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Influence of Arsenic on Rice Growth and its Mitigation with Different Water Management Techniques

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ABSTRACT
Rice is the staple food for more than half of the world's population. Arsenic (As) contaminated irrigation water (groundwater) is a threat to irrigated rice cultivation. A pot study was conducted to mitigate Arsenic (As) problem in rice by two water managements (aerobic and anaerobic) practices. In this study two rates of As (0 and 40 mg kg^{-1}) were applied as; aerobic As_0, anaerobic As_0, aerobic As_{40} and anaerobic As_{40} and other two treatments were kept as aerobic As_{40a} (before flowering anaerobic and after flowering aerobic) and anaerobic As_{40b} (before flowering aerobic and after flowering anaerobic). A rice variety, BRRI dhan28, was grown. Application of 40 mg kg^{-1} As significantly affected plant growth in anaerobic condition. The higher seedling survibility, filled grain and yield were in aerobic condition. The tissue analysis showed that plant grown in aerobic condition contained less contents of As in root (20%), straw (97%) and grain (61%). Thus, aerobic rice cultivation can be a sustainable technology to mitigate arsenic problem in rice at arsenic contaminated soil.

Key words: Aerobic condition, anaerobic condition, contaminated soil, ground water, irrigation

INTRODUCTION
Food security is the condition of having enough food to provide adequate nutrition for a healthy life and nowadays it becomes a critical issue for the developing world. Nearly half the world's population depends on rice for their survival. In Asia as a whole, much of the population consumes rice in every meal. Rice is also the staple food crop of Bangladesh and farmed in watery conditions. Farmers are applying imbalanced doses of fertilizer, insecticide and pesticide to their rice field to harvest maximum yield from their small piece of land which is creating environmental pollution as well as one of the causes of climate change. Besides these consequences rice cultivation with arsenic contaminated irrigation water brought another risk for human health. The higher arsenic contents have been observed in the top layer of soil (Haq *et al.*, 2013). The major cause of As contamination in soil is the mining of coal and oil as well as mining and metallurgy of non-ferrous metals (Lozna and Biernat, 2008). Furthermore, drinking water is the source of arsenic-rich rocks through which the water has filtered. The practice of groundwater for irrigation purpose has increased As concentrations and approximately 80% of pumped groundwater is being applied for crops. Therefore, it has a possibility of arsenic accumulation in rice and rice plants from irrigation water (Delowar *et al.*, 2005). Long term use of arsenic contaminated ground water for irrigation

accumulating As in the agricultural soil and eventually in rice plant. The added arsenic gradually accumulates in the topsoil and amounts now appear to be reaching toxic levels to rice in some soils that had been irrigated with highly As contaminated water for 10-20 years or more (Brammer, 2007).

Rice plant grown in As contaminated soil accumulated arsenic in grain and straw. In some cases, human As intake from the consumption of rice exceeds that from drinking water (Williams *et al.*, 2006). So rice is a major source of dietary intake of inorganic As among the rice feeding population. Therefore, necessary steps should be taken to mitigate the excessive accumulation of As in rice plant. Many areas of Bangladesh are already contaminated with arsenic and in dry season and there is no other option for irrigation except As contaminated ground water. Some studies showed that rice grown under flooded conditions accumulated more As than that grown under aerobic condition. Contamination by As occurs greater in rice plant than other upland crops because of the anaerobic conditions that lead to arsenic mobilization causing enhanced bioavailability to rice (Takahashi *et al.*, 2004). Although, rice plant need trace amount of arsenic for its growth and development, excessive amount causes physiological disorder that finally reduced crop yield. Study reports proved that higher amount of As significantly reduced plant height, effective tiller number, straw weight and grain yield of rice (Wang *et al.*, 2006; Hu *et al.*, 2013).

Bangladesh is a densely populated agriculture based country and facing intense pressure to grow more food using existing facilities. There is no other option for rice production except in arsenic contaminated soil or contaminated irrigation water. A solution is needed in order to grow rice in this soil and water with minimum arsenic accumulation in paddy and straw. The main objective of the present study was to investigate the effect of As on rice growth and its mitigation by water management techniques.

MATERIALS AND METHODS

Experimental site, soil and treatments: Experiment was conducted in September, 2013 at a greenhouse of the Soil Science Division (SSD), Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh. Two rates of arsenic (As_0 and As_{40} mg kg^{-1}) with two different water management practices (aerobic and anaerobic) were selected. The treatments were: (1) Aerobic As_0, (2) Anaerobic As_0, (3) Aerobic As_{40}, (4) Anaerobic As_{40}, (5) Aerobic As_{40a} (before flowering anaerobic and after flowering aerobic) and (6) Anaerobic As_{40b} (before flowering aerobic and after flowering anaerobic). The last two treatments were selected to evaluate the effect of irrigation after flowering on grain yield of rice grown in high soil As condition. The experiment was conducted with a completely randomized design with 4 replications. The pot size was 22.5 cm high×28.5 cm diameter and each filled with 6 kg air dried; sieved (2 mm) soil. Soil of the experimental field is clay loam in texture. Initial properties of the surface soil (0-15 cm depth) were as follows: pH 6.04, organic carbon 1.2%, available phosphorus (P) 8.7 mg kg^{-1} (0.5 M $NaCO_3$ extracted) and exchangeable potassium (K) 0.26 meq/100 g soil (Neutral 1.0 N NH_4OAc extracted). Soil for the pot study was collected from an As contaminated area in Bangladesh contains 3.21 mg kg^{-1} As. Five seedlings of BRRI dhan46 (Twenty five days old) were uprooted and transplanted in each pot. A concentration of 40 mg kg^{-1} arsenic was prepared by dissolving $AsNaO_2$ in distilled water and mixed in to the soil before transplanting the rice seedlings. The greenhouse was kept almost outside temperature, light and moisture.

Water management and fertilizer application: Water was applied to all pots for seedling establishment and maintained according to respective. For aerobic condition, water was maintained at field capacity by drain out excess water and for anaerobic the pots were maintained flooded throughout the growth. Fertilizers were given as equivalent (calculated by the soil weight basis) of 115, 20, 60 kg ha^{-1} of N, P and K in the form of urea, Triple Super Phosphate (TSP) and Muriate of Potash (MOP), respectively. The full amount of TSP and MOP were applied during soil preparation and one third of urea was applied after 7 days of transplanting. The remaining quantity of urea was applied at plant tillering and heading stages.

Harvesting: Rice plants were harvested at physiological maturity stage. The quantitative characters like plant height, tiller number, effective tiller number, panicle length, number of spikelets and number of filled grain, sterility percentage, 1000 grain weight and yield were recorded.

Arsenic determination in the rice plant and soil: At harvesting time, root, straw and grain samples were collected and dried by keeping in oven (80°C) for three days. Total As content was determined by digesting the soil, root, paddy and straw samples with tri-acid mixture (HNO_3:$HClO_4$:H_2SO_4, 5:2:1) until it became whitish or clear. Both the initial and post-harvest soil samples were collected for As analysis following the same procedure.

Sample preparation and digestion: Rice samples were sun-dried and about 1 g samples were taken separately into digestion tube and 10 mL of 69% concentrated nitric acid and 70% of perchloric acid mixture at the ratio of 5:3 was added. The samples were left to react overnight in a chemical hood and then heated in a block digester (M-24 plazas/samples, JP Selecta, Spain) at 120°C until colourless clear watery fluid appeared. Tubes were gently shaken several times to facilitate destroying all the carbonaceous material. This digestion converts all arsenicals to inorganic arsenic for FI-HG-AAS determination. Tubes were removed from the digestion block, cooled, diluted to 50 mL adding Millipore water, filtered through filter paper and stored in 50 mL plastic bottles.

Clean and oven-dried rice straw samples were digested as described by Wang *et al.* (2006) with modifications. About 0.45-0.50 g rice straw was weighed after further drying at 60°C to constant weight. It was taken separately into digestion tube and 7 mL of 69% concentrated nitric acid was added and similar procedures were followed as before.

Detection of arsenic: The digest was cooled and then filtered and finally the volume was made up to 50 mL. Concentrations of arsenic in digested samples were determined using Atomic Absorption Spectrophotometer (AAS), model PG-990 equipped with a computer with Atomic Absorption (AA) Win software (PG Instruments Ltd., UK) as described by Samanta *et al.* (1999). Briefly, samples were spiked with standards at different concentrations. For constructing standard curve, working standards of 0, 2.5, 5, 10, 15 and 20 ppb were prepared immediately before use by serial dilution of the stock in 10% hydrochloric acid. Samples exceeding the standard curve range were diluted again and analyzed further. The concentration of arsenic in those samples was calculated by multiplying the appropriate dilution factor. Sample solution concentrations were determined by direct comparison with the calibration curve and the reading was automatically transferred to AA Win software. Concentration of arsenic in the sample was calculated from the following equation:

$$\text{As concentration (ppm)} = \frac{\text{Concentration of As in sample solution (L)}\times\text{mL of sample}}{\text{Sample weight (g)}}\times 1000$$

Statistical analysis: The data was analyzed using SPSS 16.0-a statistical software and Microsoft Excel 2010. The analysis of variances (ANOVA) for rice growth and yield parameters of BRRI dhan46 were done for Least Significance Difference (LSD). Duncan's Multiple Range Test (DMRT) was used for mean comparisons of the treatments at 5% probability level.

RESULTS AND DISCUSSION

Survivability of transplanted seedling and vegetative growth of rice: Transplanted seedlings were grown in each experimental pot with the selected treatments and found in various responses. Normal vegetative growth and development was observed among all the seedlings grown in aerobic and anaerobic conditions at As control treatment. On the other hand, abnormal growth or even mortality of seedling was noticed in both water management conditions at 40 mg kg^{-1} arsenic (As_{40}) treated soil. Compared with both water management systems the seedling mortality rate was higher in the anaerobic than aerobic condition. At 21 Days after Transplanting (DAT) only 3 seedlings were survived at 40 mg kg^{-1} arsenic in anaerobic condition, however at the same time, more than 4 seedlings were survived in aerobic treatment. With the time being, the toxic effect of As become more prominent in both the aerobic and anaerobic condition and at 45 DAT only 2-3 seedlings per pot were alive (Fig. 1). Moreover, comparatively better growth and survivability of the rice plant was found in aerobic condition with 40 mg kg^{-1} arsenic than that of anaerobic state with the same level of applied arsenic (Fig. 2).

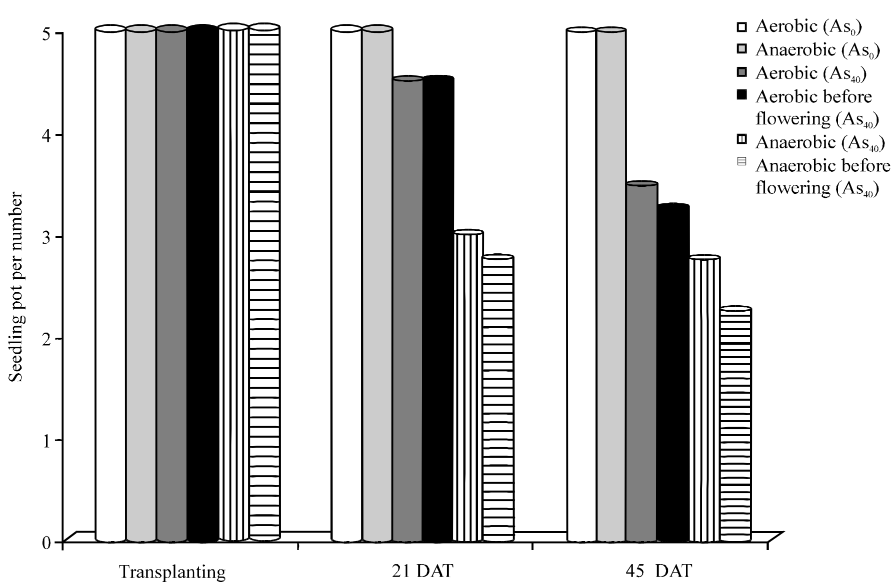

Fig. 1: Survivability of transplanted seedling with and without As treated soil at different water management up to 45 DAT

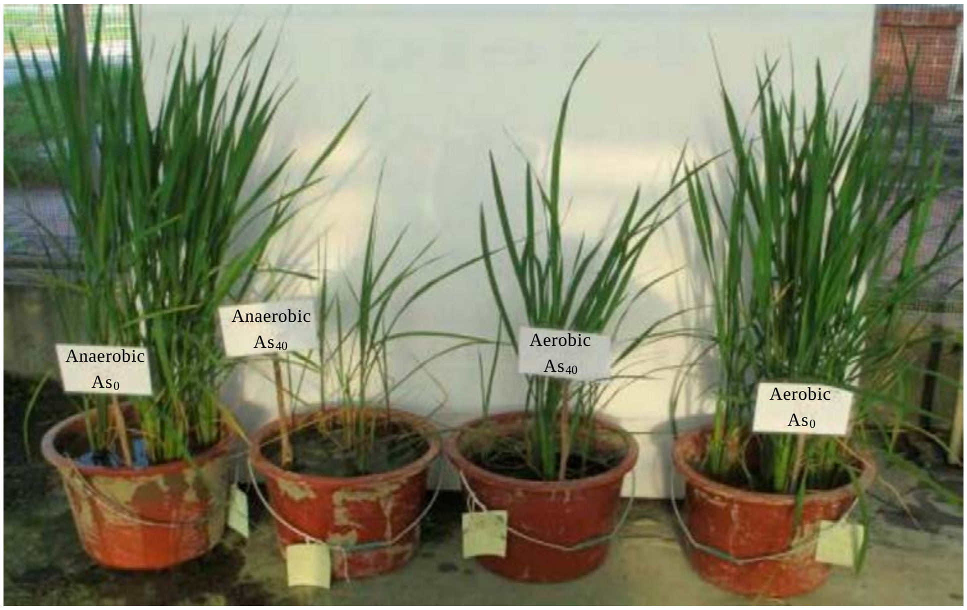

Fig. 2: Effect of arsenic on vegetative growth of rice at different water management practices

Table 1: Effect of different levels of As on different parameters of BRRI dhan46

Treatment	Plant height (cm)	No. of tiller per pot	No. of panicle per pot	Grain wt. per pot (g)	Straw wt. per pot (g)
Aerobic (As_0)	93.0^a	17.0^b	16.0^a	21.0^a	66.0^a
Anaerobic (As_0)	94.0^a	18.0^a	16.0^a	20.0^a	69.0^a
Aerobic (As_{40})	81.0^b	11.0^d	9.0^b	13.0^b	37.0^d
Anaerobic (As_{40})	55.0^d	6.0^e	3.0^c	3.0^c	13.0^e
Anaerobic before flowering* (As_{40a})	64.0^c	5.0^e	3.0^c	2.0^c	14.0^e
Aerobic before flowering** (As_{40b})	78.0^b	14.0^c	10.0^b	13.0^b	43.0^c
CV (%)	6.0	7.9	11.8	13.5	7.9

Mean values without common letters are statistically significant (DMRT, $p \leq 0.05$), *Before flowering anaerobic and after flowering aerobic, **Before flowering aerobic and after flowering anaerobic

Arsenic contamination reduced tiller production in rice plant. Maximum tiller number (18) was found in the pots treated with As_0 in anaerobic state, on the contrary minimum tillers (5) per pot was observed with anaerobic As_{40} which was followed by anaerobic before flowering As_{40b} treatment. It indicated that higher arsenic content significantly decreased tiller number per pot (Table 1 and Fig. 3). Similar findings were observed by the Azad *et al.* (2012) that the highest values of plant height and straw yield was observed at lower As concentrations however, the highest tillers number, panicles number, panicle length and grain yield were found in control treatment.

High soil As had profound effect on the plant height reduction. Similar reports were observed by Abedin *et al.* (2002) that As contaminated irrigation water significantly reduced the plant height. In our study, the plant height was found to be reduced significantly in 40 mg kg^{-1} of As treated soil in both water management practices. More significant reduction was observed in anaerobic As_{40} (55 cm) and anaerobic before flowering As_{40b} (64 cm) treatments than that of aerobic treatments with all the conditions. However, the tallest plant height was observed in anaerobic As_0 treated pot where the inherent soil arsenic was 3.21 mg kg^{-1}.

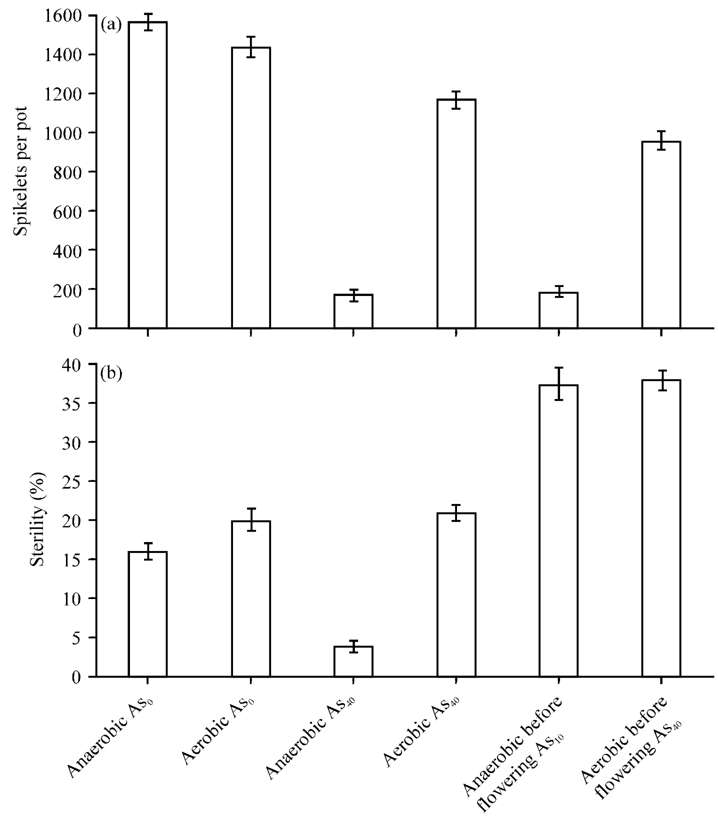

Fig. 3(a-b): Effects of different As treatments on spiklets and% sterility of rice. Bars indicate standard error, n = 4

Yield and yield contributing characters: Arsenic significantly affected rice yield and yield contributing characters. Rice yield decreased when grown in As contaminated soils. Similarly, Dilday *et al.* (2000) reported more straight head with blank florets and distorted lemma and palea of rice due to arsenic (6.8 ka ha^{-1}) contamination. In general, detrimental effect was more prominent when rice was grown in anaerobic environment compared to aerobic state (Fig. 4). Application of As 40 mg kg^{-1} in each pot showed a strong negative influence on the panicle number, filled spikelet, grain and straw yield as compared to As_0 treatment (Table 1). Detrimental effect of As application on panicle number and grain yield was also reported by Islam *et al.* (2004) and Liu and Goa (1987). In contrast the adverse effect of As contamination on yield was lower in aerobic condition than anaerobic condition. Even though, the yield in As treatment under aerobic condition was significantly less than in control treatment, the yield was higher than that in anaerobic condition. This indicates that, high level of As had suppressing effect on rice yield, but aerobic condition alleviated the suppressing effect to some extent. Irrigation after flowering of plant initially grown in aerobic condition affected the yield. Higher number of spikelets per pot and less sterility percentage were obtained under aerobic condition compared to anaerobic condition and this ultimately reflected on higher rice yield (Fig. 5).

Plant uptake and soil arsenic content: Arsenic uptake by plant depends on its availability in the soil system and As accumulation in different plant parts increasing with increased the As

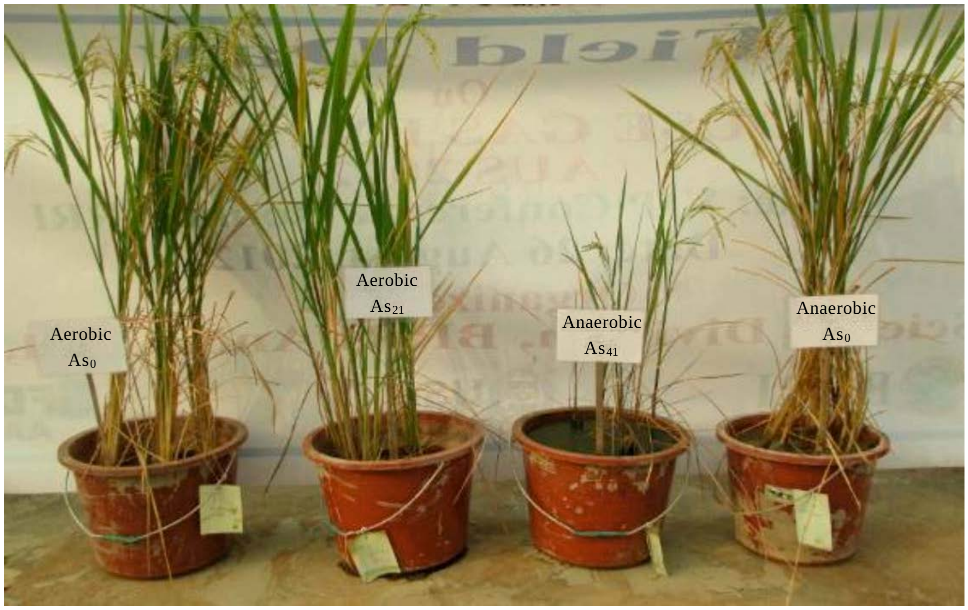

Fig. 4: Effect of arsenic on reproductive growth of rice at different water management practices

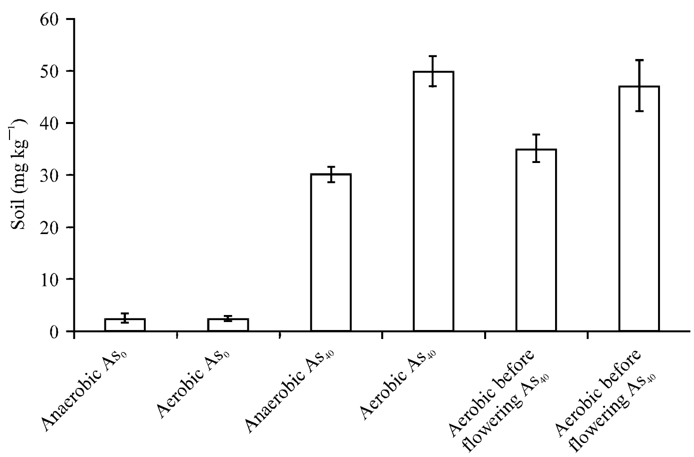

Fig. 5: Concentration of As in soil at harvest at different treatment

concentrations (Wang *et al*., 2006; Hu *et al*., 2013). It was reported that flooded paddy soil lead to soil As mobilization and enhanced bioavailability for plant (Xu *et al*., 2008; Takahashi *et al*., 2004). In the present study, higher As content was found in rice root than straw followed by the grain irrespective of As addition (initial soil As was 3.21 mg kg^{-1}) (Table 2). In anaerobic condition the accumulation of As in the rice roots and show significantly higher than that in aerobic condition even without addition of As in soil. Plants grown in aerobic before flowering also uptake less As (13% in grain, 94% in straw and 23% in grain) compared to plant subjected to anaerobic condition after flowering (Table 3). Obviously, the increase of As in different parts of rice plant was very high in anaerobic condition with aerobic situation which ultimately influenced rice yield parameters and drastically decreased yield. It was found that the anaerobic condition in soil released iron oxides

Table 2: Arsenic content in root, straw and grain of BRRI dhan46 with and without As treated soil

	Arsenic content (ppm)		
Treatment	Root	Straw	Grain
Aerobic (As_0)	10 ± 0.83^e	0.39 ± 0.10^b	0.10 ± 0.01^c
Anaerobic (As_0)	38 ± 22.63^d	1.14 ± 0.83^b	0.11 ± 0.06^c
Aerobic (As_{40})	208 ± 2.28^{bc}	4.79 ± 1.76^b	0.41 ± 0.13^{bc}
Anaerobic (As_{40})	259 ± 24.06^a	180.00 ± 65.57^a	1.04 ± 0.29^a
Anaerobic before flowering* (As_{40a})	232 ± 23.79^b	154.00 ± 99.28^a	0.60 ± 0.37^b
Aerobic before flowering** (As_{40b})	201 ± 2.76^c	10.00 ± 2.31^b	0.46 ± 0.11^b

Values (Mean± SD) without common letters are statistically significant (DMRT, $p\leq0.05$), *Before flowering anaerobic and after flowering aerobic, **Before flowering aerobic and after flowering anaerobic

Table 3: Plant tissue and grain arsenic content decreased (%) in aerobic condition compared to anaerobic cultivation method

	As decrease in aerobic compared to anaerobic (%)		
Treatment	Root	Straw	Grain
Aerobic (As_0)	74	66	9
Anaerobic (As_0)	-	-	-
Aerobic (As_{40})	20	97	61
Anaerobic (As_{40})	-	-	-
Anaerobic before flowering* (As_{40a})	-	-	-
Aerobic before flowering** (As_{40b})	13	94	23

*Before flowering anaerobic and after flowering aerobic, **Before flowering aerobic and after flowering anaerobic

that may also increase inorganic As content. Li *et al.* (2009) proved a rapid rise in As concentration as Eh dropped below 200 mv. On the other hand, in the oxidative condition and also due to rice root respiration, iron plaque form in the rhizosphere resulting oxidation of ferrous iron to ferric iron and the precipitate iron oxide on the root surface. It might be due the retention of applied As is generally important, since most of the As remained in the rooting zone, where it would have the greatest direct impact on plant processes (Khan *et al.*, 2009). The Fe oxides have strong adsorptive capacity for arsenate that reduce the availability for plant uptake (Chen *et al.*, 2005). Arsenic uptake by rice root can be influenced by the presence of dominant species in soil solution, root morphology, presence of Fe, P and Si (Sun *et al.*, 2008; Abedin *et al.*, 2002).

Higher amount of As was retained in post-harvest soil of aerobic condition compared to anaerobic condition (Fig. 5). This indicates that anaerobic condition may increase the soil As availability and create a congenial situation for higher uptake by the plants resulting in poor plant growth and yield.

CONCLUSION

It may be concluded that As has a detrimental effect on plant growth and yield of rice. In high As condition, the growth of rice plant was comparatively better under aerobic than anaerobic condition because of less As uptake by plant and higher As retention in soil. Aerobic condition does not create a congenial atmosphere of bio-available As in rice root rizosphere for plant uptake. Thus, in high As prone area rice can be cultivated under aerobic condition to minimize As uptake and obtained better yield compared to under flooded condition.

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