International Journal of Farm Sciences **5(3)** : 41-50, 2015

Arsenic induced physiological changes in improved varieties of rice grown in Malwa region of Punjab

R KAMBOJ, S SHARMA, GS VISHWAKARMA and S MITTAL

Centre of Environment Science and Technology
Central University of Punjab, Bathinda 151001 Punjab, India
Email for correspondence: sunil.cevs@gmail.com

ABSTRACT

Rice is a major staple food throughout the world. However metal contamination of rice has been recognized as a new disaster on large scale. Two rice cultivars viz PR 116 and PR 118 were grown in vitro to study the effect of increasing arsenic concentration (50, 100, 250 and 500 µM) on germination, root and shoot length, biomass dry weight, chlorophyll content and per cent cellular respiration. Results showed reduction in all these parameters thereby proving the toxic nature of arsenic. Arsenic accumulation in roots and shoots of both the varieties was monitored by atomic absorption spectrophotometer (AAS) and it was concluded that arsenic is translocated to above ground tissues and ultimately reaches the grain. The status of arsenic concentration of rice and its affect on human health need to be further investigated.

Keywords: Arsenic; rice; concentration; toxic

INTRODUCTION

Arsenic concentration in earth crust is about 3 mg/kg and 1-8 µg/l in sea water (Cullen and Reimer 1989, Smedley and Kinniburgh 2002). Arsenic is present both in inorganic as well as organic form. However organic form is less toxic as compared to inorganic form. Arsenate (+5) is the main form present under aerobic conditions whereas under anaerobic conditions arsenite (+3) is commonly found (Smith et al 1998). Toxicity and solubility of arsenite is more than arsenate (Oremland and Stolz 2003). Human population gets exposed to this heavy metal by ingestion of plants produced in the contaminated areas (Dudka and Miller 1999). Crop cultivation for human or livestock consumption can lead to the uptake and accumulation of this metal in edible plant parts with a resulting risk to human and animal health (Gupta and Gupta 1998, Lim et al 2008). Toxicity symptoms of arsenic on plants include decrease in plant height, reduction in leaf area and photosynthesis, wilting and necrosis of leaf blades (Carbonell-Barrachina et al 1999). Average concentration of arsenic in rice has been reported to be about 0.5 mg/kg

(Zhao et al 2010) and per day consumption of rice is 200 g (Zhu et al 2008). As a result the amount of ingested arsenic exceeds the daily limit which has been set for drinking water.

Arsenic in low concentrations induces stimulatory effect on the plant growth while at higher concentrations exerts negative effect on the plant growth which includes reduction in grain yield, grain weight and root biomass (Panaullah et al 2009).

Thus the present study was conducted to find out the effect of different concentrations of arsenic on germination, seedling growth of PR 116 and PR 118 varieties of rice in terms of per cent germination, shoot length, root length and biomass dry weight, arsenic accumulation in roots and shoots of the two varieties, total chlorophyll content and per cent cellular respiratory ability.

MATERIAL and METHODS

Germination studies: Rice seeds of cultivars PR 116 and PR 118 procured from Punjab Agriculture University, Ludhiana were grown in vitro in plastic trays lined with filter paper and adsorbent cotton. Seeds were sterilized with 5 per cent sodium hypochlorite solution and rinsed with distilled water thrice. 60 seeds were placed in each tray and treated with different concentrations of arsenate (50, 100, 250 and 500 µM). Trays were incubated at 35^{o}C under dry and hygienic conditions. Germinated seeds were counted after 7 days (Abedin and Meharg 2002). Germination was considered to be complete after the emergence of plumule and radicle. Root and shoot length was measured using metric scale. Biomass dry weight was estimated by drying the roots and shoots at 60^{o}C for 48 hours.

Determination of arsenic accumulation in plant material by atomic absorption spectophotometer (AAS): Plant material (100 mg) was digested in concentrated HNO_3 at 70^{o}C and was preserved and analysed by graphite furnace atomization (GFA) method. Accumulated arsenic in roots and shoots was measured by standard graph of arsenic.

Chlorophyll estimation (Hiscox and Israeltam 1979): Shoots (100 mg) were taken in test tube and 4 ml of DMSO reagent was added to it. The mixture was kept in a water bath for 1 hour. Absorbance was taken at the wavelength of 645 nm and 663 nm. Blank was prepared using DMSO. Total chlorophyll content was calculated according to the method of Arnon (1949) modified by Rani and Kohli (1991).

$$\text{Total chlorophyll content } (\mu g/ml) = 6.45 \times A663 + 17.72 \times A645 \quad \ldots\ldots A$$

$$\text{Total chlorophyll content } (\mu g/ml) = \frac{\text{A x volume of DMSO (ml)}}{\text{Dry weight of tissue}}$$

Per cent cellular respiration (Steponkus and Lenphear 1967): Leaf tissue (100 mg) of test plant material was dipped in 1.5 ml of freshly prepared triphenyl tetrazolium chloride (TTC) solution. Sample was incubated at room temperature in dark for 18 hours. After incubation TTC solution was drained off from test tubes which were rinsed with distilled water twice. Extraction of samples was carried out with 5 ml of absolute alcohol and the extracted samples were kept in water bath for 20 minutes. Absorbance was recorded at 530 nm and expressed in terms of dry weight equivalent. The cellular respiratory ability was expressed as per cent with respect to control.

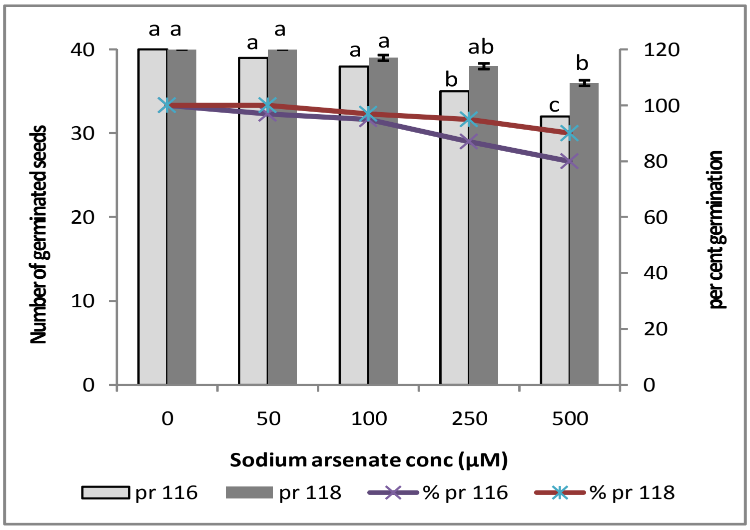
RESULTS

Germination: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 1). 100% germination was reported in control. Arsenic exposure did not affect the seeds of improved varieties (PR 116 and PR 118) of rice to a greater extent. Decline in per cent germination for PR 116 was 3, 5, 13 and 20 at 50, 100, 250 and 500 µM arsenic treatment respectively over the control. However no decline in germination was observed up to 50 µM concentration. Thereafter 3, 5 and 10 per cent decline was reported at 100-500 µM concentration. It shows that heavy metal exposure had negligible effect on improved varieties of rice.

Shoot length: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 2). Gradual decrease in shoot length was observed at higher concentrations (250 and 500 µM). However lower concentrations had no significant effect on rice seedlings of both the varieties. Decline in shoot length for PR 116 was 3, 13, 18 and 24 per cent whilst for PR 118 it was 0.5, 9, 16 and 25 per cent at 50, 100, 250 and 500 µM respectively over the control.

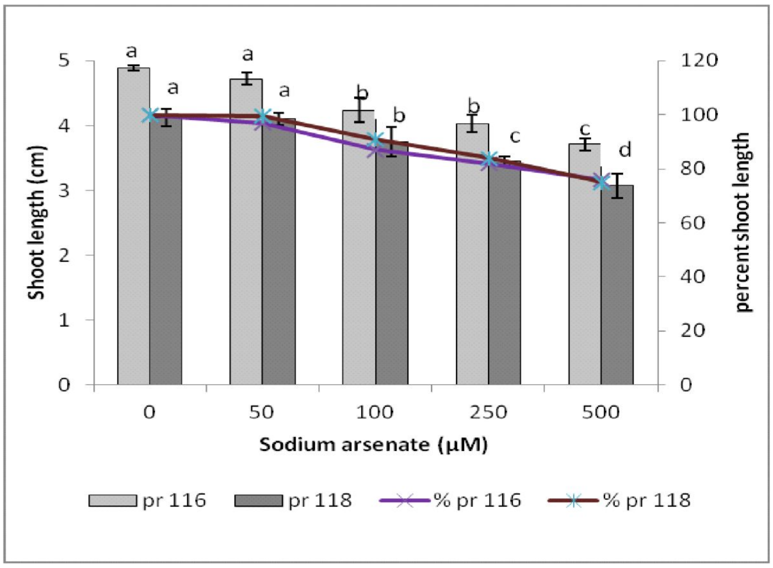
Root length: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 3). Significant reduction in root length was reported for both the varieties. As compared to control decline in root length for PR 116 and PR 118 ranged from 47-93 and 39-96 per cent respectively at 50-500 µM treatment.

Biomass dry weight: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 4). Studies reported significant effect of arsenic exposure on dry biomass in both the rice varieties. Lowest treatment (50 µM) showed 27 and 16 per cent while highest



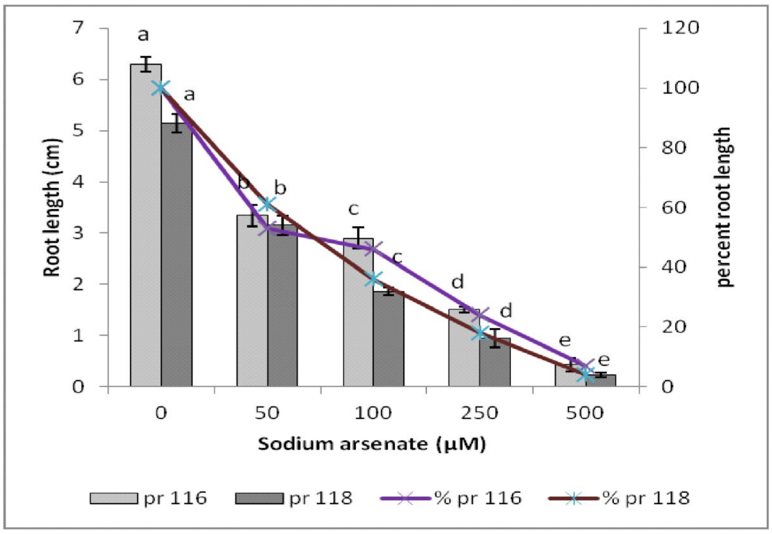
Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 1. Germination of rice seedlings grown on different arsenate concentrations



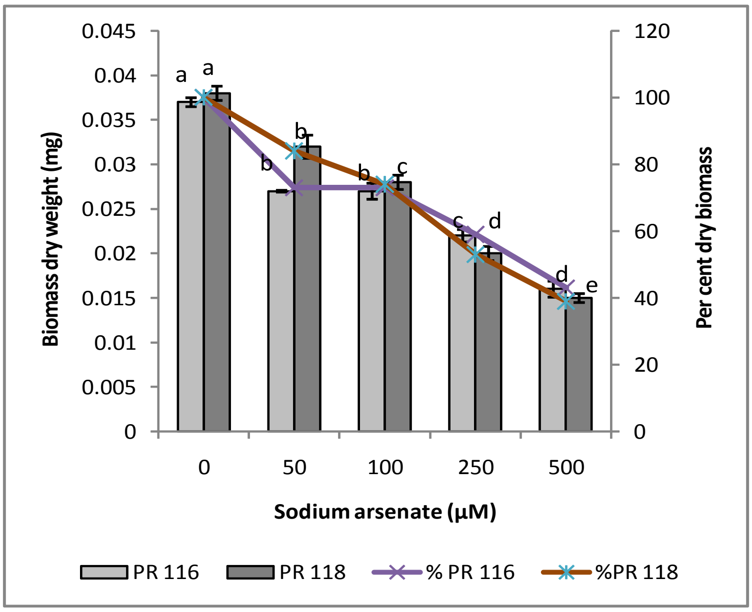
Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 2. Shoot length of rice seedlings grown on different arsenate concentrations



Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 3. Root length of rice seedlings grown on different arsenate concentrations



Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 4. Biomass (DW) of rice seedlings grown on different arsenate concentrations

treatment (500 µM) showed 57 and 61 per cent decline in biomass for PR 116 and PR 118 respectively over the control.

Arsenic accumulation in roots and shoots: Arsenic accumulation in roots was higher than shoots. Average arsenic concentration in roots of PR 116 and PR 118 was 410.11 and 428.38 mg/kg respectively. Similarly shoots of PR 116 and PR 118 accumulated 36.06 and 30.41 mg/kg of arsenic respectively. Arsenic accumulation in above ground tissue (shoot) showed the translocation of this heavy metal into aerial parts (Fig 5).

Chlorophyll content: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 6). Photosynthetic pigments were inhibited in presence of arsenic. Reduction in chlorophyll content was observed in both the varieties. In PR 116 chlorophyll content decreased to 92, 80, 60 and 44 per cent at 50, 100, 250 and 500 µM respectively over the control. Similarly decrease in chlorophyll content for PR 118 was observed to be 94, 84, 63 and 54 per cent respectively at 50-500 µM asrenic treatment over the control.

Per cent cellulart respiration: Data were analyzed by one way ANOVA post hoc multiple comparison Tukey's test (Fig 7). 100 per cent cellular respiration was reported in control of both the rice varieties. In PR 116 cellular respiration was reduced to 84, 71, 59 and 46 per cent at 50-500 µM arsenic treatment respctively over the control. Similar results were observed with PR 118 showing 83-39 per cent reduction at 50-500 µM arsenic treatment as compared to control.

DISCUSSION

Growth and physiology of rice seedlings was affected upon arsenate exposure. The study was carried out to investigate the effect of heavy metal (arsenic) exposure on two improved varieties of *O sativa* L viz PR 116 and PR 118 and led to the conclusion that each parameter was affected with increased arsenic. To test metal toxicity germination is not the commonly used technique. Vegetative response such as root length, shoot height and total biomass are used to study the resistance of plants towards metal toxicity. In the present study germination of rice seedlings was not affected to a considerable extent in response to metal toxicity. Arsenic in higher concentration (500 µM) inhibited the seed germination. The results are in accordance to the work done by previous researchers (Abedin and Meharg 2002, Shri et al 2009) in rice varieties. The results showed inhibitory effect of arsenic on root length and shoot height. Effect of arsenic was more conspicuous on roots as compared to shoots since roots are the first site of contact with arsenic. Similar results were shown by Das et al (2004) and Choudhury et al (2011)

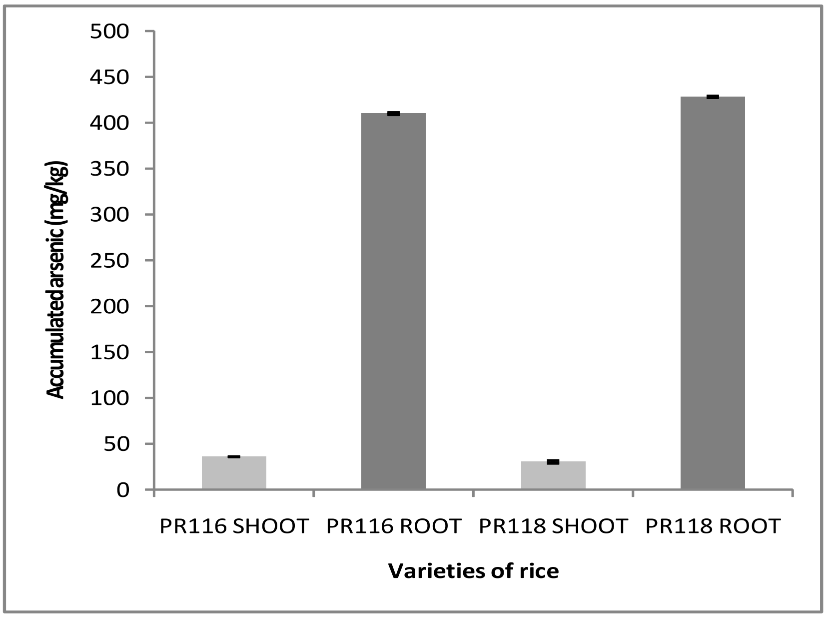
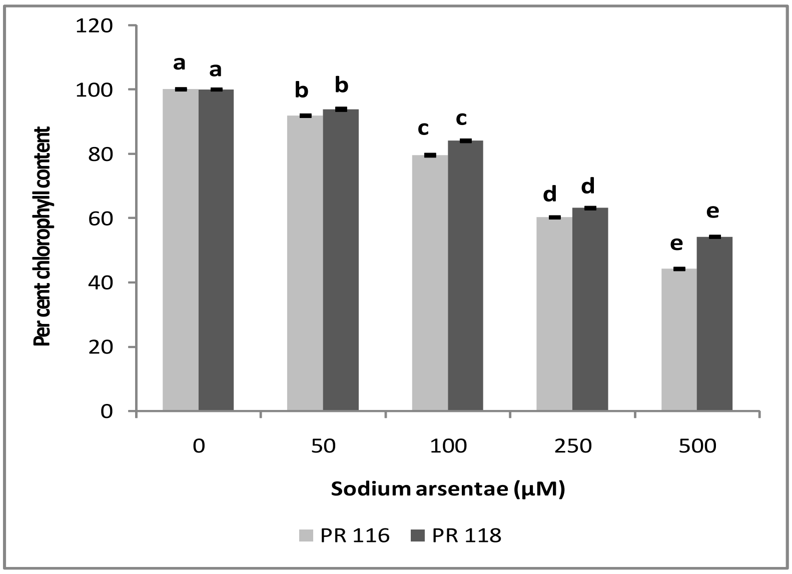
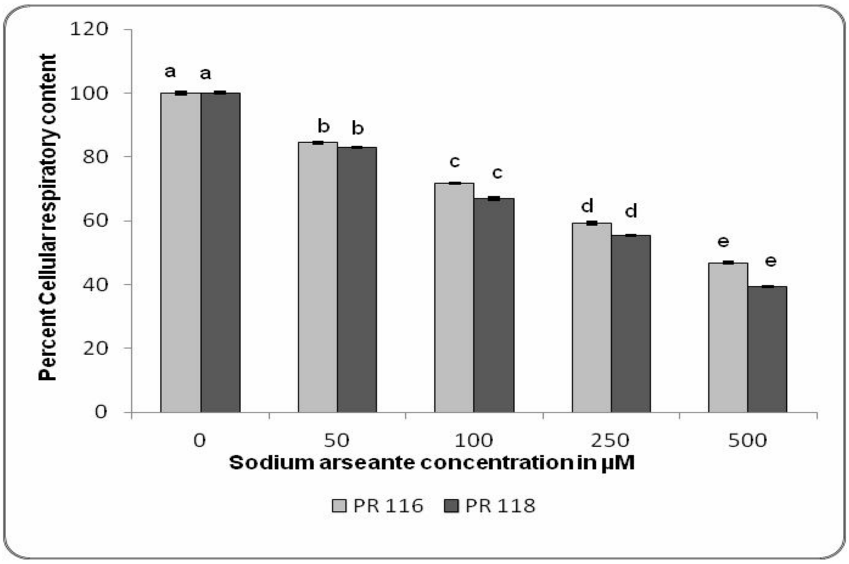


Fig 5. Arsenic accumulation in rice seedlings grown on different arsenate concentrations



Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 6. Chlorophyll content of rice seedlings grown on different arsenate concentrations



Means followed by the same letter columns are not significantly different by Tukey's test at 0.05% level

Fig 7. Per cent cellular respiratory content of rice seedlings grown on different arsenate concentrations

where the inhibition effect was more on roots than shoots. Moreover decrease in plant height leads to biomass reduction. Pigna et al (2008) and Panaullah et al (2009) reported biomass reduction at higher concentration of arsenate. The above findings are based upon the fact of arsenic interference with plant metabolic processes such as phosphorylation and ATP synthesis. ATP production gets reduced which leads to plant growth inhibiton and ultimately to plant death (Garg and Singla 2011). Root and shoot tissue of test plants were tested for arsenic accumulation. Results showed more arsenic accumulation in roots compared to shoots. Similar findings were reported by Rahman et al (2007) showing arsenic accumulation 20 to 75 times higher in roots than shoots. Similar results were supported by previous findings of Liu et al (2004), Liu et al (2006) and Lee et al (2013).

Photosynthetic pigments (chlorophyll content) of the test plants PR 116 and PR 118 showed reduction which was more significant at higher arsenic concentration than at lower level of arsenic. Shaibur et al (2006) and Azad et al (2013) reported similar observations in their studies showing reduced chlorophyll content in plants under arsenic stress. The possible reason could be that As binds with thiol group of protein and gets accumulated in the cell which is responsible for chloroplast disruption and leads to reduced chlorophyll content.

Results of cellular respiratory ability were reported to be in accordance with the findings of Singh et al (2007) and Shao et al (2011). Physiological and biochemical processes were disturbed in presence of heavy metals thereby altering the cellular respiratory ability of the plant.

REFERENCES

Abedin MJ and Meharg AA 2002. Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L). Plant and soil **243(1):** 57-66.

Arnon DI 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant physiology **24(1):** 1-16.

Azad M, Monda A, Hossain I and Moniruzzaman M 2013. Experiment for arsenic accumulation into rice cultivated with arsenic enriched irrigation water in Bangladesh. American Journal of Environmental Protection **1(3):** 54-58.

Carbonell-Barrachina AA, Burl OF, Valero D, Lopez E, Martinez-Romero D, Martinez-Sanchez F 1999, Arsenic toxicity and accumulation in turnip as affected by arsenic chemical speciation. Journal of Agriculture and Food Chemistry **47:** 2288-2294.

Choudhury B, Chowdhury S and Biswas AK 2011. Regulation of growth and metabolism in rice (*Oryza sativa* L) by arsenic and its possible reversal by phosphate. Journal of Plant Interactions **6(1):** 15-24.

Cullen WR and Reimer KJ 1989. Arsenic speciation in the environment. Chemical Reviews **89(4):** 713-764.

Das H, Mitra AK, Sengupta P, Hossain A, Islam F and Rabbani G 2004. Arsenic concentrations in rice, vegetables and fish in Bangladesh: a preliminary study. Environment International **30(3):** 383-387.

Dudka S and Miller W 1999. Accumulation of potentially toxic elements in plants and their transfer to human food chain. Journal of Environmental Science and Health, Part B **34(4):** 681-708.

Garg N and Singla P 2011. Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. Environmental Chemistry Letters **9(3):** 303-321.

Gupta UC and Gupta SC 1998. Trace element toxicity relationships to crop production and livestock and human health: implications for management. Communications in Soil Science and Plant Analysis **29(11-14):** 1491-1522.

Hiscox JD and Israelstam GF 1979. A method for extraction of chlorophyll from leaf tisue without maceration. Canadian Journal of Botany **57:** 1332-1334.

Lee CH, Hsieh YC, Lin TH and Lee DY 2013. Iron plaque formation and its effect on arsenic uptake by different genotypes of paddy rice. Plant and Soil **363(1-2):** 231-241.

Lim HS, Lee JS, Chon HT and Sager M 2008. Heavy metal contamination and health risk assessment in the vicinity of the abandoned Songcheon Au–Ag mine in Korea. Journal of Geochemical Exploration **96(2):** 223-230.

Liu WJ, Zhu YG, Hu Y, Williams PN, Gault AG, Meharg AA, Charnock JM and Smith FA 2006. Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (*Oryza sativa* L). Environmental Science and Technology **40(18):** 5730-5736.

Liu WJ, Zhu YG, Smith FA and Smith SE 2004. Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L) grown in solution culture? Journal of Experimental Botany **55(403):** 1707-1713.

Oremland RS and Stolz JF 2003. The ecology of arsenic. Science **300(5621):** 939-944.

Panaullah GM, Alam T, Hossain MB, Loeppert RH, Lauren JG, Meisner CA, Ahmed ZU and Duxbury JM 2009. Arsenic toxicity to rice (*Oryza sativa* L) in Bangladesh. Plant and soil **317(1-2):** 31-39.

Pigna M, Cozzolino V, Violante A and Meharg AA 2008. Influence of phosphate on the arsenic uptake by wheat (*Triticum durum* L) irrigated with arsenic solutions at three different concentrations. Water, Air and Soil Pollution **197(1):** 371-380.

Rahman MA, Hasegawa H, Rahman MM, Rahman MA and Miah M 2007. Accumulation of arsenic in tissues of rice plant (*Oryza sativa* L) and its distribution in fractions of rice grain. Chemosphere **69(6):** 942-948.

Rani D and Kohli R 1991. Fresh matter is not an appropriate relation unit for chlorophyll content: experience from experiments on effects of herbicide and allelopathic substances. Photosynthetica **25:** 655-657.

Shaibur MR, Kitajima N, Sugawara R, Kondo T, Huq SI 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.

Shao Y, Jiang LN, Zhang DJ, Ma LJ and Li CX 2011. Effects of arsenic, cadmium and lead on growth and respiratory enzymes activity in wheat seedlings. African Journal of Agricultural Research **6(19):** 4505-4512.

Shri M, Kumar S, Chakrabarty D, Trivedi PK, Mallick S, Misra P, Shukla D, Mishra S, Srivastava S, Tripathi RD and Tuli R 2009. Effect of arsenic on growth, oxidative stress and antioxidant system in rice seedlings. Ecotoxicology and Environmental Safety **72(4):** 1102-1110.

Singh HP, Batish DR, Kohli RK 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(1):** 65-73.

Smedley P and Kinniburgh D 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Applied Geochemistry **17(5):** 517-568.

Smith E, Naidu R and Alston AM 1998. Arsenic in the soil environment: a review. Advances in Agronomy **64:** 149-195.

Steponkus PL and Lanphear FR 1967. Refinement of the triphenyl tetrozolium method for determining cold injury. Plant Physiology **42:** 1423-1426.

Zhao FJ, McGrath SP and Meharg AA 2010. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Annual Review of Plant Biology **61:** 535-559.

Zhu YG, Williams PN and Meharg AA 2008. Exposure to inorganic arsenic from rice: a global health issue? Environmental Pollution **154(2):** 169-171.

Received: 23.2.2015 ***Accepted: 13.5.2015***