$ for 2 weeks. The concentration of 6.67 μmol As L^{-1} or 13.3 μmol As L^{-1} was chosen because a preliminary experiment showed that these doses did not cause acute toxicity to rice seedlings. These concentrations were also comparable to some contaminated irrigation water, and were lower than the highest As-contaminated irrigation water (26.7 μmol As L^{-1}) reported in some areas (Abedin et al., 2002b). The nutrient solutions were changed twice a week, and solution pH value was adjusted to 5.5 using 0.1 *M* KOH or HCl. The experimental design was completely randomized with each treatment replicated four times and there were 48 pots in total.

Experiment II: Measurement of changes in arsenic speciation in nutrient solution

Uniform seedlings were selected and transplanted to PVC pots containing 500 mL of 1/3 strength nutrient solution (see above). After 1 week, seedlings were divided into two groups. Fe plaque was induced on the root surface of one group of rice plants using the same method as in Experiment I. No plaque was induced on the rice root of another group. After Fe plaque induction, all of plants were exposed to 1/3 strength nutrient solutions with 13.3 μmol As L^{-1} as $NaAsO_2$ for 72 h; Control pots with no plants were also included to check changes of arsenic speciation in nutrient solution. After treatment, 10 mL nutrient solution from each pot was sampled at 24 and 72 h. Each treatment replicated three times and there were nine pots in total.

After sampling, arsenite in solution was analyzed at once to prevent the further changes in arsenic speciation during storage. Arsenite and total arsenic concentrations were measured by atomic fluorescence spectrometry (2202E, Beijing Haiguang Analytical Instrument Co., Beijing, China). For the determination of arsenite, 4 mL sample solution was diluted with deionized water to 10 mL. The reductant is 10 g L^{-1} KBH_4 and the carrier acid solution used was 1.0 *M* HCl. For total arsenic determination, the same procedure as described above was carried out after adding 2 mL of 10 g L^{-1} thiourea solution and 1.7 mL concentrated HCl (Yang et al., 2003). The concen-

trations of arsenate were calculated by subtracting arsenite concentrations from total arsenic concentrations. The recovery of arsenite was carried out by spiking standard solution of arsenite in sample solution and determination of arsenic concentration in mixed solution. The recovery rates were between 90 and 108% in this experiment.

DCB extraction of plaque from roots

At harvest, Fe plaque and Mn plaque on fresh root surfaces were extracted with dithionite–citrate–bicarbonate (DCB) using the method of Taylor and Crowder (1983b) and Otte et al. (1991). The whole root system of each seedling, including controls with no plaque, was incubated for 60 min at room temperature (20–25 °C) in 40 mL of a solution containing 0.03 *M* sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) and 0.125 *M* sodium bicarbonate ($NaHCO_3$), with the addition of 1.0 g sodium dithionite ($Na_2S_2O_4$). Roots were rinsed three times with deionized water that was then added to the DCB-extract. The resulting solution was made up to 100 mL with deionized water. After DCB extraction, roots and shoots were oven dried at 70 °C for 3 days and weighed.

Plant analysis

Dried plant materials were ground and about 0.25 g weighed accurately into clean, dry Teflon tubes (100 mL) for digestion and 5 mL of concentrated HNO_3 was added. The tubes were placed on a microwave accelerated reaction system (Mars5, CEM Microwave Technology Ltd., USA). The procedure of digestions is: 15 min ramping to 160 °C, and holding at this temperature for 15 min. A reagent blank and standard reference plant material (GBW07603 from the National Research Center for Standards in China) were included, to verify the accuracy and precision of the digestion procedure and subsequent analysis using recovery. After digestion the solutions were cooled, diluted to 50 mL with ultra-pure water (Easy-pure, Dubugue, Iowa, USA) and transferred into acid-washed plastic bottles. The concentrations of Fe, Mn, P and As in the DCB-extracts and in the acid digests were measured by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer, Optima 2000 DV, Perkin Elmer, USA) and atomic fluorescence spectrometry (AF-610A, Beijing Ruili Analytical Instrument Co., Beijing, China), respectively.

Data analysis

Element concentrations in DCB-extracts, roots and shoots were calculated on the basis of dry weight. Total As (T_{As}), percentages of As in DCB-extracts, roots and shoots were calculated as follows:

$$T_{As} = T_{DCB\text{-}extract\text{-}As} + T_{Root\text{-}As} + T_{Shoot\text{-}As}$$

$$T_{DCB\text{-}extract\text{-}As} = C_{DCB\text{-}extract\text{-}As} \times Root_{biomass}$$

$$T_{Root\text{-}As} = C_{Root\text{-}As} \times Root_{biomass}$$

$$T_{Shoot\text{-}As} = C_{Shoot\text{-}As} \times Shoot_{biomass}$$

$$DCB\text{-}As\% = (T_{DCB\text{-}extract\text{-}As}/T_{As}) \times 100$$

$$Root\text{-}As\% = (T_{Root\text{-}As}/T_{As}) \times 100$$

$$Shoot\text{-}As\% = (T_{Shoot\text{-}As}/T_{As}) \times 100$$

Where $T_{DCB\text{-}extract\text{-}As}$, $T_{Root\text{-}As}$ and $T_{Shoot\text{-}As}$ represent the total As in DCB-extracts, roots and shoots, respectively; $C_{DCB\text{-}extract\text{-}As}$, $C_{Root\text{-}As}$ and $C_{Shoot\text{-}As}$ are As concentrations in DCB-extracts, roots and shoots, respectively.

Specific arsenic uptake: $(T_{Root\text{-}As} + T_{Shoot\text{-}As})/Root_{biomass}$

Statistical analysis

Analysis of variance (ANOVA) on plant biomass and concentrations of metals were performed using Windows-based Genstat (6th edition, NAG Ltd., England).

Results

Experiment I: Effects of Fe and Mn plaques on arsenate and arsenite uptake and arsenic translocation

Plant growth and plaque formation

In general, there was no significant difference in root growth between plaque treatments.

Table 1. Dry weights (g per pot) of roots and shoots of rice seedlings with Fe plaque, Mn plaque and no plaque, supplied with nutrient solutions containing 6.67 or 13.3 μmol L^{-1} arsenite or arsenate for 2 week, and analysis of variance by three-way ANOVA

Plaque treatments	As concentrations in solution (μmol L^{-1})		Roots	Shoots
Fe plaque	Arsenite	6.67	0.38 ± 0.02 bc*	0.80 ± 0.06 de
		13.3	0.41 ± 0.02 bc	0.73 ± 0.02 e
	Arsenate	6.67	0.36 ± 0.01 c	0.82 ± 0.03 cde
		13.3	0.38 ± 0.03 bc	0.77 ± 0.04 de
Mn plaque	Arsenite	6.67	0.38 ± 0.01 bc	0.92 ± 0.07 bcd
		13.3	0.44 ± 0.05 ab	0.74 ± 0.09 e
	Arsenate	6.67	0.36 ± 0.01 c	1.05 ± 0.04 ab
		13.3	0.38 ± 0.01 bc	1.00 ± 0.02 ab
No plaque	Arsenite	6.67	0.35 ± 0.02 c	1.04 ± 0.11 ab
		13.3	0.49 ± 0.01 a	0.98 ± 0.06 abc
	Arsenate	6.67	0.38 ± 0.02 bc	1.12 ± 0.05 a
		13.3	0.41 ± 0.02 bc	1.10 ± 0.06 a
Analysis of variance				
Concentration of As (C)			$P < 0.001$	NS
Plaque (P)			NS	$P < 0.001$
Speciation of As (S)			$P < 0.05$	$P < 0.001$

*The different letters in the same line indicated the results of multiple comparisons according to LSD test; data are means ± SE, $n = 4$.

Although the overall effects of concentrations ($P < 0.001$) and species of As ($P < 0.005$) in nutrient solutions on root dry weights were significant (Table 1), the results of multiple comparison showed that root dry weight of only arsenite treatment (13.3 μM) in no plaque treatment was significantly higher than those of other 11 individual treatments. Shoot dry weights of rice seedlings with iron plaque were significantly lower than those of Mn plaque and no plaque with the tendency Fe plaque < Mn plaque < No plaque ($P < 0.001$). Shoot dry weights of Plants with arsenite were statistically lower than those treated with arsenate only for Mn plaque treatments, not for control and Fe plaque (Table 1).

Fe and Mn plaques were clearly visible as reddish or brown coatings on the root surface after 12 h induction, and Fe plaque was much more apparent than Mn plaque. Control (no plaque) plant roots were white, and no colored coating was visible. Plaques on the root surface were removed with DCB-extracts. Fe and Mn concentrations in DCB-extracts indicated the amounts of Fe and Mn accumulated on the root surface (Table 2). The results showed that Fe concentrations in DCB-extracts for Fe plaque treatment were significantly higher than those for Mn plaque and no plaque treatments. Although Mn concentrations in DCB-extracts for Mn plaque treatment were higher than those for Fe plaque and no plaque treatments, the amount of Mn plaque was significantly lower than that of Fe plaque.

Table 2. Fe and Mn concentrations in DCB-extracts (DCB-Fe and DCB-Mn) (mg kg^{-1}) of rice roots with Fe plaque, Mn plaque and no plaque

Plaque treatments	DCB-Fe	DCB-Mn
Fe plaque	42067 ± 1543 a	26.77 ± 1.99 b*
Mn plaque	6710 ± 595 b	223.7 ± 15.5 a
No plaque	5263 ± 1009 b	24.15 ± 1.75 b

*Different letters in the same line indicated a significant difference ($P < 0.001$) according to LSD multiple comparison test; data are means ± SE, $n = 16$.

As concentrations in DCB-extracts

Concentrations and species of As supplied, plaque formation, and the interactions between

them had significant effects on As concentrations in DCB-extracts (Table 3). As concentrations in Fe plaque when arsenite or arsenate was supplied were significantly higher than those in Mn plaque and no plaque. However, in Mn plaque and no plaque treatments, As species added to the solution had little effect on DCB-As concentrations. In iron plaque treatments, As concentrations in iron plaque on the roots exposed to solution with arsenate were significantly higher than those exposed to solution with arsenite. These results indicated that Fe plaque has higher affinity to arsenate than to arsenite.

Arsenic concentrations in plant tissues

The concentrations of arsenic in roots and shoots increased significantly with increasing of arsenic concentrations in nutrient solutions when arsenate and arsenite were supplied ($P < 0.001$, Table 3). Arsenic concentrations in roots were significantly lower when arsenate was supplied than when arsenite was supplied, especially with Fe plaque ($P < 0.001$, Table 3). Fe plaque decreased As concentration in roots when 13.3 μmol L^{-1} arsenate was supplied compared with Mn plaque or no plaque, and the difference was significant for no plaque treatment. Overall, there were no significant differences in shoot-As concentrations between arsenate and arsenite treatments. As concentrations in shoots with iron plaque were higher than those with Mn plaque and control (no plaque) when 13.3 μmol L^{-1} arsenate was supplied, but the differences were not significant for no plaque (Table 3). However, As concentrations in shoots with Fe plaque were significantly higher than those with Mn plaque and no plaque when 13.3 μmol L^{-1} arsenite was supplied.

Specific arsenic uptake (SAU)

Specific arsenic uptake (SAU) at 13.3 μmol L^{-1} was significantly higher than those at 6.67 μmol L^{-1} for both arsenite and arsenate

Table 3. As concentrations (mg kg^{-1}) in dithionite–citrate–bicarbonate (DCB) extracts, roots and shoots of rice with Fe plaque, Mn plaque and controls (no plaque), exposed to nutrient solutions with arsenite or arsenate at 6.67 or 13.3 μmol L^{-1} for 2 week, analysis of variance by three-way ANOVA

Plaque treatments	As concentrations in solution (μmol L^{-1})		DCB-As	Root-As	Shoot-As
Fe plaque	Arsenite	6.67	276 ± 16.7 d	356 ± 19.9 de	16.9 ± 1.2 def *
		13.3	353 ± 9.6 c	547 ± 68.9 ab	30.4 ± 4.6 a
	Arsenate	6.67	451 ± 19.7 b	244 ± 6.27 f	15.3 ± 2.3 ef
		13.3	590 ± 4.9 a	403 ± 18.9 cd	26.4 ± 1.0 abc
Mn plaque	Arsenite	6.67	164 ± 6.7 fg	378 ± 18.7 cd	15.9 ± 2.7 ef
		13.3	172 ± 17.6 fg	580 ± 97.8 a	19.2 ± 2.2 cde
	Arsenate	6.67	136 ± 14.4 g	250 ± 16.4 f	11.6 ± 0.9 f
		13.3	168 ± 6.8 fg	470 ± 24.9 bc	19.7 ± 1.4 cde
No plaque	Arsenite	6.67	130 ± 12.4 g	391 ± 16.1 cd	14.8 ± 0.7 ef
		13.3	219 ± 23.8 e	623 ± 24.7 a	22.8 ± 2.0 bcd
	Arsenate	6.67	146 ± 28.0 g	264 ± 15.5 ef	15.6 ± 1.2 ef
		13.3	193 ± 5.8 ef	536 ± 16.2 ab	24.3 ± 1.4 abc
Analysis of variance					
Concentration of As (C)			$P = 0.033$	$P < 0.001$	$P < 0.001$
Plaque (P)			$P < 0.001$	NS	$P = 0.002$
Speciation of As (S)			$P < 0.001$	$P < 0.001$	NS
C × P			$P < 0.001$	NS	NS
C × S			$P = 0.024$	NS	NS
P × S			$P < 0.001$	NS	NS

*The different letters in the same line indicated the results of multiple comparisons according to LSD test; data are means ± SE, $n = 4$.

treatments ($P < 0.001$; Figure 1). Arsenic species ($P < 0.001$) also affected significantly the SAU by rice roots. SAU of roots exposed to arsenite solution was significantly greater than those exposed to arsenate (534 mg kg^{-1} and 409 mg kg^{-1} for plaque treatments combined, respectively). There was no significant difference in SAU between Fe, Mn plaques and no plaque treatments when arsenite was supplied (Figure 1). However, when 13.3 μmol L^{-1} arsenate was supplied SAU with Fe plaque was statistically lower than that of no plaque ($P = 0.013$). Mn plaque also decreased SAU compared with no plaque, but the difference was not significant. The ranking of SAU for the treatments was as follows: no plaque (600 mg kg^{-1}) > Mn plaque (523 mg kg^{-1}) > Fe plaque (457 m g kg^{-1}) ($LSD_{0.05}$: 93.5 mg kg^{-1}).

As distribution in different components

Dithionite–citrate–bicarbonate (DCB)-As% in Fe plaque and arsenate treatments was about 59%, and the highest among other five treatments ($P < 0.001$, Figure 2). It indicated that most arsenic was accumulated in Fe plaque when arsenate was supplied. However, Root-As% in other treatments was greater than 50%, suggesting that most arsenic sequestrated in roots in the other five individual treatments. Iron plaque treatments gave the lowest proportions of As in shoots when arsenate was supplied ($P < 0.001$). Shoot-As% was significantly higher when arsenate was supplied than when arsenite was supplied for Mn plaque and no plaque treatments ($P < 0.001$).

Experiment II: Measurement of changes in arsenic speciation in nutrient solution

Arsenate was detected in nutrient solutions originally spiked with arsenite in all three treatments (Fe plaque, no plaque and no plant, respectively) at 24 and 72 h . There were no significant difference between Fe plaque and no plaque treatments in arsenate and arsenite concentrations in nutrient solutions at 24 h, while at 72 h, there was more arsenite oxidation in the no plaque treatment than that of Fe plaque ($P < 0.001$). Arsenate concentrations in pots with no plant were significantly higher than those with plants ($P = 0.007$). Overall, arsenate formed in nutrient solutions during the 72 h experimental period only accounted for a small fraction of the total arsenic (22.2%, Figure 3). There was an increasing depletion of total arsenic in nutrient solution containing plants at 24 h than at 72 h , and there

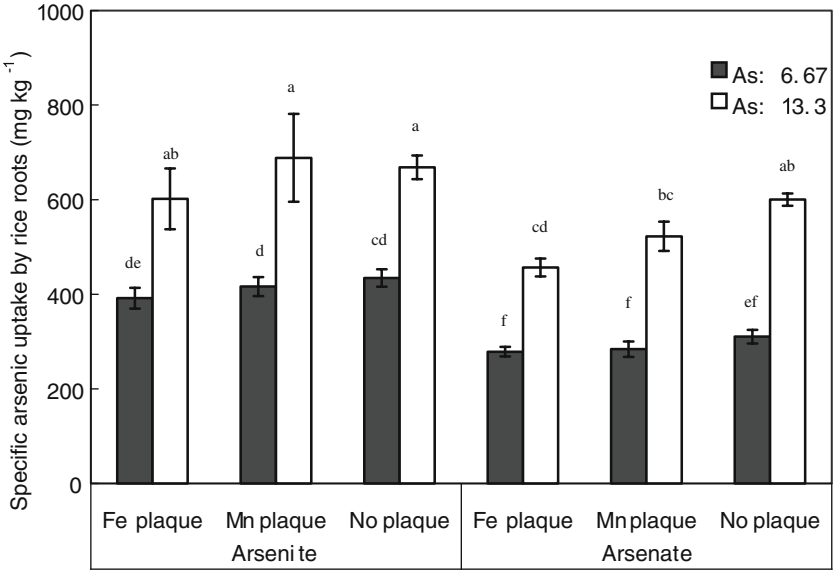

Figure 1. Specific arsenic uptake (mg kg^{-1}) by rice roots with Fe, Mn plaque and no plaque, exposed to nutrient solutions with arsenite or arsenate at 6.67 μmol L^{-1} (closed columns), or 13.3 μmol L^{-1} (open columns) for 2 week (means ± SE, $n = 4$). The different letters indicated the results of multiple comparisons according to LSD test.

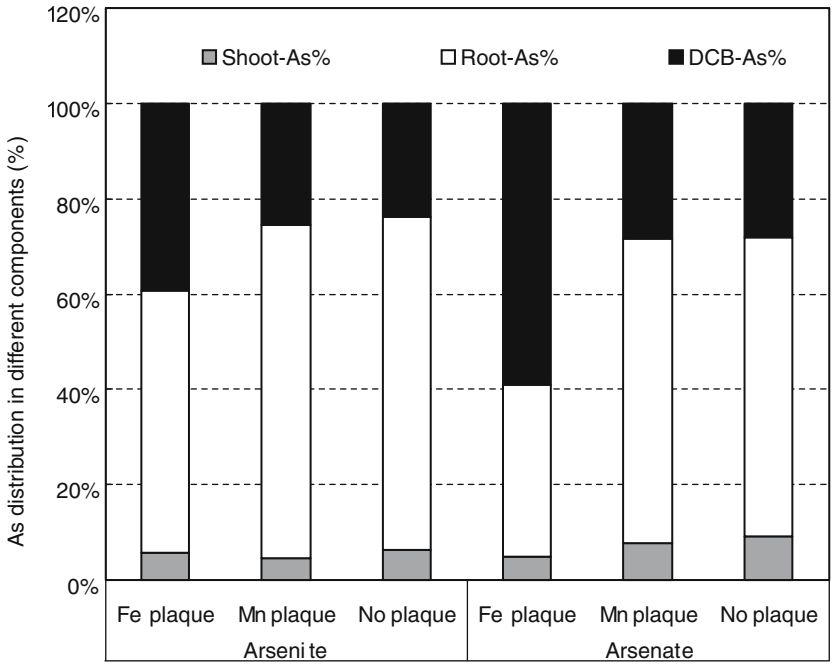

Figure 2. Percentages of arsenic in different components of rice plants with Fe plaque, Mn plaque and no plaque grown in nutrient solutions with 6.67 μmol L^{-1} or13.3 μmol L^{-1} arsenite or arsenate for 2 week (means, $n = 4$).

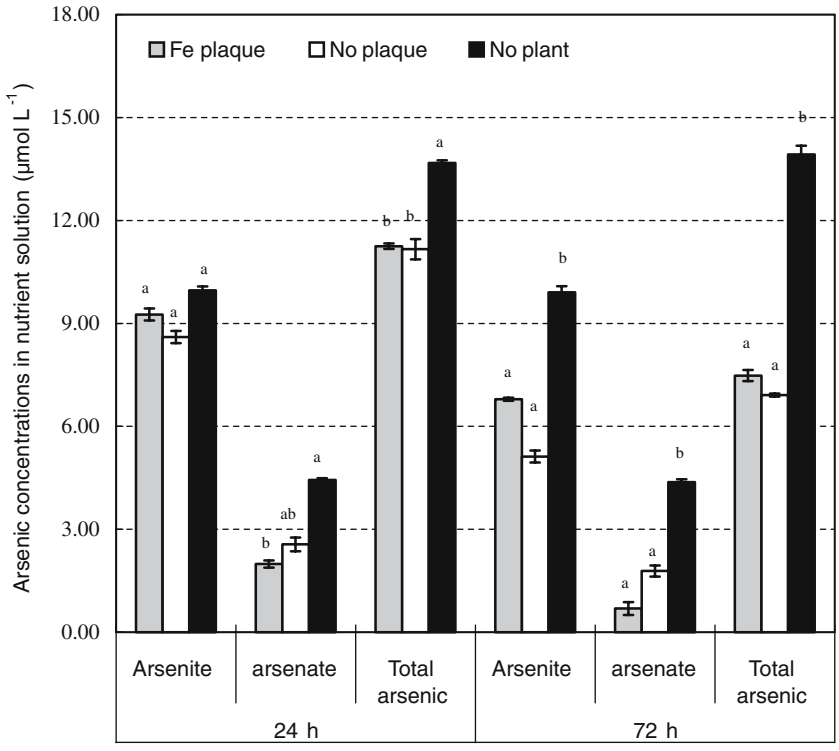

Figure 3. The concentrations of Arsenite, arsenate and total arsenic in nutrient solution originally spiked with 13.3 μmol L^{-1} arsenite (means ± SE, $n = 3$). Solution samples were taken 24 and 72 h after the experiment started. The different letters within the same group indicated the results of multiple comparisons according to LSD test.

was a near 100% recovery of total arsenic irrespective of the exposure period in the no plant treatment.

Discussion

Overall, Fe and Mn plaques had little effect on biomass of rice roots (Table 1). However, shoot dry weights of rice seedlings with Fe plaque were slightly lower than those with Mn plaque and controls with no plaque. This agrees with the results obtained by Ye et al. (1997, 2001), but differs from those of Christensen and Sand-Jensen (1998), Greipsson and Crowder (1992). Greipsson (1994, 1995) suggested that the formation of iron plaque could enhance the growth of plants and reduce excessive Cu, Ni and Zn toxicity to rice seedlings. The different effects of Fe plaque on plant growth may depend on the age of plaques, species of plants or types of heavy metals. In addition, our result showed that the higher concentrations of arsenate or arsenite (13.3 μmol L^{-1}) slightly increased root dry weight. It is possible that the higher concentration of As inhibited P uptake and indirectly stimulated root elongation in order to take up more P.

In this study, although the same concentrations of Fe and Mn were added to the nutrient solution to induce Fe and Mn plaque, the amounts of Mn on the root surface were much less than Fe amounts (Table 2), which was similar to other aquatic plants (Ye et al., 2001). This might be due to the fact that Fe and Mn ions respond differently to changes in pH and redox potential, and at any given pH, iron oxides and hydroxides may precipitate at lower redox potentials than Mn oxides. Similarly, under fixed Eh, Fe starts to precipitate as an oxide at a considerably lower pH than Mn (St-Cyr and Crowder, 1990). Bacha and Hossner (1977) reported a ratio of 43:1 (Fe:Mn) in plaque of rice roots under laboratory conditions. In the present investigation, the ratio of amounts of Fe to Mn on the root is about 190:1. The different ratio may depend on different conditions for inducing plaque formation.

Arsenic concentrations in Fe plaque on the roots treated with arsenate were significantly higher than with arsenite. This supports our previous conclusion that Fe plaque has stronger affinity to arsenate than to arsenite (Liu et al., 2004a, b). Hansel et al. (2002) also showed that arsenate and arsenite co-exist in Fe plaque on the root surface of two common aquatic plant species, reed canarygrass (*Phalaris arundinacea*) and cattail (*Typha latifolia*), being comprised predominantly of arsenate (82%) with lesser amounts (18%) of arsenite–Fe (hydro-) oxide complexes, although the predominant species of arsenic is arsenite in flooded soil. In paddy soil this difference also applies to chemical properties of As in soil, where both arsenite and arsenate are adsorbed on Fe oxide surfaces but have very different adsorption behaviors depending on oxidation state, the mineralogy of the iron oxides and pH values (Manning and Goldberg, 1997; Pierce and Moore, 1982). According to the acid dissociation constants of H_3AsO_4 and H_3AsO_3, the predominant species of arsenate at pH 4.6 and 9.2 are $H_2AsO_4^-$ and $HAsO_4^{2-}$, respectively. The predominant species of arsenite are $H_3AsO_3^0$ at pH 4.6 and approximately an equimolar mixture of $H_3AsO_3^0$ and $H_2AsO_3^-$ at pH 9.2 (Sadiq et al., 1983). Dixit and Hering (2003) reported that sorption of arsenate onto amorphous iron oxide and goethite is more favorable than that of arsenite below pH 5–6, whereas above pH 7–8.5, arsenite has a higher affinity, which agrees with our results.

Arsenic concentrations in DCB-extracts of rice roots with iron plaque are higher than those of Mn plaque and no plaque when arsenite or arsenate was supplied. There are some possibilities to explain this result. First, DCB-extracts not only removed coatings on the root surface, but also extracted some elements accumulated in the apoplast of roots (Otte et al., 1989; Strasser et al., 1999). Although no plaque is formed on the root surface for controls, Fe, Mn and As in the apoplast would still be extracted by DCB solution. The amounts of Fe, Mn and As extracted in Mn plaque and no plaque treatments were less than in Fe plaque treatment (Tables 2, and 3). Second, it may be that Fe plaque not only adsorbs arsenate, but also sequesters arsenite (Dixit and Hering, 2003; Hansel et al., 2002). In our experiment, the pH was 5.5 in the nutrient solution, and may have been lower at the root surface due to organic acid excretion or imbalance of ion uptake. If so, the predominant species of arsenite would be $H_3AsO_3^0$ (Sadiq

et al., 1983). It is more likely that arsenite could be partly oxidized to arsenate, which has higher affinity for iron plaque, co-precipitates with Fe(III) and adsorbs to plaque.

There are a number of reports showing that Fe plaque can act as a barrier to the uptake of toxic metals (Batty et al., 2000; Chen et al., 2005; Christensen and Sand-Jensen, 1998; Greipsson, 1994, 1995; Otte et al., 1991; Zhang et al., 1998). The present study showed that specific uptake of As by rice roots with Fe plaque was significantly lower than in roots with no plaque when arsenate was supplied. Mn plaque also decreased SAU compared to controls (no plaque), but the effect was much less obvious (Figure 1). This suggests that plaque on the root surface indeed is a major barrier to arsenate uptake. Therefore, the effect of Fe plaque on the uptake of arsenate by rice plants is important for understanding the accumulation and metabolism of arsenic by rice and for the development of practical approaches to reducing As accumulation. However, some investigations have shown that the formation of Fe plaque did not inhibit the uptake of heavy metals (Ye et al., 1997, 1998), which agrees with the effect of root plaque on arsenite uptake by rice roots in the present study. It is clear that there was no significant difference in SAU for arsenite between treatments with Fe, Mn plaques and no plaque (Figure 1). This may be because the root surface pH was lower than 5.5 and the predominant species of arsenite was $H_3AsO_3^0$ (uncharged). The transport of this As species into roots might be via aquaporins (Meharg and Jardine, 2003). In this case, the effect of Fe plaque on arsenite uptake would be small compared to that on arsenate uptake. Iron plaque is thus not a barrier to arsenite uptake.

One may also argue that substantial amounts of arsenite may be oxidized to arsenate during the 72 h nutrient change intervals, our results did show that arsenite could be oxidized to arsenate in the nutrient solution at room temperature. However, the amounts of arsenate formed during the nutrient change interval were not substantial (Figure 3). The oxidation could be mediated by microbial activities in the nutrient solution. Cullen and Reimer (1989) reported that sterile water samples were less susceptible to speciation changes than non-sterile samples. Laboratory studies also showed that the kinetics of oxygenation of arsenite were slow in the slightly acid range, around pH 5 (Eary and Schramke, 1990).

In conclusion, our results demonstrated that the role of Fe plaque on uptake and translocation of arsenite and arsenate is different. The Fe plaque acted as a ''buffer'' to arsenate accumulation in rice plants. However, when arsenite was supplied the main barrier to As uptake may be the root tissue, rather than Fe plaque. Furthermore, under our experimental conditions, the amounts of Mn plaque formed on the root surface was much less than those of Fe plaque, and Mn plaque did not exert any measurable alteration of As accumulation in rice plants irrespective of the arsenic species. Future more realistic experiments to examine the effects of the mixture of Fe/Mn plaque formed on root surface of rice plants grown from different soil types may be warranted.

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