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Arsenic Toxicity in Aerated Condition and Its Subsequent Effects on Metal Micro-Nutrients in Hydrponic Rice

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Abstract

Effect of aeration on arsenic (As) and its subsequent effect on metal micronutrients in rice (*Oryza sativa* L. cv. Akihikari) were studied. The treatments were: (1) T1 (control), (2) T2 (aeration), (3) T3 (13.4 µM As) and (4) T4 (13.4 µM As + aeration) for 21 days in the greenhouse. After harvesting, the samples were dried, digested with H_2SO_4-H_2O_2 mixture and iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were measured. Arsenic with aeration (T4) had more toxic effete as compared to As treatment (T3). Arsenic at 13.4 µM (T3) level induced chlorotic symptom in the fully developed young leaves. Iron, Mn and Zn concentrations decreased in shoots but increased in roots at T3 and T4 treatments. Copper concentration decreased in shoots and in roots at T3 and T4 treatments. Translocation of Fe, Mn and Zn; and shoot height and root length decreased at T3 and T4 treatments. In presence of aeration, As-toxicity did not decrease, rather As showed further negative effects on rice growth.

Keywords: Arsenic species, chlorophyll, dry matter yield, iron, shoot height.

Introduction

The toxic metalloid arsenic (As) is found almost everywhere in nature. Though it is toxic but, it has been proved as an essential element for animal. Yet scientists could not prove As is essential for plants (Liebig 1966). High concentrations of As can interfere metabolic process and inhibit plant growth, sometimes may cause cellular death (Sizova *et al.* 2002). Arsenic has been used as pesticides, insecticides, fungicides, herbicides and soil sterilizers (Lepp 1981). It is known to us as phytotoxic element and the people of Bangladesh know As as "*bish*" which can produce cancer. Arsenic-toxicity largely depends on chemical forms rather than their amount present (Woolson *et al.* 1971). The trivalent compounds (arsenite, As^{3+}) are more phytotoxic than the pentavalent form (arsenate, As^{5+}), while both are more toxic than their organic compounds (Ochiai 1985). The uptake of As is ranked as As_{org}>> As^{5+}> As^{3+} for radish (Tlustos *et al*. 1998), however, the toxicity was ranked as As^{3+}> As^{5+}>> As_{org} in same plants (Tlustos *et al*. 1998). The toxicity of arsenate is less than arsenite to roots; therefore, arsenate is easily absorbed and translocated to the shoots (Wauchope 1983). Where non-lethal amounts of arsenate are available, translocation may result in comparable concentration in foliage and in roots (McLean *et al.* 1944).

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It is considered that As calamity in Bangladesh is the largest known mass poisoning in the history, with an estimated 35-77 million people are exposed to As-contaminated underground water (Rabbani *et al*. 2002). Groundwater of different countries like Bangladesh, India, Canada, Japan and Taiwan contains As. People of Bangladesh and India (the neighboring country of the western boarder of Bangladesh) use As contaminated water for drinking, home consumption as well as for irrigation for production of rice, wheat, and vegetables. Arsenic in groundwater is mainly inorganic with arsenate comprising about 50% of the total (Samanta *et al.* 1999) and the remaining portion may be arsenite and others. The groundwater As can be accumulated in soils through irrigation and its subsequent entry into food chain through various food materials (Huq and Naidu 2003) and ultimately enter into human body. In some areas of Bangladesh, where soils receive As-contaminated ground water irrigation, the concentration has been found to be as high as 80 mg L^{-1} (Huq *et al*. 2003). Arsenic contaminated soil is being practiced for crop cultivation, resulting in less crop production due to As-phytotoxicity. Depending on the phytotoxicity, plants can be divided as:- (1) As sensitive plants:- growth is mostly affected at very low concentrations and showing the toxicity symptoms both in shoots and roots i.e- rice and barley; (2) moderately As sensitive plants or As accumulator:- upon absorption, As is mostly concentrated in root without showing toxicity symptoms in shoots i.e.- arum (*Colocasia antiquorum*, Araceae *family*); and (3) As tolerant or hyperaccumulator plants:- upon absorption, As is readily translocated to the aerial parts and As is mostly concentrated in shoot without showing toxicity symptom i.e.- Chinese brake fern (*Pteris vittata* L.). Some vegetable crops like arum and *Amaranthus gangeticus* etc. (Huq *et al*. 2003), kalmi *(Ipomoea reptans*, Convolvulaceae family; Shaibur *et al*. 2009a) and spinach (Shaibur and Kawai 2009) were reported to be As accumulators. In arum, the concentration of As have been found to be as high as more than 150 mg kg^{-1} dry weight (DW; Huq *et al*. 2003).

Extensive experimental results related to As-toxicity in higher plants have been reported e.g. rice (Shaibur *et al.* 2006; Shaibur *et al.* 2008a; Shaibur *et al.* 2011; Shaibur and Kawai 2011a, b), barley (Shaibur *et al.* 2008b; Shaibur *et al.* 2009b, c; Shaibur *et al*. 2012a), sorghum (Shaibur *et al.* 2008c), spinach (Shaibur and Kawai 2009; Shaibur *et al.* 2010), kalmi (Shaibur *et al.* 2009a) and baranuniya (Shaibur *et al*. 2012b, c). We reported that As induced chlorosis in the fully developed young leaves of rice (Shaibur and Kawai 2011a, b; Shaibur *et al.* 2011) and barley (Shaibur *et al.* 2008b; Shaibur *et al.* 2009b; Shaibur *et al.* 2012a). However, As enhanced Fe concentration in the shoots of barley grown in Fe-depleted medium (Shaibur *et al.* 2009c). The analysis report had provided a comprehensive data base for future research on metal-complex or the organic constituents that are produced under As-toxicity.

There are several reports on As distribution and accumulation in plant parts including rice but there are very little information on studies about the effects of aeration on As and its subsequent effect on physiological properties. Therefore, the present study was conducted. The main objectives of this study were to observe the effect of aeration on As and its subsequent effect on physiological properties including metal micronutrients in rice.

Materials and Methods

Seedling preparation: Rice (*Oryza sativa* L. cv. Akihikari) seeds were surface sterilized with 2% chlorinated lime [$Ca(OCl)_2$] for 45 minutes and washed for 1 hour with tap water. After washing, the seeds were wrapped between moistened towels and were kept in a seed growth chamber at 25 ± 2 ˚C for 72 hours. The germinated seeds were transferred on plastic net in a box containing 2% $CaCl_2$ for 9 days. The seedlings were transferred in half-strength nutrient solution for another 9 days.

Seedling cultivation: When the seedlings were suitable for transplantation (18 days after germination, at 3rd leaf stage), the treatments were started with full-strength nutrient solution (Table 1). Each bunch contains 5 seedlings and each bucket (10 liter) containing 16 bunches. The treatments were: (1) T1 (control), (2) T2 (aeration), (3) T3 (13.4 µM As) and (4) T4 (13.4 µM As + aeration) for 21 days. The pH (5.5) was adjusted daily with a digital pH meter and with 1 M HCl and/or 1 M NaOH at around 4 pm during the experiment (22 September, 2004 - 1 November, 2004). We choose this pH because, at this pH iron (Fe) content together with other mineral elements are available for plant growth. Solution was renewed every week. We reported that 13.4 µM As gave the most conspicuous visible results (Shaibur *et al.* 2006). Arsenic was used as sodium meta-arsenite ($NaAsO_2$; Kanto Chemical Company, Tokyo, Japan).

Table 1 Composition of full-strength the modified nutrient solution used in this study (Alam *et al.* 2003).

Salt	Strength	Salt	Strength
NH_4NO_3	1.0 mM	$MnSO_4$	10.0 µM
K_2SO_4	1.0 mM	$CuSO_4$	1.0 µM
$MgSO_4$	0.8 mM	$ZnSO_4$	1.0 µM
NaH_2PO_4	0.5 mM	H_3BO_3	3.0 µM
$CaCl_2$	0.5 mM	H_2MoO_4	0.05 µM
----------	---------	Fe^{3+}-citrate	10.0 µM

Sample collection and preparation: After 21 days after treatments (DAT), 3 bunches (5 seedlings/bunch as 1 replication) were taken for taking physiological data and other 3 bunches were taken for digestion to determine Fe, Mn, Zn and Cu. We concentrated our study

at this stage, where nutrient deficiencies or inhibitory effects likely to be the most apparent and therefore, differences among treatments would be the easiest to observe. The samples were washed with deionized water properly, separated into shoots and roots with sterilized stainless steel scissor and were dried for 48 hours at 55-60˚C in an electric oven (Isuzu Seisakusho Company, Tokyo, Japan). After that, the samples were cut into small pieces and were digested.

Physiological parameter: Chlorophyll in the fully expanded young leaves (5^{th} leaf) was recorded by taking SPAD reading with a SPAD-502 chlorophyll meter (Minolta Camera Company, Tokyo, Japan). We also took the data of DW, shoot height and root length, width of leaf blade, leaf number and tiller number.

Digestion procedure: The individual sample was taken in an acid washed 100 mL glass beaker, 3 mL analytical grade of H_2SO_4 was added, covered with glass coverer, heated at 100˚C for 1.5 hours, at 140˚C for 1.5 hours and at 180˚C for 2 hours on an electric hot plate (model NF-HG 59, National Electronics Company, Tokyo, Japan). The samples were cooled and 2 mL analytical grade of H_2O_2 was added to each sample and heated at 180˚C for 5 hours. The samples were kept for over night for cooling. In the following day, another 3 mL H_2SO_4 and 2 mL H_2O_2 was added to each sample and heated at 180˚C for 9 hours continuously. At the last stage of the digestion, all samples were clear. After cooling, the samples were volumed at 50 mL and were transferred in 50 mL acid washed plastic bottle. This solution was used for Fe, Mn, Zn and Cu determination.

Used reagents: Chemicals used were of analytical reagent grade. Solutions were prepared previously with pure water. Stock solution of As was prepared by dissolving $NaAsO_2$ (Kanto Chemical Company, Tokyo, Japan) in pure water and was kept at room temperature.

Elemental analysis: After digestion, the samples were analyzed for Fe, Mn, Zn and Cu with atomic absorption spectroscopy (AAS; Hitachi 170-30, Tokyo, Japan). Arsenic was measured by Hydride Generation Technique (HG-AAS, Hitachi HFS-3 instrument, Tokyo, Japan; Shaibur and Kawai 2011a, b).

Experiment set up and statistical analysis: The experiment was a completely randomized blocks with 3 replications. Analysis of variance (ANOVA) and the Ryan-Einot-Gabriel-Welsch multiple range test were calculated using SAS Proc. GLM (SAS 1987). Quality control measures for each batch including calibration with reference samples, blanks and replicate analysis were followed through the analysis in order to ensure reliable analytical data. Two blank samples were run with each set of samples to check the purity of the reagent and possibility of contamination. Precision and analytical accuracy of the methods were

evaluated by analyzing a standard reference certified Oyster tissues. We used international standard in As measurement using HG-AAS, and an internal laboratory standard to estimate analytical reproducibility.

Calculation for used terminologies: Concentration in µg of element g^{-1} DW; accumulation in shoot in µg of element $plant^{-1}$ shoot; accumulation in root in µg of element $plant^{-1}$ root; and translocation % in nutrient accumulation in shoot/ total accumulation (shoot + root) × 100 (Shaibur and Kawai 2011a, b).

Results and Discussion

Visible symptoms: Interveinal chlorosis (failure of chlorophyll formation) in young leaves, necrosis (death associated with discoloration and dehydration of old leaves) and wilting were recorded both in As (T3) and As plus aeration (T4) treated plants (Odanaka *et al*. 1987). Chlorosis was most probably due to the lack of Fe concentration in shoot (Table 2). We could not find any significant difference of visible symptom between T3 and T4 treatments (Figure 1). Discoloration of roots was observed in T3 and T4 treatments but no distinct differences between them were observed.

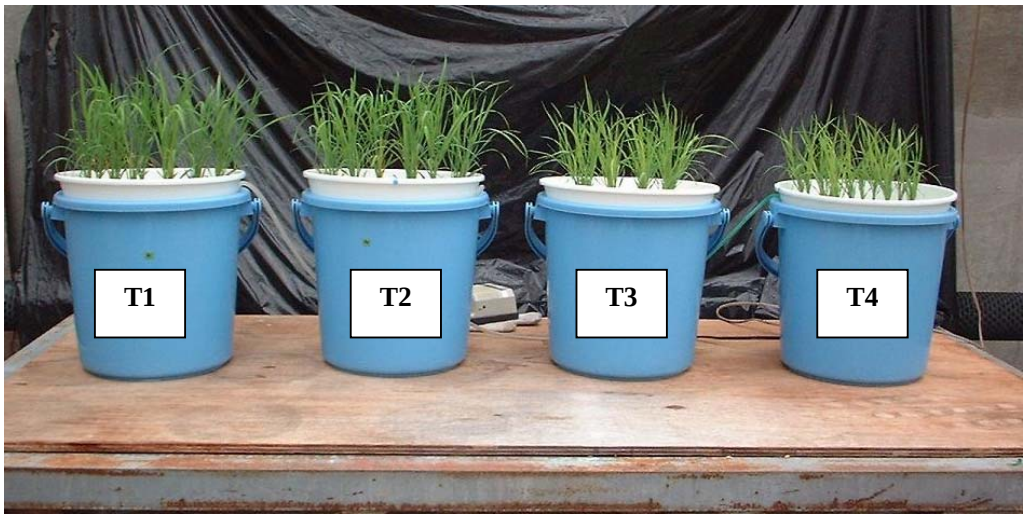

Figure 1: Figure showing the visible symptoms of rice seedlings in the different of concentrations of As. Arsenic showed visible toxicity symptom. T1 (control); T2 (aeration); T3 (13.4 µM As); and T4 (13.4 µM As + aeration). This picture was taken in a sunny day on 21 days after treatments (1 November, 2004).

Dry weight (DW): Dry weight of shoots and roots decreased in T3 and T4 treatments (Figure 2). The more severe effects were observed in T4 treatment (Shaibur *et al.* 2008a). Arsenic

reduced shoot and root growth (Tang and Millar 1991; Shaibur *et al.* 2011a, b). In this experiment, growth of rice seedlings seems to be dependent on the As (T3) and As plus aeration (T4) treatments. Arsenic may damage the rice roots, resulting in the inhibition of nutrient uptake (Abedin *et al*. 2002). The DW reduction was most probably due to the fact that in presence of As, roots may not produce calcium pectate, resulting in lower biomass production. This supposition however needs to be verified. The other probable cause may be that As may reduced photosynthesis activity which ultimately leads less carbohydrate production and dry matter yield (Knauer *et al*. 1999).

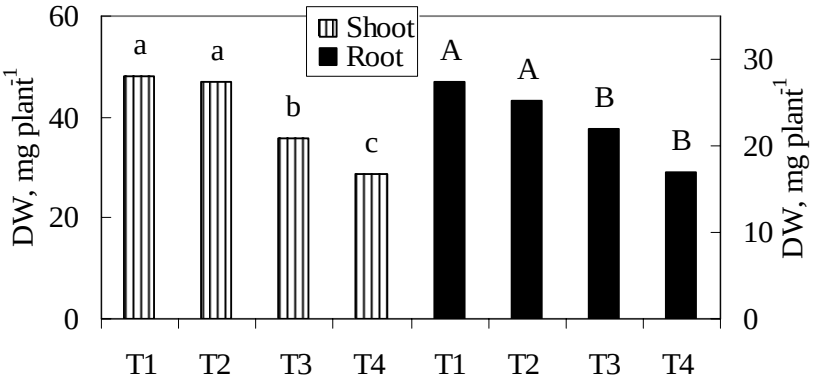

Figure 2: Growth of rice seedlings in presence of As or As + aeration. Bars with different letters are significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test. T1 (control); T2 (aeration); T3 (13.4 µM As); T4 (13.4 µM As + aeration) and DW (dry weight).

Arsenic with aeration (T4) was found to be more toxic as compared to As treatment (T3) for shoot and root DW. Aeration may convert some arsenite to arsenate. However, this result does not confirm to the normally held views regarding the relative toxicity of two treatments (T3 and T4). This opposite result was not surprising as there are reports of higher toxicity of arsenate over arsenite in mycorrhizal fungus (*Hymenoscyphus ericae*) biomass (Sharples *et al*. 2000); and algal and phytoplankton communities (Blanck *et al*. 1989; Knauer *et al*. 1999). However, no differences in root biomass (Marin *et al*. 1992) and total DW (Onken and Hossner 1995) were observed when the rice plants were treated with arsenite and arsenate.

Shoot height: Shoot height decreased significantly in T3 or T4 treatments as compared to others (data not shown). However, there were no differences between these two treatments (T3 and T4), indicating that aeration could not show any significant effect on As-toxicity reduction. Shoot growth decreased in T3 treatment as compared to control (T1), this was most probably due to the fact that As might decrease the activity of plant growth regulators like hormone or enzyme by blocking the activity of sulfhydryl groups of protein in shoot. Our

result supported by the experimental result of Abedin *et al.* (2002) and Tsutsumi (1980) who observed 60% reduction of height at 312.5 mg As kg^{-1} dry soil. Marin *et al*. (1992) also observed that shoot height decreased at 0.8 mg L^{-1} arsenate and monomethylarsonic acid in solution culture. Presence of As in irrigation water or in soil at an elevated level could hamper normal growth and development of plants. Arsenic inhibits seed germination (Liebig 1966; Abedin and Meharg 2002) and plant height (Tsutsumi 1980; Shaibur *et al.* 2006).

Root length: Aeration (T2) did not change the root length but As (T3) or As plus aeration (T4) decreased the root length as compared to control (T1; data not shown). There were no differences between T3 and T4 treatments (data not shown). The root length reduction was most probably due to the fact that As disrupt the root function. Arsenic reacts with the sulfhydryl groups of protein in roots membranes (Speer 1973; Webb 1966). Trivalent arsenite has great affinity for thiol groups and combination or chelation of As with thiols, effectively inhibits key enzymes containing active thiols (Schroeder and Balassa 1966) and ultimately reduces the root length. Significant reduction of root length caused by As was due to the fact that plant roots were the first point of contact for the toxic As in the rooting medium.

Chlorophyll indices: Aeration (T2) significantly increased chlorophyll content as compared to control (T1, without aeration; Figure 3a). This was most probably due to the fact that in presence of aeration; rice took up higher content of Fe from rooting medium and translocated to the shoots (Table 2). Iron concentration and accumulation decreased in shoot in As-treated plants (T3) as compared to T1 and T2 treatments (Table 2). In As-treated plants, translocation of Fe decreased, resulting in lower chlorophyll content and showing the slight whitish chlorosis in young leaves (Figure 1). We could not find any significant difference between T3 and T4 treatments regarding chlorophyll data (Figure 3a). The chlorophyll content decreased in T4 treatment as compared to T1 and T2 treatments.

Tiller number: We could not find any new tiller even in control treatment (Figure 3b). This was most probably due to the fact that the duration of the experiment was not sufficient to form new tiller (total 39 days = 9 days in $CaCl_2$ solution + 9 days in half strength solution + 21 days after treatments). The cold temperature of the greenhouse may also be another cause. Abedin *et al.* (2002) found that the tiller number decreased significantly due to different arsenate treatments. They did their experiment up to yield (Abedin *et al.* 2002).

Leaf number and width leaf blade: The treatments were started at 3rd leaf stage of the seedlings. At 21 DAT, five leaves were found in each plant in all treatments, indicating that there was no effect of the treatments on the formation of new leaves especially in this short time (Figure 3c). But actually the length of new leaves was shorter in As-treated plants as compared to control (T1) indicating toxic effect of As on leaves formation. The width of the leaf blade decreased significantly (Figure 3d), indicating that enlargement of leaf blade

decreased due to As-toxicity. The reduction of leaf blade was most probably due to the fact that As may inhibits the formation of auxin. The primary physical effect of auxin in plants is to stimulate the elongation of cells in shoots. This supposition demands further study. It has already been reported that As-reduced leaf area in baranuniya (Shaibur *et al.* 2012c) and photosynthesis (Knauer *et al*. 1999).

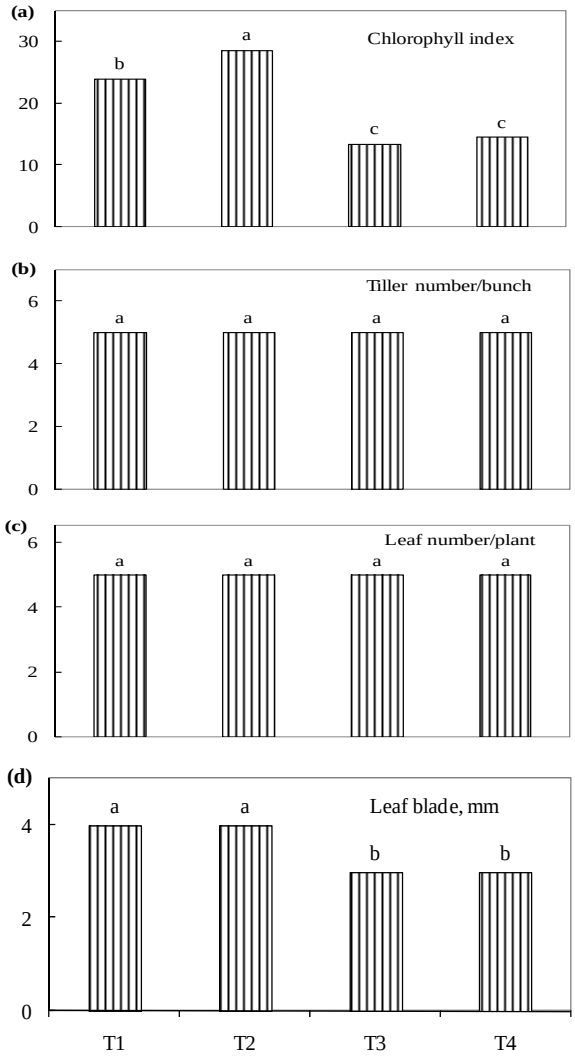

Figure 3: (a) Chlorophyll index; (b) Tiller number (c) Leaf number; and (d) Leaf blade as affected by As or As plus aeration. T1 (control); T2 (aeration); T3 (13.4 µM As); and T4 (13.4 µM As + aeration). Bars with the different letters are significantly different ($p<0.05$) according to Ryan-Einot-Gabriel-Welsch multiple range test.

Effect of aeration on As: In shoots, As concentrations were higher in T4 treatment (43.1 µg g^{-1}) as compared to T3 treatment (34 µg g^{-1}; Figure 4a). Similar result was also found in roots (Figure 4a). In presence of aeration, some arsenite (AsO_2^-) might be converted to arsenate (AsO_4^{3-}) and plant may absorb slightly higher content of As and easily translocated to the shoots, resulting in reduced plant growth (Figure 2). This hypothesis needs to be verified. Arsenic accumulation also increased both in shoots and roots in T4 treatment as compared to T3 treatment (Figure 4b). Chemical kinetics play an important role in the conversion between As^{5+} and As^{3+} but considerable amounts of As^{5+} and As^{3+} can be found under highly reduced and oxidized conditions, respectively (Masscheleyn *et al.* 1991). Therefore, to draw a conclusion in this experiment As species in the medium needs to be determined. In highly reduced condition e.g.- in groundwater As may be present as arsenate by 50% of the total (Samanta *et al.* 1999). Masscheleyn *et al.* (1991) found 60-90% As species was present as As^{5+} in a field soil of bean plants (redox potential 500-200 mV). Translocation was 7.51% in T3 treatment, but the value was 10.64% in T4 treatment, indicating that aeration increased As translocation.

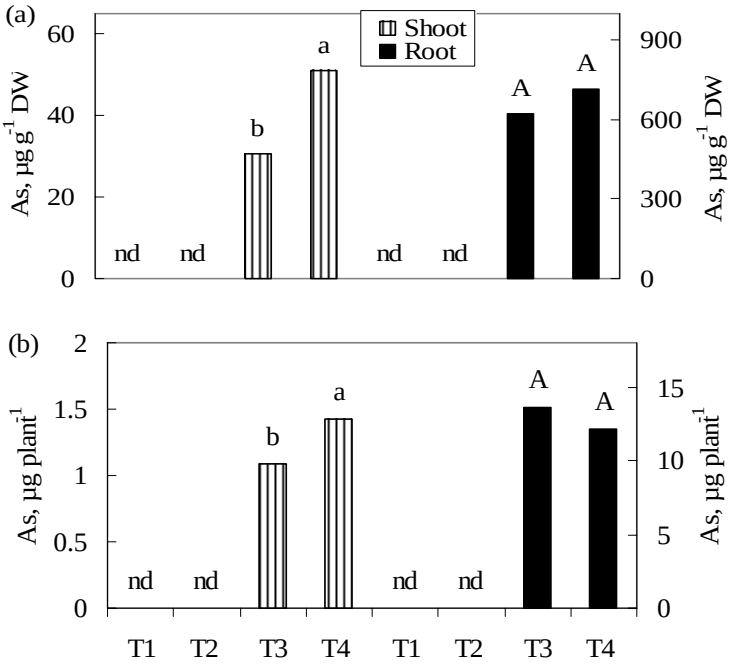

Figure 4: Arsenic concentrations (a) and accumulations (b) in rice seedlings in presence of As or As + aeration. Bars with the same letters are not significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test. T1 (control); T2 (aeration); T3 (13.4 µM As); T4 (13.4 µM As + aeration); DW (dry weight); and nd (not detected).

Effect of As or As plus aeration on Fe: In shoots, Fe concentration increased in T2 treatment as compared to T1 treatment (Table 2) but accumulation did not increase, implied that a concentration effect might be happened in the shoot systems for these two treatments. Iron concentration decreased in shoots of As treatment (T3) as compared to control (T1). The reduction of Fe concentration in T3 treatment might be responsible for the reduction of chlorophyll content (Shaibur and Kawai 2011a, b). Iron concentrations were almost similar in T3 and T4 treatments but were lowered as compared to T1 and T2 treatments (Table 2). In roots, Fe concentrations were similar in T1 and T2 treatments but were higher in T3 and T4 treatments as compared to T1 and T2 treatments (Table 2). Similar results were found for accumulation. The higher concentrations and accumulations of Fe in the roots of T3 and T4 treatments may result in the lower translocation, which ultimately results for the lower concentration of Fe in the shoots of T3 and T4 treatments. The low concentration of Fe in the young leaves is responsible for the whitish chlorosis in rice (Shaibur and Kawai 2011a, b).

Effect of As or As plus aeration on Mn: Manganese concentration decreased in shoots in T2, T3 and T4 treatments as compared to T1 (control; Table 2). The lowest value was obtained for T4 treatment, indicating that As showed the more toxic effect in presence of aeration. However, opposite results were obtained in roots for the same treatments. Not only concentrations but also accumulations in shoots and translocation from root to the shoots decreased significantly with the treatments. In roots, the accumulation of Mn increased in T2, T3 and T4 treatments as compared to T1 (control treatment; Table 2), resulting in lower concentrations in shoots. Manganese is necessary in various biochemical reactions (Phosphoglucomate, enolase, phosphokianase, Phosphotransferase, etc.), however, it does not form part of prosthetic groups of enzyme and it acts as an inorganic ion (Clarkson and Hanson, 1980; Lehninger 1975). These reactions may be reduced or limited by lower concentration of Mn in shoot tissues, may result in lower growth (Figure 2).

Effect of As or As plus aeration on Zn: Zinc concentration decreased in shoots significantly due to aeration (T2), As (T3) and As plus aeration (T4) treatments as compared to T1 treatment (Table 2). The lowest value was obtained for As plus aeration (T4) treatment. But in root opposite results were obtained (Table 2). In shoots, Zn accumulation was also negatively influenced by the T3 and T4 treatments where the lowest value was recorded for T4 treatment. In root, there were no distinct differences of Zn accumulations (Table 2). Arsenite has shown antagonistic interactions with P in nutrient and soil solution (Woolson *et al*. 1973) and within the plants (Wallace *et al*. 1980). Arsenic also shows antagonistic interaction with Zn (Marin 1989) which are in agreement with our result. The most studies showed that Zn deficiency was induced by P (Marschner 1998). It is now well known that arsenate is chemically similar to phosphate which can substitute in much metabolic processes. The antagonism between As and Zn is clearly apparent in Table 2. It can be seen that Zn level

decreased in T3 and T4 treatments, shown more severe toxic effect in shoots. Zinc accumulation in shoots decreased due to application of As (T3) in rooting medium. Similar result was also obtained by Marin *et al*. (1993).

Table 2 Concentration (µg g^{-1} DW; dry weight), accumulation (µg $plant^{-1}$ shoot or root) and translocation (%) of metal micronutrients of young rice seedlings grown in As-containing nutrient solution.

Treatment	-------Concentration in shoot---------				---------Concentration in root---------			
	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
T1	75 b	1327 a	87 a	32 a	1312 c	167 d	58 b	303 a
T2	80 a	1194 b	77 b	33 a	1325 c	214 c	56 b	300 a
T3	68 c	940 c	70 c	29 b	1945 b	237 b	62 a	186 b
T4	69 c	697 d	58 d	28 b	2448 a	300 a	65 a	167 c
	---------Accumulation in shoot-------				---------Accumulation in root---------			
	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
T1	3.55 a	63.73 a	4.16 a	1.54 a	36.08 b	4.54 b	1.57 a	8.31 a
T2	4.08 a	55.98 b	3.36 a	1.54 a	37.87 b	5.36 a	1.41 a	7.60 a
T3	2.55 b	33.53 c	2.50 b	1.04 b	42.58 a	5.17 a	1.35 a	4.07 b
T4	2.54 b	19.93 d	1.66 c	0.80 b	41.58 a	5.08 a	1.10 a	2.83 c
	----------Translocation (%) from roots to shoots----------							
	Fe		Mn		Zn		Cu	
T1	9.20 b		93.3 a		73 b		15.8 a	
T2	10.6 a		91.2 a		92 a		16.9 a	
T3	7.30 c		86.6 b		65 c		16.5 a	
T4	5.80 d		80.0 c		60 d		16.5 a	

Note: Means followed by same letters in each row are not significant (p = 0.05) according to Ryan-Einot-Gabriel-Welsch Multiple Range test. T1 (control); T2 (aeration); T3 (13.4 µM As); and T4 (13.4 µM As + aeration).

Effect of As or As plus aeration on Cu: In shoots, Cu concentrations were similar in T1 and T2 treatments. However, the concentrations decreased in T3 and T4 treatments as compared to T1 and T2 treatments (Table 2). There was no significant difference between T3 and T4 treatments, indicating that aeration did not have significant effect on As-toxicity reduction. Similar results were found in the case of roots regarding concentration. Arsenic with aeration (T4) showed more toxic effect on Cu concentration as compared to only As treatment (T3) in roots. We did not find any difference of Cu accumulation between T1 and T2 treatments as well as between T3 and T4 treatments both in shoots and in roots. The fact is that Cu uptake was significantly decreased with As treatment (T3), resulting in a significant reduction of root growth (Figure 2).

Conclusions

In presence of aeration, As showed the worst result as compared to other treatments. The threshold value of As in rice could be between 0 to 13.4 µM (1 ppm As in nutrient solution) which can reduce more than 10% dry matter yield, shoot height and width of leaf blade. More sophisticated research is needed to clarify reason why As showed more toxic effect on rice in aerated condition. The effect of aeration on arsenite in solution needs to be determined. The concentration of Fe in shoot decreased but in roots increased in T3 and T4 treatments. The concentrations of Mn, Zn and Cu decreased in shoots at T3 and T4 treatments, resulting in lower translocation. Not only concentration but also accumulation and translocations of those elements were also decreased with As treatment (T3). Arsenic in aerated condition was found to be more toxic as compared to without aeration. This indicates that in presence of aeration, arsenite may be transferred into arsenate which is more toxic than arsenite for rice (Abedin *et al*. 2002).

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