Effect of Iron (Fe2+) Concentration in Soil on Arsenic Uptake in Rice Plant (Oryza sativa L.) when Grown with Arsenate [As(V)] and Dimethylarsinate (DMA)

Effect of Iron (Fe^{2+}) Concentration in Soil on Arsenic Uptake in Rice Plant (*Oryza sativa* L.) when Grown with Arsenate [As(V)] and Dimethylarsinate (DMA)

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Abstract

Being inorganic arsenicals predominant, methylarsenicals also occur in anaerobic paddy soils. Therefore, this study investigated the influence of Fe^{2+} concentrations and arsenic speciation (arsenate; As(V) and dimethylarsinate; DMA) in paddy soils on arsenic uptake in rice plant. Rice seedlings were grown in soil irrigated with a Murashige and Skoog (MS) growth solution containing As(V) or DMA with or without 1.8 mM Fe^{2+} in excess to the background concentration of total Fe (0.03 mM) in the soil. Arsenic concentration in rice roots increased initially and then decreased gradually when the seedlings were grown with excess Fe^{2+} and As(V). In contrast, arsenic concentration in the roots increased steadily ($P < 0.01$) when the seedlings were grown without excess Fe^{2+} and As(V). When the form of the arsenic was DMA, total arsenic (tAs) concentration in rice roots increased gradually ($P < 0.01$), and was not affected by the addition of excess Fe^{2+} in the soil. When rice seedling was grown with As(V), tAs concentration in rice roots and shoots increased steadily ($P < 0.01$) for gradual increase of Fe^{2+} concentrations in soil. However, tAs concentration in roots and shoots was independent of Fe^{2+} concentrations in soil when the form of arsenic was DMA. The tAs concentrations in rice shoots also increased significantly ($P < 0.01$) with increasing exposure time for both As(V) and DMA. Thus, Fe^{2+} concentrations in soil affects arsenic uptake in rice plant depending on the speciation of arsenic.

Keywords: Arsenate [As(V], Dimethylarsinate (DMA), Ferrous iron (Fe^{2+}), Rice seedling, Soil.

Introduction

Arsenic is a toxic environmental pollutant that has chronic and epidemic effect on humans through widespread water and crop contamination from geogenic sources in Bangladesh (Hossain, 2006; Smith et al., 2000) and West Bengal (India) (Chowdhury et al., 2000). Arsenic-contaminated groundwater is used not only for drinking purpose but also for crop irrigation in arsenic-affected Asian countries (Meharg and Rahman, 2003; Ninno and Dorosh, 2001). Groundwater is used extensively to irrigate paddy rice in Bangladesh, particularly during the dry season, and about 75% of the total cropped area is given over to rice cultivation in the country (Meharg and Rahman, 2003). Background levels of arsenic in paddy soils range from 4 to 8 mg kg^{-1}, and can reach up to 57 mg kg^{-1} in areas where the crop land has been irrigated with arsenic-contaminated groundwater (Alam and Sattar, 2000). A recent study has been reported that groundwater irrigated paddy fields in Bangladesh are the net sinks of arsenic from groundwater, and very little arsenic delivered by irrigation returns to the aquifer (Neumann et al., 2011). In Bangladesh, it is estimated that irrigation removes up to 1400 tons of arsenic from the aquifer each year and deposits this arsenic onto paddy fields (Ali et al., 2003). Arsenic levels in soil can also be increased by the use of arsenical chemicals such as lead arsenate ($PbHAsO_4$) and calcium arsenate ($Ca_3(AsO_4)_2$) in agriculture (Murphy and Aucott, 1998). Increasing arsenic levels in agricultural soils leads to the elevation of arsenic in rice, vegetables, and other food crops (Meharg and Rahman, 2003; Williams et al., 2006).

Currently, the mechanisms involved in arsenic accumulation by rice are still poorly understood. Therefore, it is important to understand the environmental, nutritional, and internal/external factors of rice that may play important roles in arsenic uptake. Iron (Fe) is an important nutrient for plants. The precipitation of ferric oxides/hydroxides (Fe-plaques) on the roots of wetland and aquatic plants at neutral or alkaline pH is a common phenomenon (Emerson et al., 1999; Wang and Peverly, 1999).

Fe(III)-oxides/hydroxides on soil particulate or root surfaces of wetland plants adsorb As(V) strongly (Zhao et al., 2010). Due to the formation of Fe-oxides/hydroxides on rice root surface and sequestration of As(V) in the Fe-oxides/hydroxides (Blute et al., 2004; Liu et al., 2006), concentration of Fe^{2+} in the growth medium may affect the As(V) uptake in rice root and subsequent translocation to shoots of rice plant and in grains. Zhao et al. (2010) have recently reviewed the interactions between inorganic arsenic (iAs) and Fe(III)-oxides/hydroxides in uptake by rice plant. However, methylated arsenic compounds such as dimethylarsinate (DMA), monomethylarsonic acid (MMAA) and trimethylarsine oxide (TMAO) are also found in soil as minor components (Huang and Matzner, 2006; Takamatsu et al., 1982). These methylated arsenicals in paddy soils is supposed to be produced from iAs through biomethylation by some soil microorganisms or algae (Bentley and Chasteen, 2002; Takamatsu et al., 1982). Most of the previous studies related to arsenic-Fe interactions in plant uptake have focused on As(V) and arsenite (AsIII) since these are the predominant species in aerobic and flooded (anaerobic) paddy soils, respectively (Meharg and Jardine, 2003; Takahashi et al., 2004). Little is known about the effect of interactions of Fe and methylarsenic species in plant uptake. Therefore, it is imperative and relevant to investigate the uptake of methylarsenic species in rice plant in relation to the Fe concentrations in soil.

In views of the adsorption of As(V) by Fe(III)-oxides/hydroxides on rice roots (Zhao et al., 2010), the occurrence of DMA in anaerobic paddy soils from microbial methylation (Takahashi et al., 2004), and the existence of both As(V) and DMA in rice tissues (Abedin et al., 2002; Marin et al., 1993), the objective of this study was to investigate the effect of Fe^{2+} on As(V) and DMA uptake in rice roots and their translocation to shoots. This study will help out in understanding the role of Fe nutrient on the uptake of iAs and methylarsenicals in rice shoots and grain.

Materials and Methods

Seed sterilization

Rice seeds of BRRI dhan28 verity were collected from the Bangladesh Rice Research Institute, Gazipur-1700, Bangladesh. The seeds were surface-sterilized before use in the experiment. For surface sterilization, about 100 g seeds were soaked in 200 mL of 1% methyl-1-butylcarbamoyl-2-benzimidazole carbonate solution for 10 min. Seeds were then washed with deionized (DI) water and soaked in DI water at 20 ºC for 24 h, and then at 45 and 52 ºC for 2 and 10 min, respectively.

Experimental setup and chemical treatments

Sterilized rice seeds were then soaked in DI water for 48 h, and were germinated on moistened filter paper placed in Petri-dishes. After 10 d when the germinated seeds produced sufficient roots and about 2 cm of shoots, they were transplanted in 500-mL polystyrene test vessels (130mm×90mm×70mm) containing 200 g soil. The chemical composition of the soil was: SiO_2 (95.5%), Al_2O_3 (2.3%), Fe_2O_3 (0.2%), CaO (0.02%), MgO (0.08%). In the soil, the background concentration of phosphorus was 5.8 mg kg^{-1} and arsenic concentration was below the instrumental limit of detection (0.01 µg L^{-1} in water). The particle size of the soil was 0.42-0.60 mm (24%) and 0.30-0.42 mm (60%). Before seedling transplantation, the soil was flooded with modified Murashige and Skoog (MS) nutrient solution (Murashige and Skoog, 1962) without Fe nutrition (Table 1). The medium was then autoclaved in order to ensure that there was not microorganism in the medium, and microbial methylation/demethylation did not occurred during the growing period of the rice seedlings. The background concentration of Fe in the soil was 0.03 mM. About 20 germinated seeds of 10 d were transplanted in each vessel, and the seedlings were allowed to grow for 10 d. Seed germination, growth of rice seedlings and subsequent steps of the experiments were performed in Japan in the laboratory.

The conditions in the plant growth chamber were set to 14:10 h light/dark schedule and 100-125 µE m^{-2} s^{-1} light intensity at 22(±2) ºC.

After growing rice seedlings for 10 d in test vessels, the seedlings were about 10 cm with sufficient root systems. To investigate the effects of exposure time and Fe^{2+} concentrations on As(V) and DMA uptake in rice, seedlings were grown in soil irrigated with MS culture solution modified in Fe and phosphate concentrations. About 1.08, 2.16 and 4.32 mM of iron was added to the modified MS culture medium for concentration-dependant uptake experiment, while its concentration in the MS solution was 1.80 mM for time-dependant uptake experiment. Phosphate was not used in this study so that it could not influence arsenic uptake. The pH of the soil was maintained to 6.5 using buffer solution.

To investigate the effect of Fe^{2+} on time-dependent arsenic uptake, rice seedlings of 10 d were continued to grow in the test vessels with (1.80 mM) or without excess Fe^{2+} (in addition to its background concentration in soil) in the soil. As(V) and DMA of 0.3 mM were applied to the soil by dissolving $Na_2HAsO_4 \cdot 7H_2O$ and $(CH_3)_2AsO_2Na \cdot 3H_2O$, respectively, in the MS solution. Samples were collected every 2 h with three replications. Arsenic concentrations in rice roots was determined over a 10-h period, and the samples were collected every 2 h. Total arsenic in roots was measured on a dry weight (d. wt.) basis.

In concentration-dependent uptake experiment, rice seedlings of 10 d were continued to grow in the test vessels by adding 0 (control), 1.08, 2.16 and 4.32 mM of Fe^{2+} to the soil by dissolving $FeSO_4 \cdot 7H_2O$ in the MS solution. 0.3 mM of As(V) or DMA was applied to the soil by dissolving salts or the respective species in the MS solution.

Extraction of Fe-plaque from rice root surfaces

After growing the rice seedling in this condition for 5 d, samples were collected randomly with three replications (3 seedlings from 3 vessels of same treatments). Rice seedlings were uprooted by hand and the plants were washed by DI water to remove all the external particles from the root surface. Fe-oxides/hydroxides from rice root surfaces were extracted by citrate-bicarbonate-ethylenediaminetetraacetate (CBE) solution (Rahman et al., 2008a). In CBE-extraction technique, rice roots were incubated in 10 mL of CBE solution for 60 min at room temperature, and washed with DI water for three times. The CBE solution was prepared from 0.03 M, 0.125 M and 0.050 M of sodium citrate, sodium bicarbonate and EDTA, respectively. CBE-extraction of Fe-oxides/hydroxides was performed to remove arsenic that was adsorbed on Fe-hydroxides of the rice root surface.

Digestion and chemical analysis of plant samples

All chemical reagents used were of analytical grade. Glassware and dishes were washed with detergent (3 times) followed by 5 M HCl solution (3 times), and rinsed with deionized water (10 times) before use them in the experiment.

The roots were rinsed with DI water, and blotted dry with tissue paper. The roots were then excised at the basal node to separate roots from shoots, and the samples were oven dried at 65 ºC for 48 h. After measuring dry weight (d. wt.) of roots and shoots, the samples (approximately 0.25 g) were taken into 50-mL polyethylene digestion tubes. About 3 mL of concentrated (65%) nitric acid (HNO_3) was added to the samples and they were allowed to stand overnight. The samples were then heated on a heating block at 95 ºC for 90 min. After cooling to room temperature, 2 mL of 30% hydrogen peroxide (H_2O_2) was added and heated again at 105 ºC for 30 min. The residues were diluted to 10 mL with DI water, and analyzed for total arsenic by graphite-furnace atomic absorption spectrometer (AAnalyst 600, Perkin Elmer, Germany).

QC/QA for arsenic analysis

In order to check the precision of arsenic analysis in the samples, two reagent blanks and a certified standard reference materials (SRM 1573a, tomato leaf from NIST, USA) were included in every analytical batch. The certified value of arsenic in SRM was 0.112±0.004 µg g^{-1} d. wt., while the measured value was 0.124±0.006 µg g^{-1} d. wt. The concentrations detected in all samples were above the instrumental limits of detection (≥ 0.01 µg L^{-1} in water).

Statistical analysis

Analysis of variance (ANOVA, one-way) on arsenic and Fe concentrations was performed using SPSS (v15.0 for windows), and regression analysis of the data was performed by GraphPad Prism (v5.0 for windows).

Results

Effect of excess Fe^{2+} in soil on As(V) and DMA uptake in rice roots

In short term experiment (10 h), total As (tAs) concentration in rice roots increased initially and then decreased gradually ($P > 0.01$) with growing time when the seedlings were grown with As(V) and 1.80 mM of Fe^{2+} in addition to its background concentration in soil (0.03 mM) (Fig. 1A). In contrast, when the seedlings were grown with As(V) without excess Fe^{2+}, tAs concentration in rice roots increased steadily ($P < 0.01$) with increasing growing time (Fig. 1A). Despite the addition of excess 1.8 mM Fe^{2+} in the soils, tAs concentration in rice roots was increased ($P < 0.01$) with growing time for DMA (Fig. 1B). Results showed that arsenic uptake in rice roots was influenced significantly for the

addition of excess Fe^{2+} and As(V), while arsenic uptake was independent of the addition of excess Fe^{2+} in soils for DMA.

When the form of arsenic was As(V), the tAs concentration on rice root surfaces was related to the amount of Fe-oxides/hydroxides on rice root surfaces (apoplastic Fe). The tAs concentration was significantly higher on roots of rice seedlings grown with As(V) and excess Fe^{2+} than that grown with As(V) and without excess Fe^{2+} (Fig. 2A). In contrast, tAs concentration on rice root surfaces was not influenced by the addition of Fe^{2+} in soil (Fig. 2B). Regardless of the addition of excess Fe^{2+} in soil, concentrations of Fe and arsenic on rice root surfaces increased significantly with growing time for As(V) treatment while tAs concentration did not increase for DMA treatment (Fig. 2). Fe concentrations were highly correlated (r^2 = 0.909 and 0.711 for 'with excess' and 'without excess' Fe^{2+}, respectively; $P < 0.01$) with tAs concentration on rice root surfaces for As(V) treatment (Figs. 3A, B) indicating that the formation of Fe-oxides/hydroxides on rice root surfaces, and that the adsorption of As(V) on the Fe-oxides/hydroxides increased with increasing growing time. However, concentrations of Fe and tAs on rice root surfaces were not correlated for DMA treatment (r^2 = 0.551 and 0.447 for 'with excess' and 'without excess' Fe^{2+}, respectively; $P > 0.01$) (Figs. 3C, D).

Rice seedlings of 10 d were grown for extended time (5 d) in soils irrigated with MS solution containing 0.3 mM of As(V) or DMA and different concentrations of Fe^{2+} (0, 1.08, 2.16 and 4.32 mM) in addition to its background concentration of 0.03 mM to investigate the effect of increasing Fe^{2+} concentrations on arsenic uptake in rice roots. The concentration of tAs in rice roots increased significantly ($P < 0.01$) with increasing Fe^{2+} concentrations in soil for As(V) treatment, while its concentration was not increased with increasing Fe^{2+} concentrations in soil for DMA treatment (Fig. 4).

Effect of excess Fe^{+2} in soil on As(V) and DMA uptake in rice shoots

Regardless of the As species and the addition of excess 1.8 mM Fe^{2+} in soil, tAs concentrations in rice shoots increased significantly ($P < 0.01$) with increasing growing time (Fig. 5) indicating that arsenic speciation and Fe^{2+} concentrations in soil affect arsenic uptake in rice shoots. The concentration of tAs in rice shoots were more than four-times higher for As(V) treatment compared to that for DMA. When rice seedlings were grown for extended time (5 d) with different concentrations of Fe^{2+} in soil, tAs concentrations in rice shoots increased significantly ($P < 0.01$) with increasing Fe^{2+} concentrations in soil for As(V) treatment, while tAs concentration did not increase significantly ($P > 0.01$) for DMA treatment (Fig. 6).

Discussions

Effect of excess Fe^{2+} in soil on As(V) uptake in rice roots

Although As(V) enters into rice roots through phosphate uptake pathway due to their similar physicochemical properties (Liu et al., 2004b; Rahman et al., 2008a; Rahman et al., 2008b; Wang et al., 2002), it has high adsorptive affinity to Fe-oxides/hydroxides (Chen et al., 2005). In paddy soils, Fe-oxides/hydroxides on rice root surfaces (Chen et al., 2005; Liu et al., 2006; Liu et al., 2004a) as well as on soil particles (Zhao et al., 2010) decrease the concentration of bioavailable fraction of As(V) in the soil that may results in low arsenic influx in rice roots. In this study, the initial increase followed by gradual decrease of the tAs concentration in roots of rice seedlings grown with excess Fe^{2+} and 0.3 mM As(V) (Fig. 1A) was related to Fe and arsenic concentrations on rice root surfaces (Figs. 3A, B). Irrespective of the addition of excess Fe^{2+} in soil, Fe concentration on rice root surfaces was low initially that might not be able to absorb high amount of As(V). Therefore, arsenic uptake in rice roots increased initially. Fe concentration on rice root surfaces increased steadily ($P < 0.01$) with growing time, and the concentration of Fe on root surface was significantly higher ($P < 0.01$) when the rice

seedlings were grown with access Fe^{2+} than without access Fe^{2+} (Fig. 2C). This result indicates that the amount of Fe-oxides/hydroxides on the root surfaces increased with growing time for the addition of excess Fe^{2+} in the soils. It has been reported that heavy metal and nutrient uptake in rice is related to the amount of Fe-plaque (Fe-oxides/hydroxides) on the roots surfaces (Zhang et al., 1998). In this case, increase of Fe-oxides/hydroxides on rice roots with growing time and the subsequent adsorption of As(V) on the Fe-oxides/hydroxides decreased arsenic uptake in rice roots. This result is in consistent with the finding of Rahman et al. (2011) that Fe concentrations in culture solution decrease arsenic uptake in hydroponic rice roots when rice seedling was grown with As(V).

The concentration of tAs in rice roots increased steadily with increasing growing time when rice seedlings were grown without excess Fe^{2+} and As(V) (Fig. 1A). This was related to the amount of Fe-oxides/hydroxides on rice root surfaces. Fe and tAs concentrations on root surfaces of rice seedlings grown without excess Fe^{2+} was significantly lower than that grown with excess Fe^{2+} (Figs. 2A, C). This might be because the amount of Fe-oxides/hydroxides was less and the bioavailable fraction of As(V) was high in the soil rhizosphere under Fe^{2+}-limited condition. The tAs concentration in rice roots increased significantly ($P < 0.01$) when the rice seedlings were treated with As(V) and different concentrations of Fe^{2+} (1.08, 2.16 and 4.32 mM for extended time (5 d) (Fig. 4). This result is not in consistent with the result of short-term uptake study (Fig. 1A) indicating that Fe concentration in soil affects arsenic uptake in rice roots differently for growing time (short-time or long-time).

Arsenic uptake mechanisms in rice is complex because of the ability of rice plant to carry oxygen from the aerial parts down to its stem and discharge the oxygen in the rhizosphere through the roots (Brammer and Ravenscroft, 2009). This creates an oxidized zone around the roots in which Fe is oxidized and precipitated to forms a coating of Fe-oxides/hydroxides on rice roots (Liu et al., 2006). Therefore, increasing Fe^{2+} concentrations in soil increased the formation of Fe-oxides/hydroxides on

the rice root and soil particulate surfaces, serving as a strong adsorbent for As(V). Increased arsenic uptake in rice roots for increasing Fe^{2+} concentrations can be explained by As(V) mobilization in soil rhizosphere. Previous studies have shown that two mechanisms are involved in the mobilization of As(V) in the soil rhizosphere; i) reduction of As(V) to As(III) followed by desorption from the adsorption surfaces of Fe-oxides/hydroxides into the solution phase, and ii) reductive dissolution of Fe-oxides/hydroxides from rice roots to the solution (Takahashi et al., 2004) mediated by phytosiderophores.

As(V) is reduced to As(III) under the anaerobic (reducing) environment in the soil rhizosphere (Xu et al., 2007), which might be created for grown rice plant for extended time under waterlogged condition. As(III) is more bioavailable than As(V) (Zhu et al., 2008), and therefore, As(III) is readily taken up by rice plant that increased the tAs concentration in rice roots. In reducing condition, Fe bioavailability and uptake in rice roots decreased for the increase of Fe-oxides/hydroxides in the soil rhizosphere that results Fe-deficiency. Being the strategy-II plant, rice roots exude phytosiderophores in the rhizosphere soil under the Fe-deficient condition to increase Fe bioavailability and uptake (Ishimaru et al., 2006; Romheld and Marschner, 1986). Some rhizospheric microbes also solubilise precipitated Fe^{3+} in the rhizosphere to enhance Fe bioavailability and uptake in rice by exuding siderophores to the root-plaque interface (Bar-Ness et al., 1992; Crowley et al., 1992; Kraemer, 2004). In addition to this reductive dissolution of Fe-oxides/hydroxides by microbial siderophores and phytosiderophores under the reducing environment, phosphate fertilizer has also been reported to decrease the amount of Fe-oxides/hydroxides on rice roots (Hu et al., 2005). Decrease of the amount of Fe-oxides/hydroxides in the soil rhizosphere by reductive dissolution of the Fe-oxides/hydroxides or phosphate fertilizer releases the adsorbed As(V) and enhances arsenic bioavailability and uptake in rice plant (Zhao et al., 2010).

The increase of arsenic uptake for the addition of Fe in the growth medium could possibly be explained by the influence of Fe in up-regulating the phosphate transporter. Ward et al. (2008) clearly showed that the addition of Fe up-regulate phosphate transporter in *Arabidopsis*. Similar situation could be in rice. The increase of tAs by Fe treatment could be due to the up-regulation of phosphate transporter since Phosphate and As(V) are homolog, and As(V) is also taken up by plants through the same transporter (Wang et al., 2002).

Arsenic uptake in rice roots was not influenced significantly ($p > 0.01$) by different Fe^{2+} concentrations in soil when the seedlings were grown with DMA (Fig. 2B). This might be due to the fact that DMA is transported through aquaglyceroporin (Rahman et al., 2010). Poor correlation (r^2 = 0.551 and 0.447 for 'with excess' and 'without excess' Fe^{2+}, respectively) between tAs and Fe concentrations on root surfaces (apoplastic) of DMA-treated rice seedling (Figs. 3C, D) also reveals that there is no biochemical interaction between DMA and Fe. Therefore, Fe^{2+} concentrations in soil do not affect DMA uptake in rice roots.

Effect of excess Fe^{+2} on DMA uptake in rice roots

Although As(III) is the major species in paddy soils, DMA was also found in small quantities (Abedin et al., 2002; Takamatsu et al., 1982), and the uptake of DMA in rice has been reported in previous studies (Abedin et al., 2002; Marin et al., 1993). Irrespective of the addition of Fe^{2+} in soils, tAs concentration in rice roots increased significantly with time ($p < 0.01$) for DMA (Fig. 1B). This might be because DMA uptake in rice roots is independent of Fe^{2+} concentrations in growing medium (Rahman et al., 2011), and can be explained by its uptake mechanisms in plants. Kinetic studies of DMA influx in rice roots showed that the glycerol transporter (aquaglyceroporin) in root plasma membrane favors DMA uptake (Rahman et al., 2010), and rice aquaporin *Lsi1* is also supposed to

mediate the uptake of un-dissociated pentavalent DMA (Li et al., 2009). In addition, Fe-hydroxide on plant root surfaces does not favour DMA adsorption (Rahman et al., 2008a; Rahman et al., 2008b). Thus, the uptake mechanisms of DMA, and the biochemistry of DMA with Fe-oxides/hydroxides in the soil rhizosphere explain the influence of Fe on DMA uptake in rice roots adequately.

The present study also showed that, regardless of the addition of excess Fe^{2+} in soil, tAs concentration in rice roots was significantly lower for DMA than that for As(V) (Fig. 1). The tAs concentration in roots of DMA-treated rice seedling was about one-third than in roots of As(V)-treated rice seedling. This result is in consistent with the findings of Rahman et al. (2011) in rice plant. Raab et al. (2007) compared arsenic uptake by 46 plant species by exposing to 13.3 μM As(V) or DMA for 24 h, and found that the plants, on average, took up about a fifth of the amount of DMA compared with As(V) uptake.

Effect of excess Fe^{+2} in soil on As(V) and DMA uptake in rice shoots

The short-term uptake study showed that tAs concentration in rice shoot did not differ significantly for growing rice seedlings with or without excess Fe^{+2} and As(V) (Fig. 5A). This result agrees with the previous findings for hydroponic rice (Liu et al., 2004a, b), and also for other wetland plant species (Christensen and Sand-Jensen, 1998; Greipsson, 1994, 1995). Despite the fact that increasing amounts of Fe-plaque increase tAs accumulation on the root surfaces, they did not affect arsenic concentrations in rice shoots (Liu et al., 2004a). The result indicates that Fe-plaque may act as a 'barrier' to decrease arsenic translocation from roots to shoots. Nevertheless, the role of Fe^{2+} concentrations in altering the translocation of arsenic from root to shoot may depend on the plant species, arsenic speciation, and exposure time. The present study showed that, tAs concentration in rice shoots increased significantly ($p < 0.01$) with increasing Fe^{2+} concentrations in soil when the seedlings

were grown with As(V) for extended time (5 d). However, tAs concentration in rice shoots did not increase significantly ($p > 0.01$) when the seedlings were grown with DMA for the same duration (Fig. 6). Regardless of arsenic species and Fe concentrations in soil, tAs concentrations in the shoots increased gradually with increasing growing time (Fig. 5). Thus, it can be hypothesized that arsenic speciation and exposure time are important determinants that may influence the effect of Fe^{+2} concentrations on arsenic translocation in rice shoots.

Irrespective of the Fe^{2+} concentrations in soil, tAs concentration was about 10-times lower in rice shoots of DMA-treated seedlings than in shoots of As(V)-treated seedlings (Fig. 5). The result indicate that As(V) translocation from roots to shoots of rice plant is higher than that of DMA. As(III) was found to be the main form of arsenic in xylem sap when rice was grown hydroponically with As(V) indicating that As(V) is rapidly reduced to As(III) inside rice roots (Xu et al., 2007). The As(III) is then taken up in rice roots mainly by *Lsi1*, a silicon influx transporter (Ma et al., 2008). *Lsi2*, another silicon influx transporter localized at the proximal side of both exodermis and endodermis cells of rice roots, is involved in the efflux of As(III) toward the xylem (Ma et al., 2008). *Lsi2* plays a significant role in arsenic transport from roots to shoots and ultimately to the grain in rice (Ma et al., 2008). This mechanisms of As(III) uptake also explain why iAs concentration in rice shoots and grains is higher than methylarsenic species.

Although DMA is traslocated from plant's roots to shoots more efficiently than MMA and iAs species (transfer factors for As(V) and DMA were 0.18 and 1.8, respectively) (Raab et al., 2007), the present study showed that translocation of tAs from root to shoot of rice plant grown with As(V) is about 10-times higher than that grown with DMA. The reasons for limited uptake and translocation of DMA in rice shoots are unclear. However, Li et al. (2009) showed that most of the methylarsenic species are dissociated at the cytoplasmic pH (approximately 7.5), and the negatively charged methyl

As may be transported to the xylem via a pathway which is different from that of As(III) (*Lsi2* transporter). Thus, lack of involvement of *Lsi2* is in the transport of methylarsenic species would be a reason for the inefficient translocation of DMA from roots to shoots of rice plant. Whatever the reasons and mechanisms are for inefficient translocation of DMA, the present study showed that Fe^{2+} concentrations in soil do not affect the translocation of As(V) and DMA from roots to shoots of rice plant.

Conclusion

Fe-oxides/hydroxides in paddy soils influence arsenic dynamics in rice plant by changing arsenic solubility, retention, and release in the soil rhizosphere. Fe-plaque on rice roots serves either as a source or as a sink for arsenic depending on specific localized conditions and arsenic speciation. At high Fe concentration in soil, As(V) is taken up by rice roots rapidly during the initial growth phase. However, an oxidised condition is created in the soil rhizosphere with increasing growing time due to radial loss of O_2 from root aerenchyma (Colmer, 2003). This oxidised condition enhances the formation of Fe- oxides/hydroxides on rice root surfaces that adsorbed a significant amount of the As(V) in the rhizosphere. As a result, As(V) uptake in rice roots decrease. However, reductive dissolution of Fe-oxides/hydroxides by microbial siderophores and phytosiderophores released by rice plant may increase As(V) bioavailability in the rhizosphere and uptake in rice plant. Thus, Fe concentration in soil plays important role in controlling the bioavailability and uptake of iAs species in rice plant. Hypothetically, increasing concentration of Fe in paddy soils through irrigation with Fe-rich groundwater in South Asian countries would decrease iAs uptake in rice, but the scenario is opposite. The concentrations of iAs species in rice straw and grain from arsenic contaminated South Asian countries like Bangladesh and West Bengal, where irrigation water is rich in Fe, have been reported to

be higher than those from other countries. This might be due to the involvement of *Lsi2* in arsenic transport from roots to shoots and to the rice grain. On the other hand, Fe-rich irrigation water does not affect methylated arsenic uptake in rice.

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Table 1: Composition of modified Murashige and Skoog (MS) nutrient solutions used to grow rice seedlings (*Oryza sativa* L.) in experimental soil

Nutrient elements	**Concentrations (mg L^{-1})**
Macronutrients	
NH_4NO_3	1650
KNO_3	1900
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
KH_2PO_4	170
Micronutrients	
KI	0.83
H_3BO_3	6.20
$MnSO_4 \cdot 4H_2O$	22.30
$ZnSO_4 \cdot 7H_2O$	8.60
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
Iron source	
$FeSO_4 \cdot 7H_2O$	Modified*
$Na_2EDTA \cdot 2H_2O$	37.2
pH	6.5

*Fe concentrations in the MS nutrient solution were 0, 1.08, 2.16, and 4.32 mM for the concentration-dependant uptake experiment, while its concentration in the MS solution was 1.80 mM for time-dependant uptake experiment.

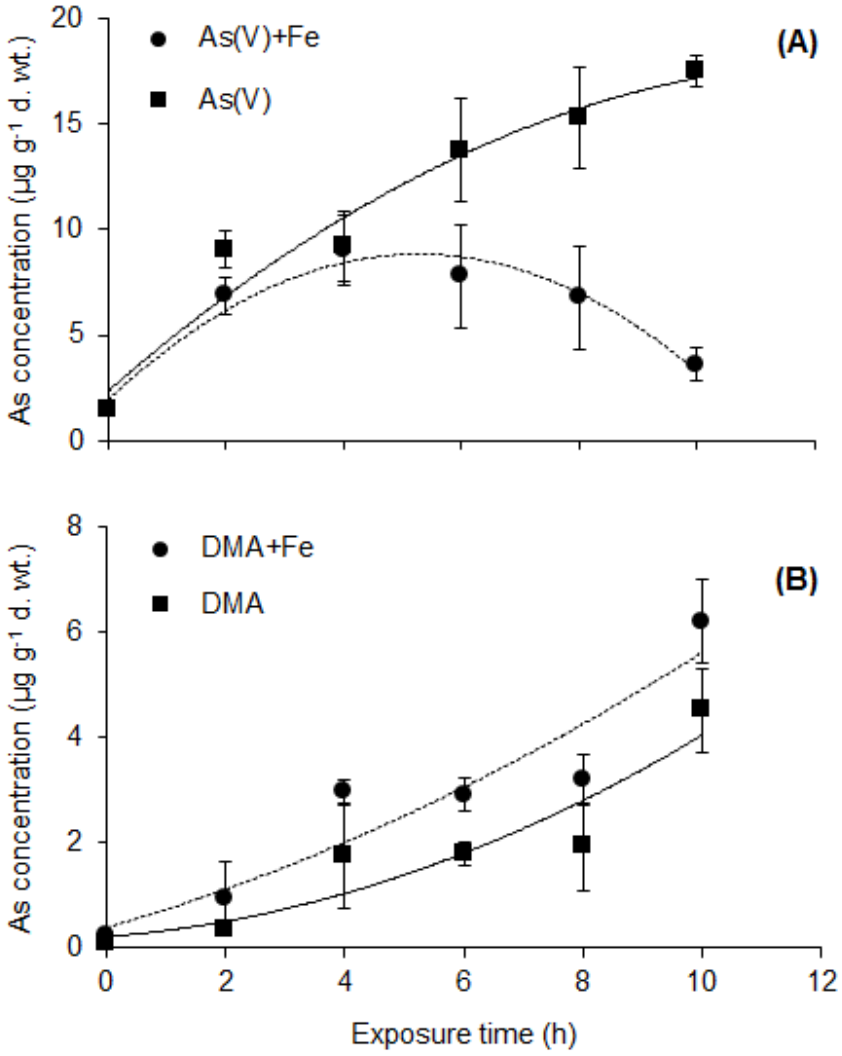

Fig. 1: Time course of arsenic uptake in rice roots affected by additional Fe^{+2} and arsenic speciation. As(V) **(A);** and DMA **(B)**. [●] with excess Fe^{+2} (Fe = 1.83 mM); [■] without excess Fe^{+2} (Fe = 0.03 mM). The soil was irrigated with MS growth solution containing 0.3 mM arsenic. Values are mean ± SD (n = 3).

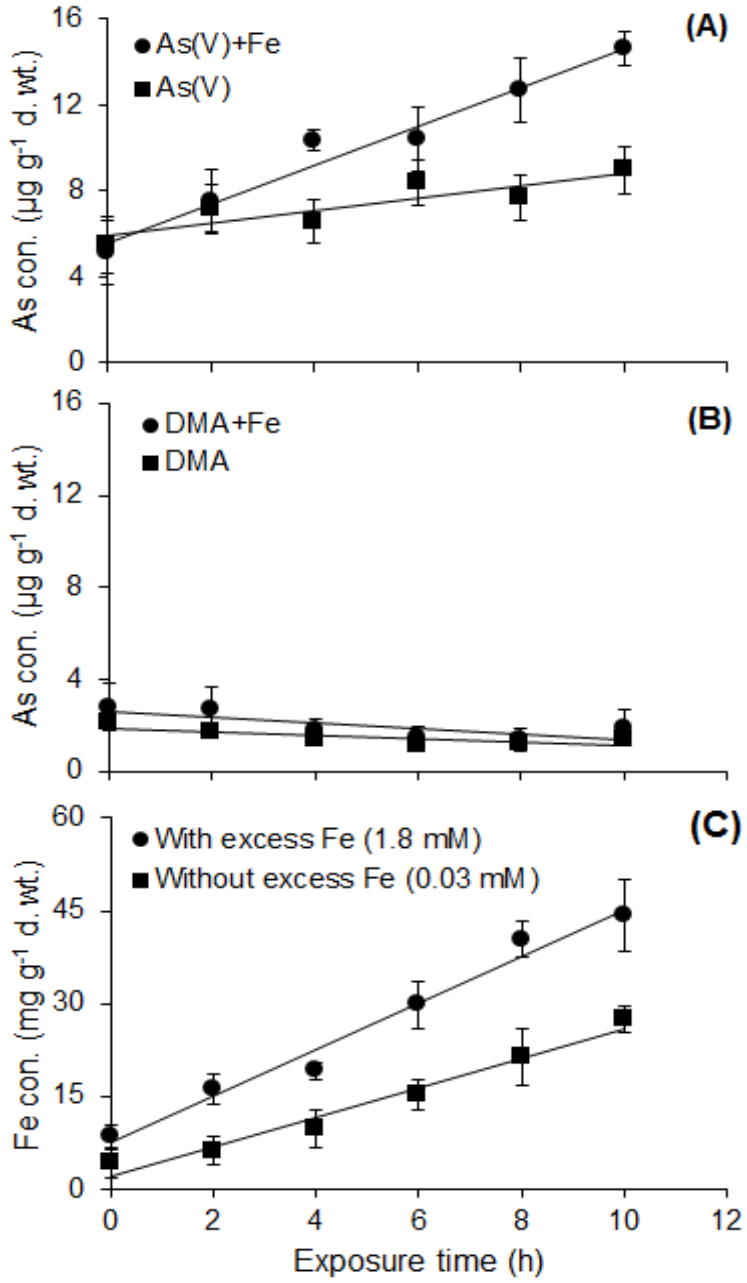

Fig. 2: Time course of arsenic concentrations on rice root surface (apoplastic) as influenced by additional Fe^{+2}. As(V) **(A)**; DMA **(B);** Fe for the system of DMA **(C)**. The soil was irrigated with MS growth solution containing 0.3 mM arsenic. [●] with excess Fe (Fe^{+2} = 1.83 mM); [■] without excess Fe (Fe^{+2} = 0.03 mM). Values are mean ± SD (*n* = 3).

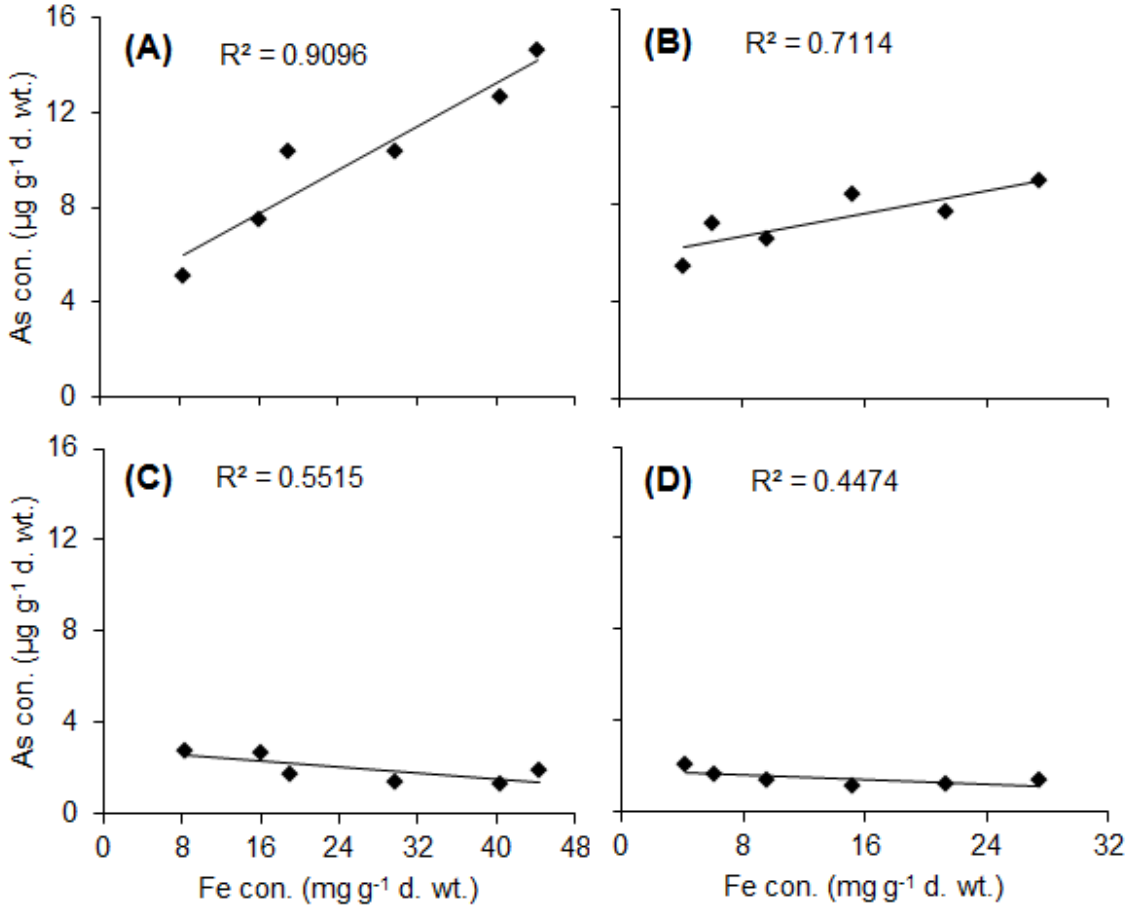

Fig. 3: Correlation between Fe and arsenic concentrations on rice roots surface grown for 10 h with As(V) and additional Fe^{+2} **(A);** with As(V) and without additional Fe^{+2} **(B);** with DMA and additional Fe^{+2} **(C);** with DMA and without additional Fe^{+2} **(D)**.

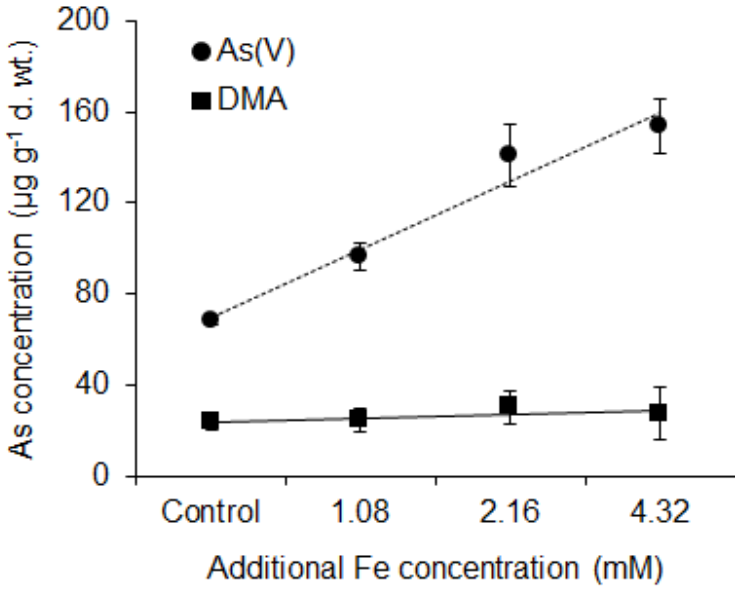

Fig. 4: Influence of Fe^{+2} concentrations and arsenic species in growth medium on arsenic uptake in rice roots. [●] As(V); [■] DMA. The soil was irrigated with MS growth solution containing 0.3 mM arsenic. Rice seedlings were grown for 5 d. Values are mean ± SD (*n* = 3).

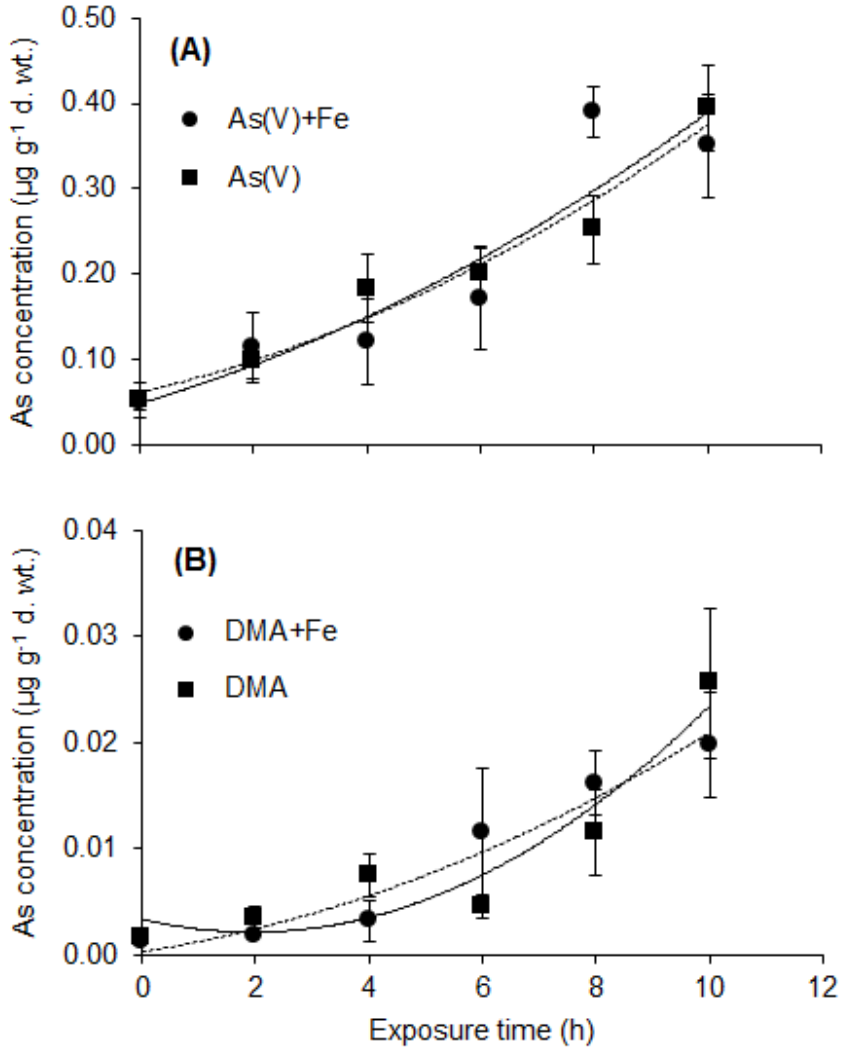

Fig. 5: Time course of As(V) **(A)** and DMA **(B)** concentrations in rice shoots as influenced by additional Fe^{+2} in the growth medium. [●] with additional Fe^{+2} (Fe = 1.83 mM); [■] without additional Fe^{+2} (Fe = 0.03 mM). The soil was irrigated with MS growth solution containing 0.3 mM arsenic. Values are mean ± SD (*n* = 3).

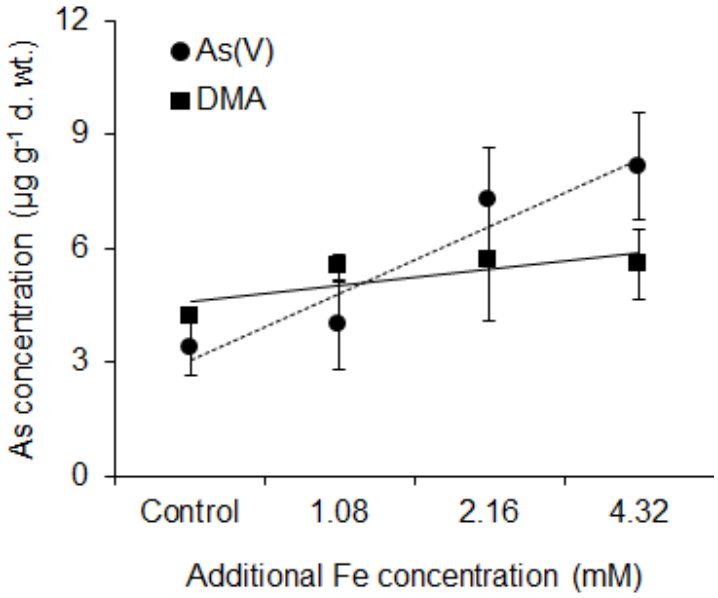

Fig. 6: Influence of Fe^{+2} concentrations and arsenic species in growth medium on arsenic uptake in shoots of rice seedlings. [●] As(V); [■] DMA. The soil was irrigated with MS growth solution containing 0.3 mM arsenic. Rice seedlings were grown for 5 d. Values are mean ± SD (*n* = 3).