

ACCUMULATION OF NUTRIENTS WITHIN RICE CROP SYSTEM UNDER ARSENIC TOXIC CONDITION: A HYDROPONIC STUDY

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ABSTRACT

A hydroponic experiment was conducted in Directorate of Research, B.C.K.V., Mohanpur to observe the effect of arsenic concentration on accumulation of minerals within rice seedlings. Seedlings were treated with 0.6, 1.2 and 1.8 ppm arsenic (As) concentration along with full Hoagland solution (1940) for 14 days in net house condition. Our experiment result indicates that arsenic was mostly concentrated in roots and a little amount was moved to shoots, indicating that arsenic was not easily translocated to shoots of rice seedlings. Accumulation of P, K, Fe, Ca, Mg, Mn, Zn and Cu content decreased in leaves significantly with the arsenic treatments, indicating that rice seedlings are As-sensitive and As-toxicity depends on the arsenic concentration in the rooting medium. In root, accumulation of P, K and Mn decreased but accumulation of Ca and Cu increased with increasing arsenic concentration. Mg accumulation increased in root at 0.6 ppm arsenic concentration and decreasing in root at high arsenic concentration (1.2ppm and 1.8ppm). Fe and Zn accumulation increased with increasing arsenic concentration and at high concentration of arsenic (1.8ppm) accumulation of Fe and Zn decreased.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major food crops in many countries. As the cultivation of rice requires huge volume of water, long term use of arsenic (As) contaminated groundwater for irrigation may result in the increase of arsenic concentration in the agricultural soil and eventually accumulation in rice plants. A huge amount of ground water loaded with arsenic is used for irrigating agricultural crops, particularly for production of boro (summer) rice. Out of nineteen districts in West Bengal, nine where cropping intensities are very high, are arsenic-affected. Five million people in West Bengal are thus affected by arsenic toxicities as they such arsenic-contaminated ground water for drinking purpose (Ghosh *et al.*, 2006). Use of such water for irrigation purposes results in an increase of arsenic concentration in soil and it subsequently enters into different parts of crops; arsenic thus ultimately takes its way to the human and animal body causing various clinical signs, *i.e.*, melanosis, hyperkeratosis and carcinogeneses (Dwivedi *et al.*, 2010). Groundwater rich in arsenic mostly occur in the Bengal Delta Plain (BDP), covering the state of West Bengal, the adjoining country of Bangladesh, extending to Bihar, Jharkhand, Uttar Pradesh, Punjab, Chattishgarh and the neighbouring country of Nepal (Bhattacharya *et al.*, 2003). In Bangladesh more than 80% of the district have arsenic levels exceeding the world health Organization guideline value for arsenic contamination in drinking water ($10\mu\text{g L}^{-1}$) (Smith *et al.*, 2006).

Uptake of As by rice is complicated by various chemical and physiological processes that occur in the rice paddy, namely (i) a change in oxidation state of As from arsenate (As-V) to

arsenite (As-III) as reduction in the paddy intensifies (Onken and Hossner, 1996; Takahashi, 2004; Panaullah *et al.*, 2009), (ii) the formation of oxidized Fe plaque on rice root surfaces that readsorbs As, but in competition with phosphate (Liu, 2004; Chen, 2005; Mei, 2009), (iii) the possible formation of insoluble As sulfur species (Reynolds *et al.*, 1999), (iv) competitive uptake of phosphate and arsenate through the same ion channel (Abedin *et al.*, 2002), (v) competitive uptake of arsenite and silicate through a general aquaporin channel (Bogdan and Schenk, 2008; Li *et al.*, 2009) and (vi) microbial methylation of inorganic As to mono- and dimethyl-As species that have different rates of uptake than those of inorganic As species (Abedin *et al.*, 2002; Raab *et al.*, 2007). Consequently we should expect that the levels and forms of As in soil solution and their uptake by rice will not be simply related to total soil As, or even available soil As content which complicates establishment of a safe level of As in soils used for flooded rice production.

The redox active forms of arsenic that cause intoxication to plants are arsenite (As III) and arsenate (As V). Both these soluble forms can be found in soil and water, but under growth conditions of paddy fields arsenite predominates, due to the partial anaerobic conditions. Consequently, the +3 oxidation state is regarded as responsible for arsenic toxicity in this environment (Meharg, 2004).

Arsenate competes directly with phosphate for uptake by the arsenic hyperaccumulator *P. vittata* (Tu and Ma, 2003; Wang *et al.*, 2002). Tu and Ma (2002) reported that Ca is effective in increasing arsenic concentrations in the fronds of *P. vittata* and enhancing arsenic translocation from the roots to the

fronds. Lombi *et al.* (2002) reported that arsenic and K in the upper epidermis were positively correlated.

In shoot, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and zinc (Zn) concentrations were limited by the 67 μ M arsenic treatment. Similar to concentrations, accumulations of these elements together with copper (Cu) were also limited in shoots by the higher arsenic concentrations. No doubt, arsenic limited the nutritional quality of JM-spinach by hampering the concentrations and accumulations of nutrient elements in plant tissues (Shaibur *et al.*, 2010).

The sequestration of arsenic by Fe plaque on the root of rice (Liu *et al.*, 2004; Chen *et al.*, 2005), macrophytes (Taggart *et al.*, 2009) and cattail (Blute *et al.*, 2004) has been demonstrated. The adsorption of arsenic by the Fe plaque may be an efficient strategy to reduce As contamination of rice grains. Dwivedi *et al.* (2010) reported that a number of elements were sequestered in the plaque in an order of Fe > Zn > P > As > Se. Other workers have also demonstrated that Fe plaque could adsorb P (Batty *et al.*, 2002), Zn and Cu (Ye *et al.*, 1998). Dwivedi *et al.* (2010) reported that amount of Fe and Zn in the Fe plaque was positively correlated in rice. Although it has been shown that excess arsenic in the soil affects a number of physiological and biochemical reactions in plants (Jha and Dubey, 2004; Mishra and Dubey, 2006), the precise mechanisms underlying arsenic phytotoxicity are poorly understood (Requejo and Tena, 2006). However, there is little data on the physiological interaction of minerals under arsenic toxicity. It is, therefore, necessary to observe the effect of arsenic toxicity on the accumulation of minerals by rice crop system.

MATERIALS AND METHODS

Plant material and culture

All experiment were conducted in Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, from March to April, 2011.

Seed of rice (*Oryza sativa* L.) cultivar, IET-4786 (Shatabdi), were surface sterilized in 0.1% HgCl₂ (w/v) solution for 2 min, thoroughly washed with deionized water and then germinated in sterilized sand. At 18 days after germination, 10 uniform seedlings (1 bunch) were selected. The sand adhering to the seedling root was washed with deionized water, and the ten seedlings were transferred to 2^{1/2}-L plastic pots containing 2-L Hoagland nutrient solution with different dose of arsenic (Fig. 1). The composition of the complete Arnon and Hoagland (1940) nutrient solution was 1.02 g/L KNO₃, 0.492 g/L Ca (NO₃)₂, 0.49 g/L MgSO₄·7H₂O, 0.23 g/L NH₄H₂PO₄ and micronutrients 2.86 mg/L H₃BO₃, 1.81 mg/L MnCl₂·4H₂O, 0.08 mg/L CuSO₄·5H₂O, 0.22 mg/L ZnSO₄·7H₂O, 0.09 mg/L H₂MoO₄·H₂O, 2.98 mg/L FeSO₄/tartaric acid.

The ten rice seedlings (1 bunch) grew normally in this nutrient solution. The pH of the Hoagland nutrient solution was adjusted to 6.5 with 0.1 M NaOH or HCl. The seedlings were inserted in to the hole of a Styrofoam plate that was floated on the nutrient solution. The plants were grown in a net house. As-V in the form of Sodium arsenate (Na₂HAsO₄·7H₂O, M. W. = 321.01)-were added to the nutrient solution at 0.6 ppm, 1.2 ppm and 1.8 ppm concentration. After 14 days of

treatment, the rice seedlings were analyzed for P, K, Ca, Mg, Fe, Mn, Zn and Cu and total arsenic.

Further, in the order to analyse the mechanism underlying the toxic effect of the As on the accumulation of nutrients in the rice seedling. The rice seedlings were cultured as same as described above. After 14 days of treatment, the rice seedling were collected and washed with deionised water. Leaves and root were separated and dried at 80°C for 3 days. Leaves and root from the rice seedling sample were digested in an Erlenmeyer flask by a mixture of concentrated acids, e.g., HNO₃ and HClO₄ (Piper *et al.*, 1942). After an overnight reaction, the content of the flask were gently boiled on an electric heater for digestion. The entire digestion process lasted 3-4 h. after complete digestion, the solution was diluted with double distilled water and filtered by Whatman No. 42 filter paper and transferred in to acid-washed plastic bottle; this solution was used for analyzing the P, K, Ca, Mg, Fe, Mn, Zn and Cu and total As content of the sample. Each treatment was performed in triplicate.

Analysis of nutrient in plant sample

The amount of Ca, Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectroscopy method, K was determined by flame photometer and phosphorus (P) was determined by Jackson (1967).

Analysis of the total arsenic in plant sample

The digest was diluted to 20mL. 2mL of the aliquot was taken in 10mL plastic tube, 1mL of concentrated HCl and 1mL of mixed reagent [5% KI (w/v) + 5% Ascorbic acid (w/v)] were added to it, kept for 45 minutes to ensure complete reaction and the volume was made up to 50 ml The resultant solution was analyzed in a PerkinElmer Atomic Absorption Spectrophotometer with Flow Injection Analysis System (FIAS 400) @ $\lambda_{max} \cong 193.7$ nm where the carrier solution was 10% v/v HCl, the reducing agent (to ensure all As species be reduced to AsH₃ and to be measured against a calibration with standard As⁺³ solution) was 0.2% NaBH₄ in 0.05% NaOH (Schmidt *et al.*, 2004).

Calculation of parameters

Accumulation of nutrient and arsenic in ten seedlings (1 bunch) was presented as mg or μ g per bunch. Bioconcentration factor is calculated by formula of Rauf *et al.* (2011).

$$\text{Bioconcentration factor} = \frac{\text{Content of nutrient in tissue}}{\text{Content of nutrient in treatment solution}}$$

Statistical analysis

Experimental data were analyzed statistically by using the windows-based SPSS 11.8 package at 95% significance level. The experimental data for the characters were subjected to the variance analysis appropriate to a CRD design for experiment.

RESULTS

Arsenic accumulation

Arsenic concentration increased both in leaves and root as a result of the applied arsenic in the nutrient solution. Arsenic

concentration in the root was 212mg/kg, 327mg/kg and 428mg/kg and in leaves was 21mg/kg, 23mg/kg and 40mg/kg at 0.6ppm, 1.2ppm and 1.8ppm arsenic treatment, respectively, indicating that arsenic was mostly concentrated in the root (Fig. 2). Our result indicating that accumulation of arsenic was higher at 1.8ppm arsenic treatment and increased concentration of arsenic increase the accumulation of arsenic.

Phosphorus accumulation

The content of P was higher in leaves compare to root. The P concentration decreased with increased concentration of arsenic in nutrient solution. Compared to the control plants, the accumulation of P decreased in both leaves and root. P content of the leaves was 0.770, 0.669 and 0.437 (mg/bunch) lower than the content in the control leaves (0.823mg/bunch) at 0.6ppm, 1.2ppm and 1.8ppm arsenic treatments. P content of the root was 0.064, 0.058 and 0.034 (mg/bunch) lower than the content in the control root (0.072mg/bunch) at 0.6ppm, 1.2ppm and 1.8ppm arsenic treatments (Fig. 4).

Potassium accumulation

The Content of K was decreased significantly in both leaves and root but percentage of K accumulation was very low at 1.8ppm (Fig.5). Percentage decrease in leaves K was low at 0.6ppm arsenic (1.85%) and higher at 1.8ppm arsenic (45.41%). Percentage decrease in root K was low at 0.6ppm arsenic (8.52%) and higher at 1.8ppm arsenic (75.26%).

Iron accumulation

The highest content of leaves Fe was recorded in control plant (6.04 μ g/bunch) and the lowest in the 1.2ppm (3.67 μ g/bunch) and 1.8ppm (2.89 μ g/bunch) arsenic treatment. The highest value of Fe was recorded at 1.2ppm (3.76 μ g/bunch) and 1.8ppm (2.76 μ g/bunch) arsenic treatment in root. Our result indicated that arsenic was responsible for accumulation of Fe in root and blocked Fe translocation from root to leaves, which was supported by Fig. 6.

Calcium accumulation

In leaves, the content of Ca was not differ significantly at 0.6ppm and 1.2ppm arsenic, but decreased significantly at 1.8ppm arsenic treatment, similar results were also found in root. But at 1.8ppm arsenic treated root Ca content (0.016mg/bunch) was higher than all treatment. Accumulation of Ca in leaves decreased significantly with increasing arsenic in nutrient solution, but accumulation of Ca in root significantly increased with increasing arsenic in nutrient solution, which was supported by Fig. 7.

Magnesium accumulation

According to this experiment the concentration of Mg continuously decreased in leaves in the range between 0.6 to 1.8ppm. Figure 8 represent the percentage decrease of leaves Mg was higher at 1.8ppm arsenic (43.3%) and minimum at 0.6ppm arsenic treatment (4.6%). In case of root, percentage increase of Mg accumulation was higher at 0.6ppm arsenic treatment (31.5%) and percentage decrease of Mg accumulation was higher at 1.8 ppm arsenic treatment (42.10%).

Manganese accumulation

From the Mn accumulation study of seedling, it was found

that the Mn content in leaves and root decreased with increase of arsenic in nutrient solution (Fig. 9). In leaves, a high Mn accumulation (131.32 μ g/bunch) was found in the control treatment. The root Mn accumulation of seedling was found to differ significantly at all arsenic concentration and highest accumulation, 3.376 μ g/bunch was observed for the control treatment; for higher arsenic concentration, the accumulation of Mn decline slowly.

Zinc accumulation

Zn accumulation in leaves and root decreased with increasing concentration of arsenic in nutrient solution. Lower concentration (0.6ppm and 1.2ppm) of arsenic in nutrient solution help in more Zn accumulation in root compared to control. Percentage decrease of Zn content in leaves was 41.1, 59.7 and 74.9 % at 0.6, 1.2 and 1.8ppm respectively compared to control. Percentage decrease of Zn content in root was 50.4 % at 1.8ppm respectively compared to 0.6ppm arsenic treated nutrient solution (Fig. 10).

Copper accumulation

Accumulation of Cu in leaves decreased with increasing As in nutrient solution. The accumulation of Cu in rice seedling depends on arsenic concentration. At 0.6 ppm As, the Cu content in leaves (3.23 μ g/bunch) less decrease compared to control leaves (3.30 μ g/bunch). At 1.8ppm As, the Cu content in leaves (1.15 μ g/bunch) highly decrease compaired to control. Root Cu content increased at 0.6ppm (4.60 μ g/bunch) and 1.2ppm (4.66 μ g/bunch) arsenic concentration. At 1.8ppm, root Cu content was decreased compared to control, 0.6ppm arsenic and 1.2 ppm arsenic (Fig. 11).

Bioconcentration factor (BCF)

Bioconcentration factor is the potential for a chemical to bioaccumulate in plant tissue and plant's ability to accumulate metals from surrounding medium. Among the macro nutrient Mg having high BCF than P, K and Ca (Fig. 12a). BCF of Cu is higher among the micro nutrient like Fe, Mn and Zn (Fig. 12b).



18 day old rice seedling



Figure 1: Rice seedlings transplanted in nutrients medium

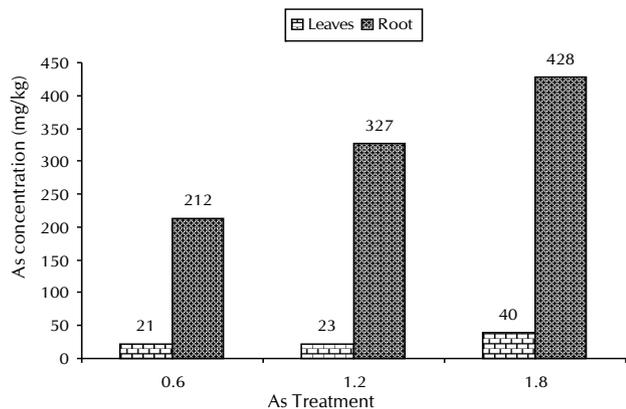


Figure 2: As concentration (mg/kg) with rice crop system

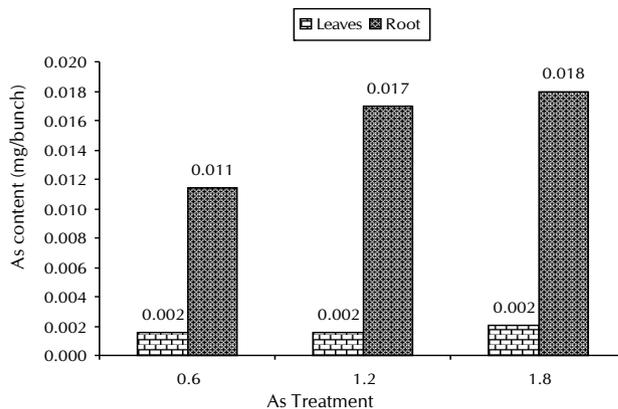


Figure 3: As content (mg/bunch) with rice crop system

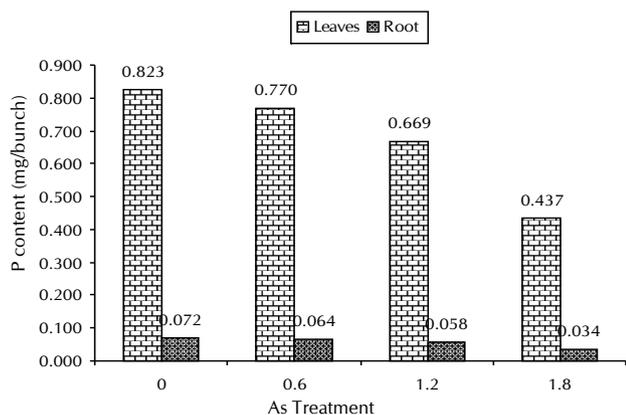


Figure 4: Phosphorus content (mg/bunch) with rice crop system

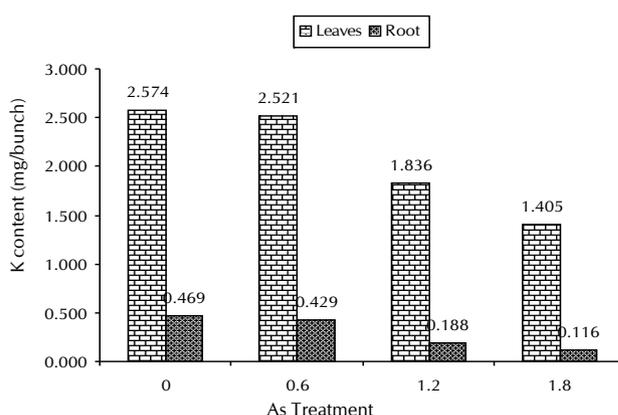


Figure 5: Potassium content (mg/bunch) with rice crop system

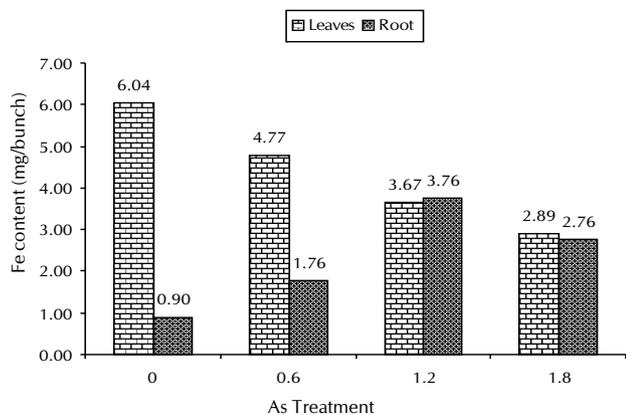


Figure 6: Iron content (µg/bunch) with rice crop system

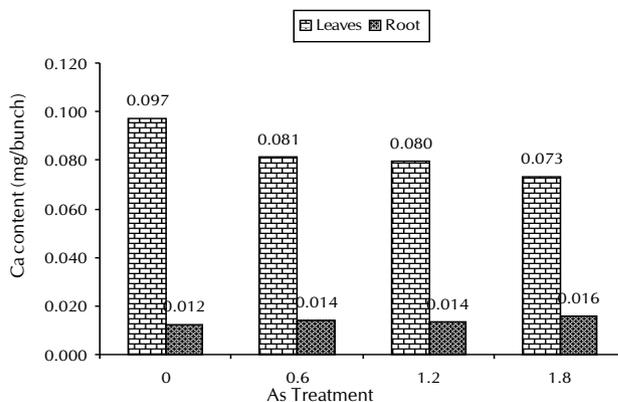


Figure 7: Calcium content (mg/bunch) with rice crop system

DISCUSSION

Arsenic concentration in leaves and roots increased significantly with increasing arsenic level in the nutrient solution. Arsenic content in the root was 7.1, 10.6 and 8.6 fold greater than the content in the leaves at 0.6, 1.2 and 1.8ppm arsenic treatment, respectively, indicating that arsenic was mostly concentrated in the root (Fig. 2). Arsenic content (mg/bunch) follow the same pattern of arsenic accumulation. Chaturvedi (2006) also reported a similar result that the arsenic is predominantly concentrated in the root, with less accumulated in the shoots. Abedin *et al.* (2002) also observed a large accumulation of arsenic by rice plants, in roots as

compared to stems. Several reports on the linear relationship between arsenic content of plant and soil arsenic concentration suggested that plants take up arsenic passively in conjunction with water flow (Abedin *et al.*, 2002). The arsenic anions may rapidly adsorb to the root surface, leading to the intense high As concentration, particularly in hydroponic culture (Wang, 2010). This may be the reason why the highest levels of arsenic are found in roots. In our experiment, percentage arsenic accumulation increased with increasing concentration of arsenic in nutrient solution. Rauf *et al.* (2011) also reported that arsenic absorption and accumulation is greatly influenced by arsenic concentration in the growth media and increases in higher arsenic levels.

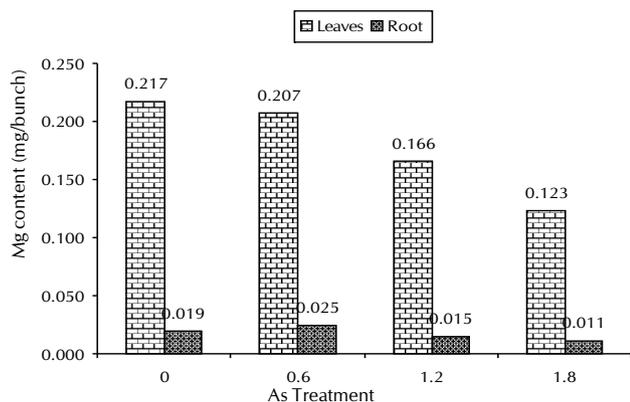


Figure 8: Magnesium content (mg/bunch) with rice crop system

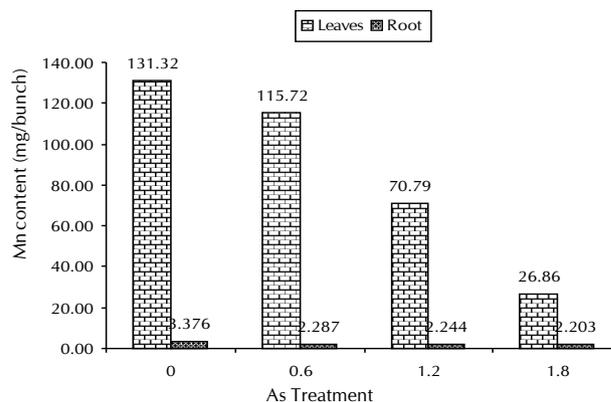


Figure 9: Manganese content (mg/bunch) with rice crop system

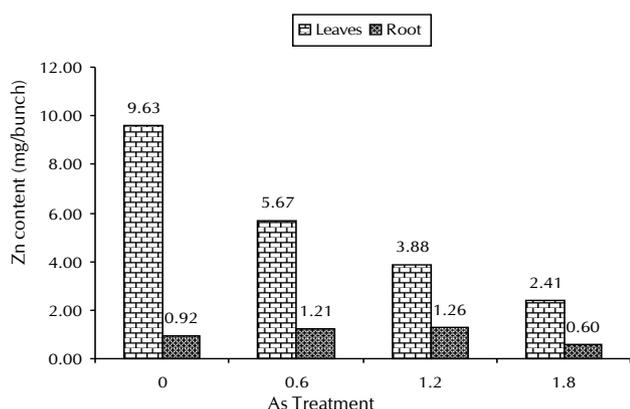


Figure 10: Zinc content (mg/bunch) with rice crop system

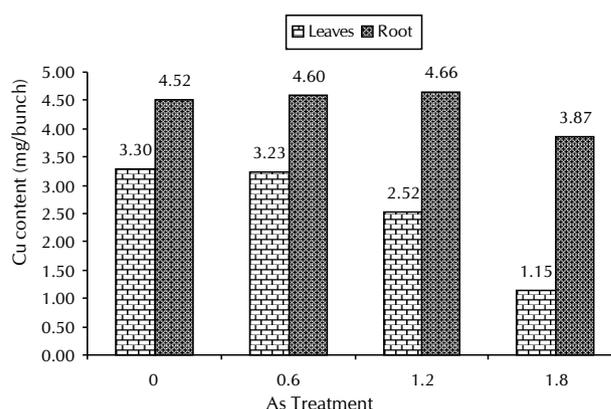


Figure 11: Manganese content (mg/bunch) with rice crop system

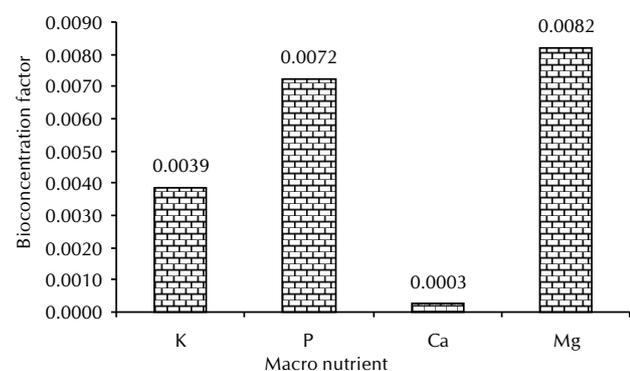


Figure 12a: Bioconcentration factor of macro nutrient

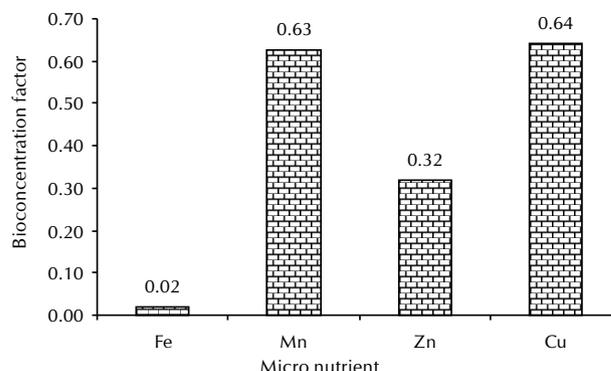


Figure 12b: Bioconcentration factor of micro nutrient

As per experiment phosphorus content (Fig. 4) of decreased with increasing arsenic concentration. The content of P was higher in leaves than root. P content of the leaves was 0.770, 0.669 and 0.437 (mg/bunch) lower than the content in the control leaves (0.823 mg/bunch) at 0.6ppm, 1.2ppm and 1.8ppm arsenic treatments. P content of the root was 0.064, 0.058 and 0.034 (mg/bunch) lower than the content in the control root (0.072mg/bunch) at 0.6ppm, 1.2ppm and 1.8ppm arsenic treatments. Shaibur *et al.* (2006) also reported that accumulation of phosphorus in leaves and root decreased with increasing concentration of arsenic in nutrient solution. Phosphate plays an important role in arsenic behaviour under aerobic conditions, where arsenate (As-V) is the dominant

arsenic form (Lambkin and Alloway, 2003). Several studies have shown that phosphate, which is an analogue of arsenate; compete with arsenate for binding sites on the iron oxides surface, resulting in a large reduction of arsenate adsorption (Manning and Golberg, 1996).

The potassium contents per bunch were 3.044, 2.951, 2.024 and 1.521 at control, 0.6, 1.2 and 1.8ppm, respectively, under treatment. Same as P, K content was higher in leaves than root. Percentage decrease in leaves K was low at 0.6ppm arsenic (1.85 %) and higher at 1.8ppm arsenic (45.41 %). Percentage decrease in root K was low at 0.6ppm arsenic (8.52 %) and higher at 1.8ppm arsenic (75.26 %). Arsenic decreased the contents of potassium both in roots and leaves

of rice seedlings (Fig. 5). Potassium is essential element for plant and they have a concerted effect on the growth of rice plants. The arsenic down-regulated the genes correlative to the absorption and utilization of K, resulting in decline of K in rice plant, and leading to stunted growth of rice seedlings (Wang *et al.*, 2010).

Figure 6 represent the accumulation of Fe in leaves was negatively influenced by arsenic and root Fe accumulation was positively influenced by arsenic concentration, but at higher arsenic treatment Fe accumulation in root was reverse. In leaves, the highest Fe content was recorded in control seedling (6.04µg/bunch) and lowest (2.89µg/bunch) at 1.8ppm arsenic treatment. The highest value of Fe content (3.76µg/bunch) in root was recorded at 1.2ppm arsenic treatment but at 1.8 ppm arsenic treatment, also having higher content of Fe (2.76µg/bunch) in root compared to control. The obtained data from our experiment suggested that Fe accumulation on the surface of root increased with increasing arsenic concentration in nutrient solution. Liu *et al.* (2004) demonstrated the sequestration of As by Fe plaque on the root surface of rice. At the root-plaque interface, siderophores by microbes or phytosiderophores exuded by rice root may form a complex with Fe³⁺ and mobilize Fe bound arsenate and be taken up through phosphate co-transporters (Liu *et al.*, 2006).

At 1.8ppm arsenic treatment, accumulation of Ca in leaves decreased with 24.47% and accumulation of Ca increased with 33.33% (Fig. 7). Marin *et al.* (1993) also found that Ca concentration increased significantly in root in the 0.8ppm arsenic treatment. Shaibur *et al.* (2006) also reported that accumulation of Ca in shoots and translocation from root to shoot decreased significantly with increasing arsenic concentration in nutrient solution.

We found that Mg content increased significantly in root in 0.6ppm arsenic treatment, but decreased at 1.2 ppm and 1.8ppm arsenic treatment (Fig. 8). Accumulation of Mg in leaves decreased significantly with increasing arsenic concentration. Carbonell-Barrachina *et al.* (1997) and Shaibur *et al.* (2006) also support that accumulation of Mg in leaves decreased significantly with increasing arsenic concentration in nutrient solution.

Toxicity of arsenic was more effective in leaves Mn accumulation than root Mn accumulation. Leaves Mn content decreased significantly with increasing arsenic concentration in nutrient solution and the lowest value of Mn accumulation was obtained for the highest arsenic concentration. Decreasing percentage of root Mn content was lower than leaves, but increasing arsenic content also decrease the Mn content of root (Fig. 9). Shaibur *et al.* (2006) also support that accumulation of Mn in leaves and root decreased significantly with increasing arsenic concentration in nutrient solution

Concentration of Zn decreased in leaves treated with arsenic, but increased in 1.2 and 1.8ppm arsenic treatment in root compared with control treatment (Fig. 10). In leaves, high content of Zn was recorded in control (9.63µg/bunch) and lowest content of Zn (2.41µg/bunch) was recorded in 1.8ppm arsenic treatment. In root, high content of Zn was recorded in 1.2ppm arsenic treatment and lowest content of Zn (0.60µg/bunch) was recorded in 1.8ppm arsenic treatment. The Zn

uptake by plants depends on the uptake capacity of root and Zn concentration in the medium (Howeler, 1973). Fe plaque sequestered higher amount of Zn on the root surface (Dwivedi *et al.*, 2010).

Copper content decreased in leaves with increasing arsenic concentration in nutrient solution. A negative correlation was observed between different level of arsenic applied and Cu content in leave. This means that increasing arsenic concentration in solution decreased the translocation of Cu in leaves. Shaibur *et al.* (2010) also reported that Translocations of Cu were limited by the applied arsenic in the nutrient solution. Lower concentrations of arsenic might be responsible for the higher uptake of Cu in root. At higher concentration of arsenic (1.8ppm) Cu uptake was reduced because higher concentration of arsenic limit the growth of seedling (Fig. 11). Micro nutrients having high potential for bioaccumulation within rice seedlings tissues compared to macro nutrients. In rice seedlings, under hydroponic experiment nutrients BCF decreases markedly in the order of Cu > Mn > Zn > Fe > Mg > P > K > Ca (Fig. 12a and 12b).

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