



EFFECTS OF AERATION ON ARSENIC AND ITS SUBSEQUENT EFFECTS ON GROWTH AND MACROELEMENTS OF RICE

Molla Rahman Shaibur, Tamanna Islam, Md. Sadiqul Amin^{1*}, S. M. Imamul Huq² and Shigenao Kawai

Department of Agro-bioscience, Iwate University, Morioka 020-8550, Japan

¹*Soil Science Discipline, Khulna University, Khulna 9208, Bangladesh*

²*Department of Soil, Water and Environment, University of Dhaka, Dhaka 1000, Bangladesh*

KUS-08/42-230408

Manuscript received: April 04, 2008; Accepted: October 30, 2008

Abstract: Effects of aeration on arsenic (As) and its subsequent effects on rice growth (*Oryza sativa* L. cv. Akihikari) and macroelements (P, K, Ca and Mg) were investigated in hydroponic condition. The treatments were control (T1), aeration (T2), 1 ppm As (T3) and 1 ppm As + aeration (T4). Arsenic was added as sodium meta-arsenite (NaAsO₂). Plants were grown up to 21 days after treatments (DAT) in greenhouse. After harvesting, roots and shoots were dried and digested separately with H₂SO₄-H₂O₂ mixture for measuring P, K, Ca, Mg and As. In T3, P concentration was more in shoots (9.67 mg g⁻¹) compared to that in roots (1.47 mg g⁻¹) with reference to T1 (7.47 mg g⁻¹) and (2.13 mg g⁻¹), respectively. Potassium concentration was less in shoots and roots as compared with T1. In T4, K concentration was increased in shoots compared to only T3 but still lowered than T1 and T2 treatments. In T4, Ca concentration was increased both in shoots and roots compared to T1, T2 and T3 treatments. There was no significant difference between T3 and T4 treatments for K, Ca and Mg accumulations in shoots, indicated that aeration did not have any significant role on As-toxicity reduction in this experimental system. In T3 and T4 treatments, P translocation was increased but did not change K, Ca and Mg translocation. Arsenic concentration in root was almost 20 and 14 times of that in shoots in with and without aerated conditions, respectively.

Key words: Aeration, arsenic, calcium, magnesium, phosphorus, potassium.

Introduction

Arsenic has achieved great notoriety because of the toxic effect of some of its compounds (O'Neill, 1995). The phytotoxicity of As depends mainly on its chemical form, e.g. arsenite (As³⁺) is more toxic than arsenate (As⁵⁺), whereas, both of them are more toxic than organic As compounds (Sachs and Michaels, 1971). Arsenic trioxide is cytotoxic, because it inhibits important sulfhydryl containing enzymes (Moriya *et al.*, 2006; Goyer, 1996).

The groundwater is used as drinking water, home consumption as well as for irrigation to rice, wheat and vegetables in Bangladesh. As concentration in Bangladesh soil was below 10 mg kg⁻¹ dry weight (DW). In some areas of Bangladesh, As concentration has been found to be as high as 80 mg kg⁻¹ DW where As-contaminated groundwater is used for irrigation (Huq *et al.*, 2003). Total concentration of 15 mg As kg⁻¹ DW is proposed as the maximum allowable limit for paddy soil (Kabata-Pendias, 2001) however, 20 mg As kg⁻¹ DW is also considered safe for crop

* **Corresponding author:** <czn_bd@yahoo.com>
DOI: <https://doi.org/10.53808/KUS.2008.9.2.0842-L>

production (Rahman *et al.*, 2007). Arsenic in groundwater is mainly inorganic with arsenate comprising about 50% of the total (Samanta *et al.*, 1999). Arsenic can be accumulated in soils through As contaminated irrigation water (Huq *et al.*, 2003; Imamul Huq and Naidu, 2003) and subsequent entry into human body through various food materials, especially the green leafy vegetables spinach (Shaibur *et al.*, 2007b) and arum (Imamul Huq *et al.*, 2005). Vegetables, like arum (*Colocasia antiquorum*, araceae family), kalmi (*Ipomea aquatica* L., Convolvulaceae family) and *Amaranthus gangeticus* etc. could concentrate high contents As in the edible parts. Arsenic concentrations have been found to be more than 150 mg kg⁻¹ DW in arum root (Huq *et al.*, 2003). Rice and wheat receiving As-contaminated irrigation water have been found to sequester the toxic metalloid into roots and stems (Huq *et al.*, 2003). The lethal concentration of As depends upon soil type, condition and plant species (Huq *et al.*, 2003).

There are several reports on As distribution in plant tissues including rice (Shaibur *et al.*, 2006; Imamul Huq *et al.*, 2007), spinach (Shaibur *et al.*, 2007b), sorghum (Shaibur *et al.*, 2008a), barley (Shaibur *et al.*, 2008b) etc. However, literature is very scarce on the effects of aeration on As-toxicity and the growth of plants and macroelements e.g. P, K, Ca and Mg distributions. Therefore, the present study was conducted to observe the effect of aeration on As and its subsequent effect on the growth and distribution of those elements in rice grown in water culture.

Materials and Methods

Seed germination: 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 continuously with tap water. After washing, seeds were wrapped between moistened towels and were kept in an electric incubator at 25 ± 2 °C for 72 hours. Germinated seeds were transferred on a net in a box containing 2% CaCl₂ for 9 days and after that the seedlings were transferred in ½-strength nutrient solution for another 9 days.

Plant culture: When the seedlings were suitable for the transplantation (18 days after germination, at 3rd leaf stage of the seedlings), the treatments were started with full-strength solution containing 1 mM NH₄NO₃, 1 mM K₂SO₄, 0.8 mM MgSO₄, 0.5 mM CaCl₂, 0.5 mM NaH₂PO₄, 10 µM MnSO₄, 1 µM CuSO₄, 1 µM ZnSO₄, 3 µM H₃BO₃, 0.05 µM H₂MoO₄ and 10 µM Fe³⁺-citrate. Five seedlings were taken in 1 bunch and each bucket (10 L) containing 16 bunches. The treatments were control (T1), containing full-strength solution, aeration (T2), 13.4 µM As (T3) and 13.4 µM As + aeration (T4). The 13.4 µM As was chosen because in our previous report (Shaibur *et al.*, 2006), we showed that this level of As gave the most visible result among the 0, 6.7, 13.4 and 26.8 µM As levels in Akihikari rice variety grown hydroponically. Arsenic was added as sodium meta-arsenite (NaAsO₂) (Kanto Chemical Company, Tokyo, Japan) for 21 d. The pH (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 – 1st November, 2004). The air temperature in the greenhouse was around 25°C at day and 20°C at night, respectively. We choose the pH 5.5 because, at this pH, the Fe content together with other mineral elements are available for plant absorption (Brady and Weil, 2002; Shaibur *et al.*, 2007a). Solution was renewed every week.

Sample collection and preparation: After 21 DAT, 3 bunches were taken for analyses. We concentrated our study at this stage of the plants, where nutrient deficiencies or inhibitory effects likely to be the most apparent and therefore, differences among treatments would be the easiest to observe. The collected plants were washed with tap water and then with deionized water and separated into shoots and roots with sterilized scissor and were dried for 48 h at 55-60°C in a fan forced electric oven (Isuzu Seisakusho Company, Tokyo, Japan). The samples were cut into small pieces and digested with H₂SO₄-H₂O₂ mixture (5:1 V/V).

Heating procedure: The individual sample was taken in acid (0.1 N HNO₃) washed 100 mL glass beaker, 10 mL analytical grade H₂SO₄ was added, covered with glass coverer and heated at 100°C for 1.5 h, at 140°C for 1.5 h and at 180°C for 2 h on electric hot plate (model NF-HG 59, National Electronics Company Limited, Japan). After that, 2 mL of analytical grade H₂O₂ was added to the cool samples and heated at 180°C for 5 h. The samples were kept for overnight for cooling. In the following day, another 10 mL H₂SO₄ and 2 mL H₂O₂ was added to the samples and heated at 180°C for 9 h continuously. At the last stage of the digestion, all the samples were clear. The digested samples were volumed at 50 mL and were transferred to acid washed plastic bottle for analyses.

Chemical reagents: All chemicals used were of analytical reagent grade. All solutions were prepared previously with MQ water [(18.2 MΩ cm⁻¹), purified by Milli-RO 60 Millipore corporation, USA]. Stock solution of As was prepared by dissolving NaAsO₂ in MQ water. This stock solution was kept in acid washed reagent bottle in the laboratory at around 25°C temperature. Working solutions of As were prepared every week by diluting the stock solution with deionized water. Glassware used for this experiment was soaked in 0.1 N HNO₃ and were rinsed with MQ water.

Chemical analyses: Total P was determined colorimetrically using a UV-visible Spectrophotometer (model UV mini 1240, Shimadzu Company Limited, Kyoto, Japan) at 420 nm wavelengths after developing the yellow color with vanadomolybdate as described by Jackson (1962). Potassium, Ca and Mg were determined with atomic absorption spectroscopy (AAS, Hitachi 170-30, Japan).

Determination of As: Arsenic was measured with Hydride Generation Atomic Absorption Spectrophotometric (HGAAS) technique by using Hitachi HFS-3 instrument. The primary solutions (50 mL) were further diluted up to 100-1000 times to determine As. The standard reference material was carried out through the analysis of As determination as a part of the quality assurance-quality control protocol. We did reagent blank and internal standards in our analysis to ensure accuracy and precision in the analysis.

Experimental design and statistical analysis: The experiment was a completely randomized blocks with 3 replications. Data were analyzed by analysis of variance (Anon, 1988). The data are presented as the means of 3 replicates (seedlings). Results were analyzed by ANOVA and with Duncan Multiple Comparison using the SAS 6.12 software package (SAS Institute, Cary, NC) using computer origin 5 at Iwate University. All statistically significant differences were tested at the $P \leq 0.05$ level.

Calculation: Concentration refers to the weight (mg) of an element per gram of DW sample while accumulation indicates total amount (mg) of element per plant shoot or root. Translocation refers to the ratio of accumulation of element in shoot to the total accumulation (shoot + root). The translocation was expressed in %.

Results

Growth as affected by As and As + aeration: In T2 treatment, shoot growth was reduced by almost 2.09% and root growth by 7.83%, compared to control (T1) treatment (Figure 1). In T3 shoot growth was reduced by 25.4% and root growth by 19.7%. In T4 shoot growth was reduced by 40.3% and root growth by 37.5%. Therefore, T4 treatment reduced shoot growth by 12.9% more and root growth by 5.19 % more compared to T3 treatment.

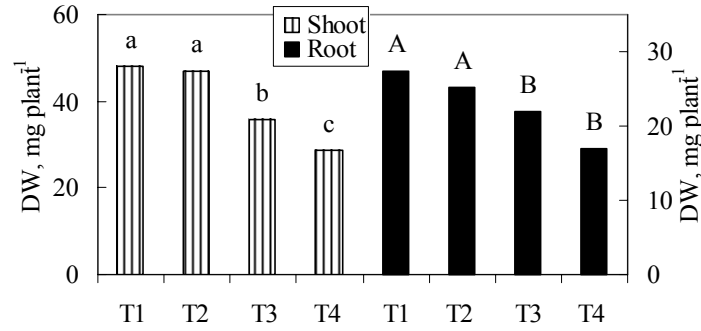


Figure 1: Growth of rice seedlings in presence of As or As + aeration. Bars with different letters are significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test. T1 (control); T2 (aeration); T3 (13.4 μM As), T4 (13.4 μM As + aeration) and DW (dry weight).

Arsenic concentration, accumulation and translocation: Arsenic concentrations seem to be higher in shoots and roots for T4 treatment compared to T3 treated plants (Figure 2a). Arsenic accumulation was also increased in shoots with T4 treatment (Figure 2b). Translocation was 7.51% for T3 treatment but it enhanced to 10.6% in T4 treatment.

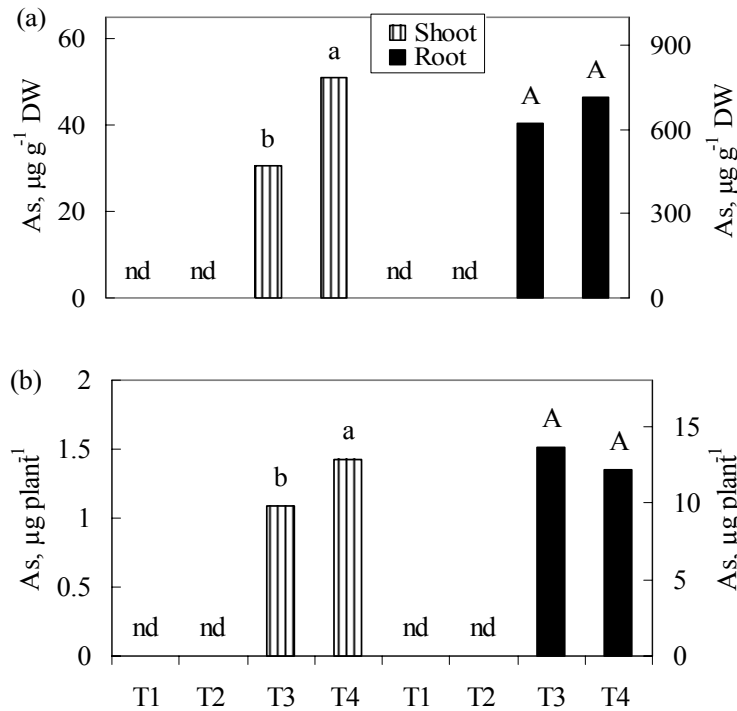


Figure 2: Arsenic concentrations (a) and accumulations (b) in rice seedlings in presence of As or As + aeration. Bars with the same letters are not significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test. T1 (control); T2 (aeration); T3 (13.4 μM As), T4 (13.4 μM As + aeration), DW (dry weight) and nd (not detected).

Potassium concentration decreased in shoot tissues in T3 and T4 treatments (Table 1). Aeration (T2) could not change the concentrations of K both in shoots and roots (Table 1). Potassium concentration increased in shoot in T4 treatment compared to T3 treatment, though the translocation was not affected significantly.

Calcium concentrations were similar in shoots of T1, T2 and T3 treatments but increased significantly in T4 treatment (Table 1). Translocations of Ca was also negatively influenced by the applied As (T3) and As + aeration (T4) treatments (Table 1).

Magnesium concentrations were similar in shoots and roots in all the treatments (Table 1). However, the accumulation decreased significantly in shoots and in roots with T3 and T4 treatments. The lowest value was recorded in T4 treatment (Table 1). For translocation, the lowest value was also recorded in the T4 treatment (Table 1).

Discussion

Growth as affected by As and As + aeration: It was found that only aeration decreased rice growth (Figure 1), probably due to the fact that rice plants can not grow well in aerobic condition (Kitagishi and Yamane, 1981; Brady and Weil, 2002). It is known that rice grows well in anaerobic condition than the aerobic condition. Reduction of shoot and root DW with 13.4 μM As was most probably due to the reduction of enzymatic activity (Mishra and Dubey, 2006) and or the activity reduction of plant growth regulators (Singh *et al.*, 2007). Root growth reduction was most probably due to the reduction of Ca pectates formation (which is important for root elongation; Hopkins, 1995) with 13.4 μM As, resulting in lower root DW production. These suppositions demand further study. It is reported that As reduced DW in rice (Shaibur *et al.*, 2006; Abedin *et al.*, 2002).

Dry weight decreased much in As + aeration treatment (Figure 1), indicating that 13.4 μM As showed severe toxic effect on rice in presence of aeration compared to no aeration. It might be due to the conversion of arsenite to arsenate because of aeration and the converted arsenate showed more toxic effect on rice growth. Sometimes, arsenate is found to be more toxic than arsenite for rice root growth (Abedin and Meharg, 2002). Our result does not confirm to the normally held views regarding the relative toxicity of arsenite and arsenate in that arsenite is more toxic than arsenate. However, there are reports of higher toxicity of arsenate than arsenite in mycorrhizal fungus (*Hymenoscyphus ericae*) (Sharples *et al.*, 2000), algal and phytoplankton (Blanck *et al.*, 1989; Knauer *et al.*, 1999). In order to clarify the reason of higher As-toxicity in aerated As condition, As species (arsenite or arsenate) in the solution culture needs to be determined. It is also important to find out the sensitivity of this particular rice variety (*Oryza sativa* L. cv. Akihikari) to arsenate and arsenite. In this experiment, it is unwise to make a generalized conclusion as there are significant variations among the plant varieties and toxicity to As species.

Arsenic concentration, accumulation and translocation: Higher As concentration in As + aeration treatment (T4) was most probably due to the fact that in presence of aeration some arsenite (AsO_2^-) might be converted to arsenate (AsO_4^{3-}) and plant accumulated slightly higher amount of As in shoot (Figure 2b) which might reduce shoot growth (Figure 1). In highly reduced condition *e.g.* - in groundwater As may be present as arsenate by 50% of the total (Samanta *et al.*, 1999). 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 As form in the medium needs to be determined. In our experiment, As concentrations in shoots and roots were correlated with the As in the nutrient solution, which, were not proportional to the As concentration in the rooting medium. Translocation was higher in aerated condition compared to no aeration condition, indicating that aeration enhanced As translocation (Figure 3).

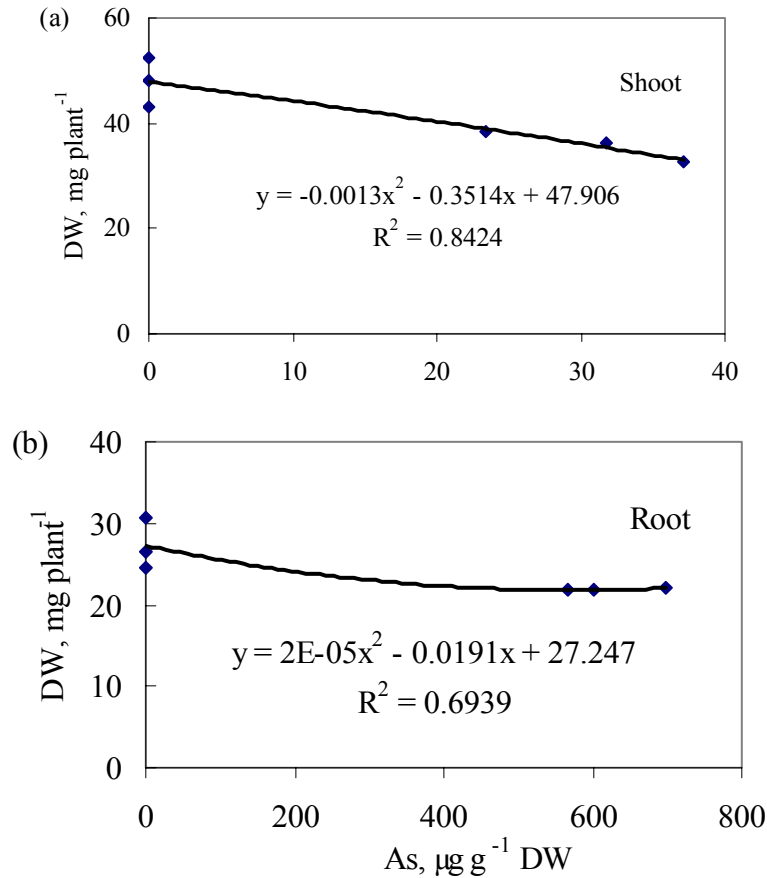


Figure 4: Two order polynomial growths curve (a) shoot and (b) root of hydroponic rice seedlings with As concentrations in plant tissues. In this case the rice seedlings were grown at 0 and 13.4 μM As levels in the greenhouse without aeration (22 September-1st November 2004). The air temperature in the greenhouse was around 25°C at day and 20°C at night, respectively.

Critical toxicity level (CTL) of As in hydroponic rice: Calculated CTL in shoots was 13.7 μg As g⁻¹ DW, however, it was 174.5 μg As g⁻¹ DW in root for 0 and 13.4 μM As treatments, respectively (Figure 4). These data indicates that the shoots were more sensitive to As-toxicity than the roots. Similarly, for 0 and 13.4 μM As + aeration treatments, the CTL was 11.5 μg As g⁻¹ DW in shoot and 75.1 μg As g⁻¹ DW in root, respectively (Figure 4) indicating that the As-toxicity was higher in aerated condition compared to no aeration condition. Growth of As treated rice seedlings was reported recently (Shaibur *et al.*, 2006), but the CTL of As in shoots and roots of rice were not reported. Based on the reported data, the calculated CTL in rice were 21.0 μg As g⁻¹ DW in shoots and 325 μg As g⁻¹ DW in roots. The relationships of the reported data (Shaibur *et al.*, 2006) were presented in Figures 6a,b. The reported data and the present data were for the similar variety of rice, but the differences were the temperature, stage of plants for As treatments, days of As exposure and the preparation time of the As solutions. The applied solutions were prepared in different times. The levels of As were also different. For the previous report (Shaibur *et al.*, 2006), we used As at the rate of 0, 6.7, 13.4 and 26.8 μM levels for 14 DAT at 4th and 5th leaf stage, but for the present case the levels of As were only 0 and 13.4 μM in presence or absence of aeration at

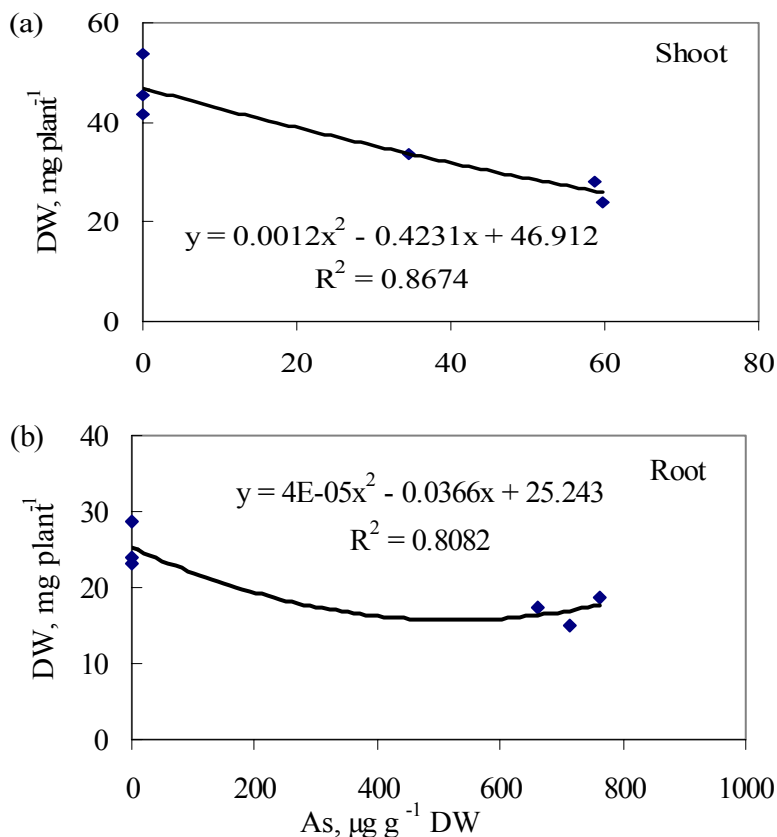


Figure 5: Two order polynomial growths curve (a) shoot and (b) root of hydroponic rice seedlings with As concentrations in plant tissues. In this case the rice seedlings were grown at 0 and 13.4 μM As levels in presence of aeration in the greenhouse (22 September-1st November 2004). The air temperature in the greenhouse was around 25°C at day and 20°C at night, respectively.

3rd leaf stage for 21 DAT. The calculated CTL of As in hydroponic sorghum was 11.7 μg As g⁻¹ DW in shoot and 367 μg As g⁻¹ DW in root (Shaibur *et al.*, 2008a). Our result suggested that the CTL of As in plant tissues may be depended on the plant species and varieties, stage of the seedlings of As application, environmental condition (especially temperature), condition of As solutions (As species) and the duration of As exposures.

Macroelement concentrations, accumulations and translocations: Phosphorus concentrations increased in shoot of the As (T3) and As + aeration (T4) treatments. It might be due to the concentration effect as the growth decreased. The accumulations were similar or decreased for those treatments compared to control (Table 1). Phosphorus accumulation did not decrease significantly at 13.4 μM As (T3) treatment, indicating that As might not hinder P absorption strictly at this concentration. Our result partially support the experimental result of Chen *et al.* (2002) who observed that P did not compete with As rather cooperate when *Pteris vittata* L. were grown in pot containing soil. However, it was also reported that the behavior of arsenate (AsO₄³⁻) resembles to that of the phosphate (PO₄³⁻) and vanadate (VO₄³⁻) and exhibit similar physicochemical behavior in soils (Kabata-Pendias, 2001).

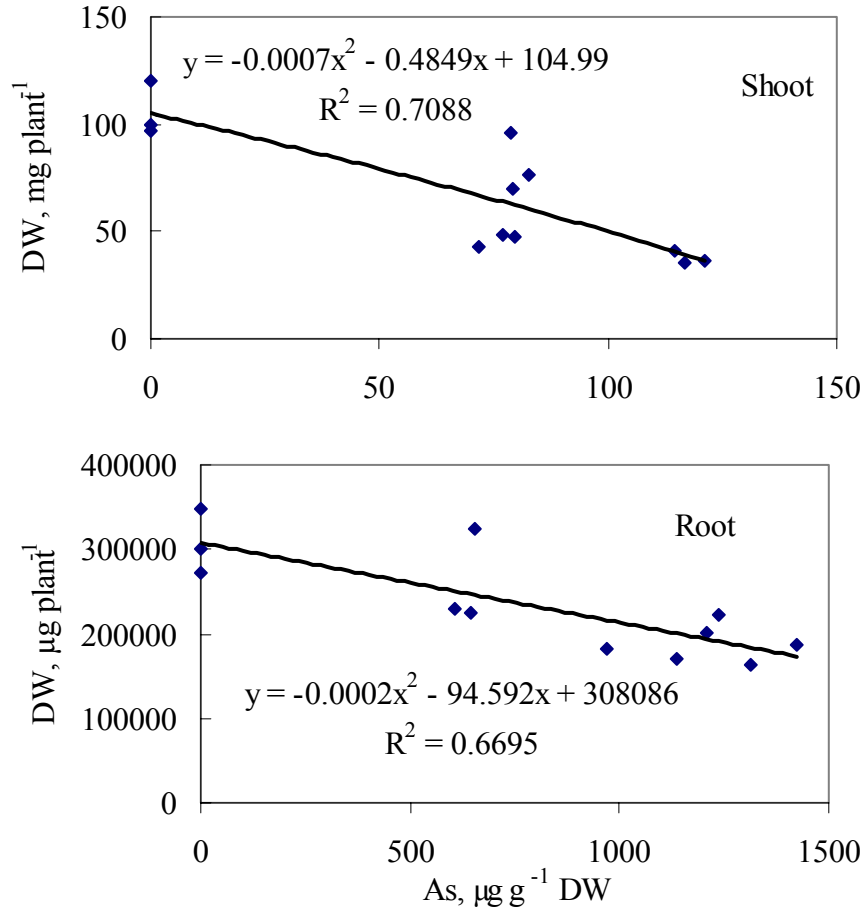


Figure 6: Two order polynomial growths curve (a) shoot and (b) root of hydroponic rice seedlings with different As concentrations in plant tissues. The growth response and As concentrations in rice seedlings were reported recently (Shaibur *et al.*, 2006) but the relationship data were not reported. In this case, the hydroponic rice seedlings were treated with 0, 6.7, 13.4 and 26.8 μM As levels at 4th or 5th leaf stage for 14 DAT without aeration in the greenhouse (August – September 2004). The air temperature in the greenhouse was around 35°C at day and 30°C at night, respectively.

They compete directly for the same sorption sites on soil particles surfaces (Hingston *et al.*, 1972) and competed with each other during the uptake in the plant system (Sharples *et al.*, 1999). The present experimental result supports the result of Marin *et al.* (1993) who found that DMAA (Dimethylarsinic acid) marginally increased P concentration in shoots and roots in hydroponic rice at 0.8 and 1.6 mg As l⁻¹ levels. Ghoshal *et al.* (2003) also found P concentration and uptake by rice straw were lower in As treated pots than their corresponding untreated counterparts. These dissimilarities are most probably due to set up of the experiments and may be due to the differences of the crop variety.

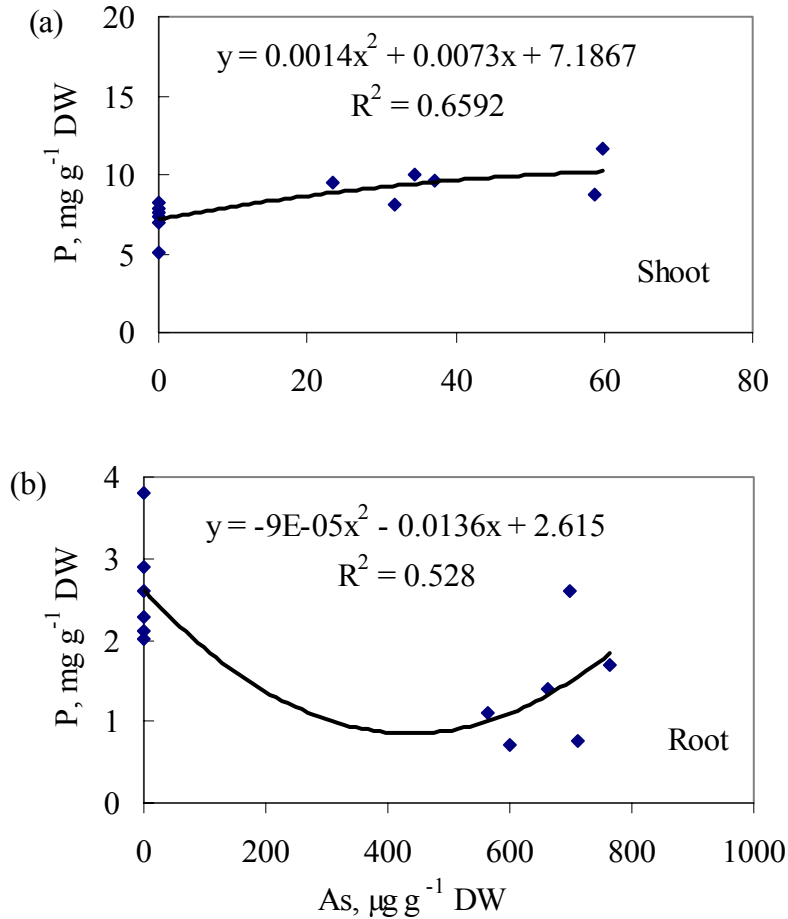


Figure 7: Polynomial two order correlation between P and As concentrations in plant tissues (a) in shoot and (b) in root.

The polynomial two order relationship between P and As concentrations in shoot tissues showed that with the increasing As concentrations, the P concentrations were also increased regardless of aeration (Figure 7a). However, this relationship was just opposite in the root tissues (Figure 7b).

Reduction of K concentration in the As (T3) and As + aeration (T4) treated plant (Table 1) was most probably due to the toxic effect of As on the plant growth (Figure 1), where As is a phytotoxic agent (Shaibur *et al.*, 2007b). The other probable cause might be the Na in the rooting medium because we used NaAsO₂ as a source of As. The competition between As and K might not be involved as K is a cation and As is an anion (arsenate or arsenite) though sometimes K may act as a cation for anion. The sharp increase in tissue Na concentration upon increased sodium arsenite is not surprising since the As was applied as its sodium salt. Our result is similar to Marin *et al.* (1993) who found that application of DMAA, K concentration decreased significantly in rice shoots (at 0.8 and 1.6 mg As l⁻¹).

The increase of Ca concentrations in shoot and root of the As (T3) and/or As + aeration (T4) treatments were most probably due to the concentration variation, because the growth decreased by those treatments. Reduction of Ca accumulations was the negative effect of As in the 13.4 μM As (T3) and 13.4 μM As + aeration (T4) treatments. In this experiment, we did not see any

additional affect of aeration on As-toxicity reduction that could increase Ca accumulation or could increase plant growth.

Magnesium concentration was not much affected by the applied treatments (Table 1). However, accumulation decreased significantly by the As (T3) and As + aeration (T4) treatments, indicated the toxic effect. Magnesium translocation was not much affected though it seemed slight negative effect.

Conclusion: It may be concluded that 13.4 μM As (1 ppm As) in the hydroponic culture can be a limiting factor for hydroponic rice which could reduce 25.4% growth in shoot and 19.7% in root, respectively. The CTL of As was 13.7 $\mu\text{g g}^{-1}$ DW in shoot and 174.5 $\mu\text{g g}^{-1}$ DW in root. Aeration did not decrease As-toxicity rather it increased in rice seedlings. Phosphorus concentration increased in shoots and decreased in roots in presence of 13.4 μM As and/or 13.4 μM As + aeration treatments, but there was no difference between these two treatments.

References

- Abedin, M.J. and Meharg, A.A. 2002. Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L.). *Plant and Soil*, 243: 57-66.
- Abedin, M.J., Cotter-Howells, J. and Meharg, A.A. 2002. Arsenic uptake and accumulation in rice (*Oryza sativa* L.) irrigated with contaminated water. *Plant and Soil*, 240: 311-319.
- Anon. 1988. *SAS/STAT User's guide*, No. 1, ANOVA, Version 6. 4th edition. Statistical Analysis System Institute, Cary, North Carolina.
- Blanck, H.; Holmgren, K.; Lander, L.; Norin, H.; Notini, M.; Rosemarin, A. and Sundelin, B. 1989. Advanced hazard assessment of arsenic in Swedish environment. pp. 256-328. In: Lander, L. (ed.) *Chemicals in the Aquatic Environment*. Advanced Hazard Assessment. Springer, Berlin.
- Brady, N.C. and Weil, R.R. 2002. *The Nature and Properties of Soils*. 13th Edition. Prentice Hall, New Jersey, USA.
- Chen, T.B.; Fan, Z.L.; Lei, M.; Huang, Z.C. and Wei, C.Y. 2002. Effect of phosphorous on arsenic accumulation in As-hyperaccumulator *Pteris vittata* L. and its implication. *Chinese Science Bulletin*, 47(22): 1876-1879.
- Ghoshal, S.K.; Mukhopadhyay, D. and Ghosh, T.K. 2003. Effect of arsenic-contaminated irrigation water on the uptake of arsenic and phosphorus by rice (cv. IET-4094). *Crop Research*, 26(2): 243-248.
- Goyer, R.A. 1996. Toxic effects of metals. pp. 691-736. In: Klaassen, C.D (ed.). *Casarett and Doull's toxicology: the basic science of poisons*. 5th edition. McGraw-Hill, New York.
- Hingston, F.J.; Posner, A.M. and Quirk, J.P. 1972. Competitive adsorption of negative charged ligands on oxide surfaces. *Faraday Discuss. Chemical Society*, 52: 334-342.
- Hopkins, W.G. 1995. *Introduction to Plant Physiology*. John Wiley and Sons Inc., Toronto.
- Huq, I.S.M.; Alam, S. and Kawai, S. 2003. Arsenic in Bangladesh environment and its impact on food chain through crop transfer. Abstract No. 22-45. Abstracts of the Annual Meeting, *Japanese Society of Soil Science and Plant Nutrition*. Vol 49. Tokyo, August 20-22, 2003. pp. 177.
- Imamul Huq, S.M. and Naidu, R. 2003. Arsenic in Ground Water of Bangladesh: Contamination in the food chain. pp. 203-226. In: Ahmed, F. (ed.). *Arsenic Contamination: Bangladesh Perspective*, International Training Network, Bangladesh.
- Imamul Huq, S.M.; Binte-Abdullah, M. and Joardar, J.C. 2007. Bioremediation of arsenic toxicity by algae in rice culture. *Land Contamination & Reclamation*, 15 (3): 327-333.
- Imamul Huq, S.M.; Joardar, J.C. and Parvin, S. 2005. Marigold (*Tagetes patula*) and ornamental arum (*Syngonia* sp.) as phytoremediators for arsenic in pot soil. *Bangladesh Journal of Botany*, 34 (2): 65-70.
- Jakson, M.L. 1962. *Soil Chemical Analysis*. Prentice-Hall, Inc., New Jersey, U.S.A.
- Kabata-Pendias, A. 2001. *Trace Elements in Soils and Plants*. 3rd edition. CRC Press, USA.
- Kitagishi, K. and Yamane, I. 1981. Eds., *Heavy Metal Pollution in Soils of Japan*. Japan Science Society Press, Tokyo.
- Knauer, K.; Behra, R. and Hemond, H. 1999. Toxicity of inorganic and methylated arsenic to algal communities from lakes along an arsenic contamination gradient. *Aquatic Toxicology*, 46: 221-230.

- Shaibur, M.R.; Islam, T.; Amin, M.S.; Huq, S.M.I. and Kawai, S. 2008. Effects of aeration on Arsenic and its Subsequent Effects on Growth and Macroelements of Rice. *Khulna University Studies*, 9(2): 291-302.
- Marin, A.R.; Pezeshkin, S.R.; Masscheleyn, P.H. and Choi, H.S. 1993. Effect of dimethylarsenic acid (DMAA) on growth, tissue arsenic, and photosynthesis of rice plants. *Journal of Plant Nutrition*, 16 (5): 865-880.
- Masscheleyn, P.H.; Delaune, R.D. and Patrick (Jr.), W.H. 1991. Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. *Environmental Science and Technology*, 25: 1414–1419.
- Mishra, S. and Dubey, R.S. 2006. Inhibition of ribonuclease and protease activities in arsenic exposed rice seedlings: Role of proline as enzyme protectant. *Journal of Plant Physiology*, 163: 927-936.
- Moriya, F.; Furumiya, J. and Hashimoto, Y. 2006. A case of fatal arsenic poisoning. *Forensic Toxicology*, 24: 88-91.
- O'Neill, P. 1995. Arsenic. pp. 106-121. In: Alloway, B.J. (ed.) *Heavy Metals in Soils*, 2nd edition. Blackie academic and professional, London.
- Rahman, M.A.; Hasegawa, H.; Rahman, M.M.; Islam, M.N.; Miah, M.A.M. and Tasmin, A. 2007. Arsenic accumulation in Rice (*Oryza sativa* L.) varieties of Bangladesh: a glass house study. *Water, Air and Soil Pollution*, 185: 53-61.
- Sachs, R.M. and Michael, J.L. 1971. Comparative phytotoxicity among four arsenical herbicides. *Weed Science*, 19: 558-564.
- Samanta, G.; Chowdhury, T.R.; Mandal, B.K.; Biswas, B.K.; Chowdhury, U.K.; Basu, G.K.; Chanda, C.R.; Lodh, D. and Chakraborti, D. 1999. Flow injection hydride generation atomic absorption spectrometry for determination of arsenic in water and biological samples from arsenic-affected districts of West Bengal, India, and Bangladesh. *Microchemical Journal*, 62: 174-191.
- Shaibur, M.R., Amin, M.S. and Kawai, S. 2007a. Effect of potassium chloride on growth and accumulation of metal micronutrients in rice seedlings grown hydroponically. *Khulna University Studies*, 8 (2): 245-252.
- Shaibur, M.R.; Imamul Huq, S.M. and Kawai, S. 2007b. Effect of arsenic on hydroponic Japanese mustard spinach. Abstracts of the Eighth Conference of the East and Southeast Asian Federation of Soil Science. Tsukuba International Congress Center, Tsukuba, Ibaraki, 22-23 October 2007. pp. 100.
- Shaibur, M.R.; Kitajima, N.; Sugawara, R.; Kondo, T.; Alam, S.; Imamul Huq, S.M. and Kawai, S. 2008a. Critical toxicity level of arsenic and elemental composition of arsenic-induced chlorosis in hydroponic sorghum. *Water, Air, and Soil Pollution*, 191: 279-292.
- Shaibur, M.R.; Kitajima, N.; Sugawara, R.; Kondo, T.; Imamul Huq, S.M. and Kawai, S. 2006. Physiological and mineralogical properties of arsenic-induced chlorosis in rice seedlings grown hydroponically. *Soil Science and Plant Nutrition*, 52(6): 691-700.
- Shaibur, M.R.; Kitajima, N.; Sugawara, R.; Kondo, T.; Imamul Huq, S.M. and Kawai, S. 2008b. Physiological and mineralogical properties of arsenic-induced chlorosis in barley seedlings grown hydroponically. *Journal of Plant Nutrition*, 31: 333-353.
- Sharples, J.M.; Meharg, A.A.; Chambers, S.M. and Cairney, J.W.G. 1999. Arsenate sensitivity in ericoid and ectomycorrhizal fungi. *Environmental Toxicology and Chemistry*, 18(8): 1848-1855.
- Sharples, J.M.; Meharg, A.A.; Chambers, S.M. and Cairney, J.W.G. 2000. Mechanism of arsenate resistance in the Ericoid mycorrhizal fungus *Hymenoscyphus ericae*. *Plant Physiology*, 124: 1327-1334.
- Singh, H.P.; Batish, D.R.; Kohli, R.K. and Arora, K. 2007. Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. *Plant Growth Regulation*, 53: 65-73.