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Bioaccumulation of arsenic (As) and phosphorous by transplanting Aman rice in arsenic-contaminated clay soils

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Abstract

Arsenic (As) pollution in soil and water has aroused a considerable attention. Inorganic forms of arsenic are associated with various cancers and cause major health problems. An experiment was conducted to investigate the effect of soil arsenic (As) concentration on arsenic and phosphorous accumulation in root, straw, husk and grain of rice (*Oryza sativa*). BRRI dhan 33 and BR11 rice varieties were planted on six levels of As-contaminated growth media (T1=3.2, T2=11.6, T3=18.7, T4=38.6, T5=57.8 and T6=80.3 ppm) in a Completely Randomized Design (CRD) with six replications. Accumulation of arsenic in root, straw, husk and rice grain significantly increased when As dose increased in soil media. It was observed that As was highly concentrated in the roots, whereas phosphorous was highest in the grain. The T6 treatment (80.3 ppm) showed the highest As accumulation in different plant tissues. Arsenic contents in grain and husk of BR 11 were found higher than those of grain and husk of BRRI dhan 33. The straw and root of BRRI dhan 33 showed higher concentration of As than straw and root of BR 11. Phosphorous concentration of straw, husk and grain were also increased in higher As concentrations. In this study, rice plant showed high bioconcentration factor (BCF) and low translocation factor (TF), whereas As content in grains did not exceed the maximum permissible limit of 1 mg As kg^{-1}, but straw As is highly risky for animal health as well as human food-chain. It could be concluded that BRRI dhan 33 can be cultivated instead of BR11 in As contaminated soil.

Keywords*:* Soil, BRRI dhan 33, BR11, Arsenic accumulation, Bioconcentration, Translocation.
Abbreviations: As_Arsenic, AEZ_Agro ecological zone, BSMRAU_Bangabandhu Sheikh Mujibur Rahman Agricultural University, MPL_Maximum permissible limit, TF_Translocation factor, BCF_Bioconcentration factor.

Introduction

Rice (*Oryza sativa*) is the principal cereal crop in Bangladesh that plays the most important role in the national economy. Rice contributes roughly 73% of the calorific and 66% of the protein intake (Alam et al., 2002). Bangladesh is a delta of high arsenic (As) contamination in groundwater and the water being widely used for irrigation. Total As concentrations ranges from 5.0 to 33 mg kg^{-1} with an average of 17 mg kg^{-1} which has been reported for some soil samples in Nawabganj, Rajarampur, Jessore, Jhenidah and Comilla regions (Islam et al., 2000). Arsenic uptake and accumulation by plant is significantly influenced by As concentration in the soil or growth media and substantially increased with increasing As levels (Marin et al., 1992, 1993b; Xie and Huang, 1998). Arsenic concentration in rice plant parts was in the order of root>straw>husk> whole grain> husked rice (Xie and Huang, 1998). Highest arsenic accumulation was reported in roots than other parts (Odanaka et al., 1987; Marin et al., 1992). Concentrations of As were reported as 5.8 to17.7 mg kg^{-1} (mean 11.2 mg kg^{-1}) in soil , 1.48-17.6 mg kg^{-1} (mean 5.88 mg kg^{-1}) in straw and 0.241-1.298 (mean 0.759 mg kg^{-1}) in rice grain (Islam et al., 2000). Rice grain generally has lower As concentration (Heitkemper et al., 2001) and the concentration remains below maximum permissible limit (MPL) of 1 mg As kg $^{-1}$. However, Xie and Huang, (1998) found higher As concentration in rice grain (husked rice) in some cultivars which exceedes 1 mg As kg^{-1} of MPL when grown in As contaminated soils. Some authors reported translocation of As in the plant systems (Benson et al., 1981; Nissen and Benson, 1982), but information on rice plant is limited. In a chemical aimed study, Odanaka et al. (1987) showed evidence of little translocation of As in rice shoot and root. However, recent studies (Schoof et al., 1998, 1999; Heitkemper et al., 2001) on rice grain show that rice grain may contain a considerable proportion of organic As forms. Uptake, accumulation, and phytotoxicity may also differ depending on the cultivar used. For example, when 11 rice cultivars were planted in As-polluted paddy field, there were significant differences among the cultivars for uptake and accumulation of As in different plant parts (Xie and Huang, 1998). Moreover, phosphate plays an important role in As behaviour under aerobic conditions, where arsenate (AsV) is the dominant As form (Lambkin and Alloway, 2003). Phosphate has been reported to displace adsorbed As from soils (Peryea, 1991). Several studies have shown that phosphate, which is an analogue of arsenate, compete with

Table 1. Physico-chemical properties of the experimental soils

Soil properties	Location					
	Gazipur soil	Faridpur soil	Elevated As contaminated soils			
Total As (µg g^{-1})	3.2	11.6	18.7	38.6	57.8	80.3
Texture	Clay	Clay	Clay	Clay	Clay	Clay
pH	5.6	7.2	7.2	7.2	7.5	7.6
Organic matter (%)	1.81	1.93	2.29	2.29	1.89	2.30
Total N (%)	0.12	0.11	0.11	0.12	0.11	0.12
Available P (µg g^{-1})	8.00	9.55	12.60	23.99	37.81	68.10
Exchangeable K (me/100g soil)	0.15	0.23	0.23	0.24	0.23	0.24
Available S (µg g^{-1})	6.90	10.18	17.99	33.43	16.17	20.71
Exchangeable Ca (me/100g soil)	11.20	20.00	20.40	21.00	21.60	21.6
Exchangeable Mg (me/100g soil)	1.37	2.03	2.28	2.18	2.08	2.08
Available Zn (µg g^{-1})	4.52	0.52	0.61	0.53	0.44	0.48
Available Cu (µg g^{-1})	2.97	4.04	4.85	4.05	4.00	4.13
Available Fe (µg g^{-1})	98.80	42.50	73.90	64.10	77.20	145.20
Available Mn (µg g^{-1})	26.20	17.60	15.40	19.20	19.70	14.70

Fig 1. Check soil and back ground soil samples collected from Gazipur and Faridpur districts of Bangladesh as shown by arrow (⇨)

arsenate for binding sites on the iron oxides surface, resulting in a large reduction of arsenate adsorption (Ryden et al., 1987; Meharg and Macnair, 1990, 1992; Manning and Golberg, 1996). Although P has increased As solubility, but desorption of As depends on the soil type. For example, As concentration in a volcanic soil solution did not change after addition of P (Peryea, 1991). Therefore, it is important to establish the relationship between As and P accumulation in rice plant parts and the As in soil and the environment of transplant Aman season. The present study was initiated with the following objectives: (1) to evaluate As absorption and translocation in *Oryza sativa* plant parts (2) to compare the As concentrations in rice plant parts between the two economically important verities and (3) to establish the relationship between As and P accumulation in rice plant parts in different levels of As in soil.

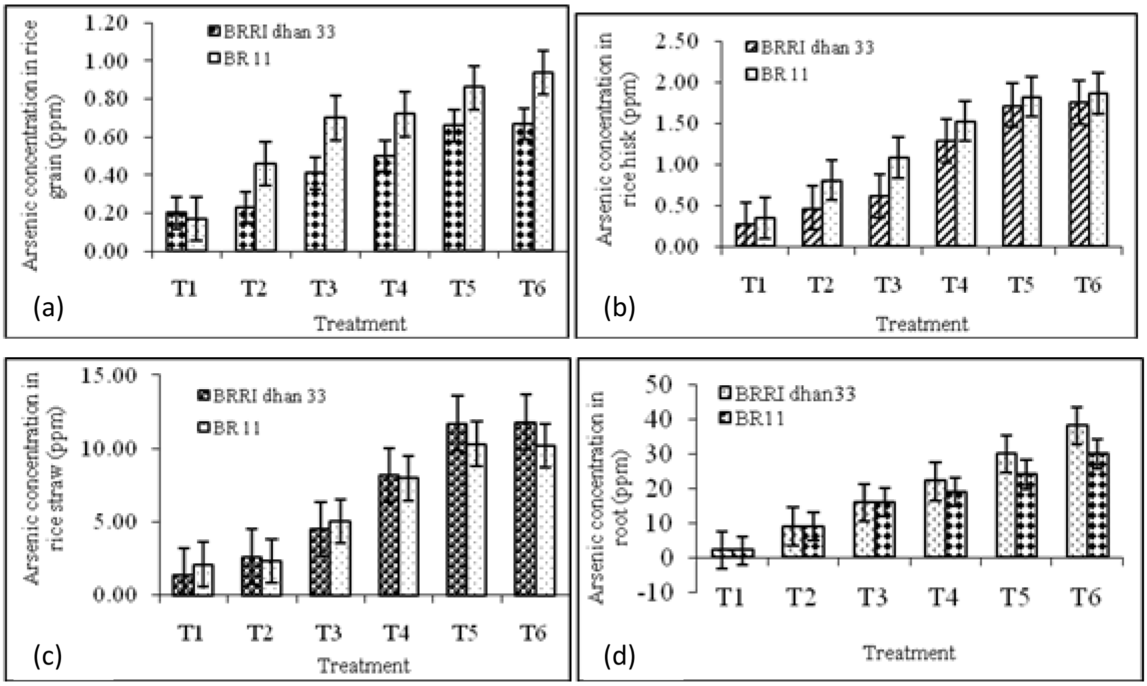

Fig 2. Arsenic accumulation in rice plant parts as influenced by different levels of arsenic in the growth media ($P \leq 0.05$).
T1 =3.2 ppm, T2=11.6 ppm, T3=18.7 ppm, T4= 38.6 ppm, T5= 57.8 ppm and T6= 80.3 ppm. Error bars represent ± SE ($P \leq 0.05$)

Results and Discussion

Arsenic accumulation in rice plant

Arsenic (As) concentrations in root, straw, husk and grain of transplant Aman rice were increased significantly ($P \leq 0.05$) when soil As concentration raised (Fig. 2). The As concentrations ranged from 2.2 to 38 mg kg^{-1} in root, 1.37 to 11.80 mg kg^{-1} in straw, 0.28 to 1.76 mg kg^{-1} in husk and 0.20 to 0.67 mg kg^{-1} in grain of BRRI dhan 33 (Figs. 2a-2d). The lowest As concentrations were observed in root, straw, husk and grains of check (blank) soil, whereas the highest found in the T6. As concentrations in BRRI dhan 33 rice root (R^2=0.97), straw (R^2=0.933), husk (R^2=0.933) and grain (R^2=0.900) were positively and significantly correlated with soil As content (Fig. 3). In BR11 cultivar, As concentrations ranged from 2 to 30.0 mg kg^{-1} in root, 2.10 to10.30 mg kg^{-1} in straw, 0.35 to 1.87mg kg^{-1} in husk and 0.17 to 0.94 mg kg^{-1} in grain (Figs. 2a-2d). The maximum As concentration in root, husk and grain was found in T6 followed by T5 and T4. The highest As concentration in the straw was recorded in T5. The minimum As concentration was observed in the check soil. The increased As accumulation in root, straw, husk and grain of the rice varieties might be due to the increase in soil As concentrations. Xie and Huang, (1998) reported that arsenic absorption and accumulation is greatly influenced by arsenic concentration in the growth media and increases in higher arsenic levels. As concentrations in BR11 rice root (R^2=0.91), straw (R^2=0.891), husk (R^2=0.872) and grain (R^2=0.776) were also positively and significantly correlated with soil As concentration (Fig. 3a-3d). It was observed that As concentration in grain and husk of BR11 was higher than grain and husk of BRRI dhan 33 (Fig. 2a-2b). Therefore, in human health, grain of BRRI dhan 33 can be consumed safer than grain of BR11. On the other hand, As concentration in straw and root of BRRI dhan 33 is higher than straw and root of BR11. Therefore, it is not recommended that straw of BRRI dhan 33 be used as fodder of animals.

Some authors reported that the absorbed As by root can readily be transported to aerial parts, causing an elevated leaf and shoot concentrations (Tsutsumi, 1980). This study showed that As contents in BRRI dhan 33 and BR 11 followed a similar order as root>straw>husk>grain. Similar findings also proposed by Xie and Huang, (1998) that arsenic concentration pattern in rice plant parts generally followed a trend as root>straw>husk>whole grain>husked rice. It was observed that concentrations of As in rice straw in all soil treatments were above 1.37 mg kg^{-1}, which can go up to 11.80 mg kg^{-1}. Abedin et al., (2002a & 2002b) found that concentrations of As in straw raised up to 91.8 mg kg^{-1} due to continuous application of As (8 ppm) contaminated irrigation water. On the other hand, As concentration in grains at all As soil treatments were 1 mg kg^{-1}, below the MPL (Xie and Huang, 1998). Islam (2002b) also found that arsenic concentration in straw was 10-15 times higher than rice grain.

Phosphorous accumulation in rice straw, husk and grain

The phosphorous (P) concentrations in straw, husk and grain of Transplant Aman rice were varied with soil As concentration (Figs.4a-4c). Phosphorous concentrations ranged from 0.042 to 0.068%, 0.048 to 0.060% and 0.261 to 0.279%

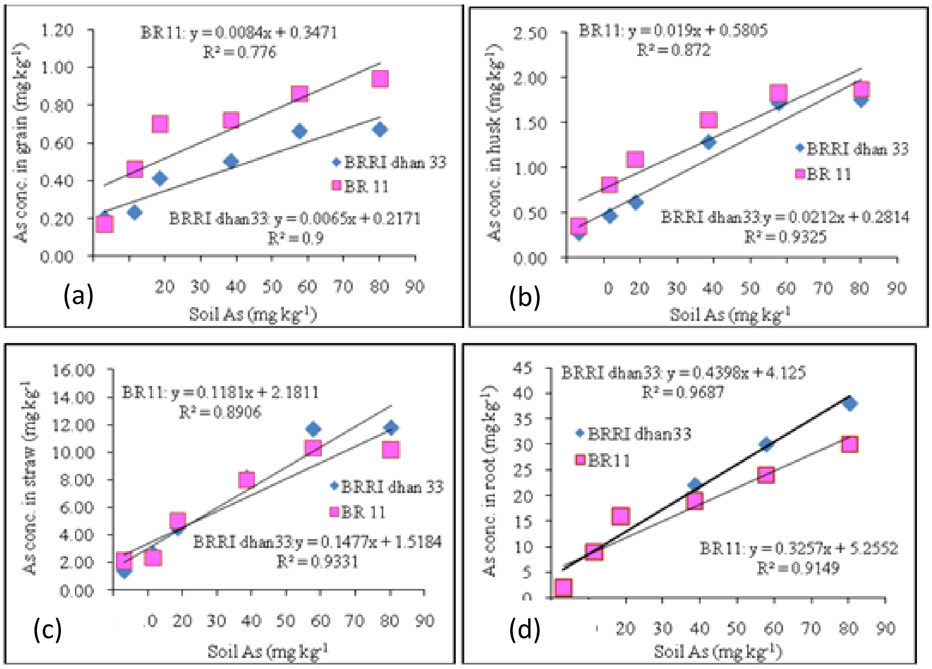

Fig 3. Relationship between soil arsenic concentration and arsenic accumulation in (a) rice grain, (b) husk, (c) straw and (d) root.

in straw, husk and grain of BRRI dhan 33, respectively. The highest P concentration in straw was found in T5 and the lowest in the T2 (blank, As free soil)(Fig. 4c). The highest P concentration in the grain was observed in T6 and the lowest was in the check (Fig. 4a). The straw P concentration in T5 was significantly higher than T2 and T1, but the P concentrations in husk and grain were not significant among treatments. Soil As was positively correlated with concentrations of phosphorous in straw (R^2 = 0.846), husk (R^2 = 0.524) and grain (R^2 = 0.877)(Fig.5a).

In BR 11, P concentrations ranged from 0.044 to 0.065%, 0.036 to 0.069% and 0.256 to 0.280% in straw, husk and grain, respectively. The highest P concentrations in straw and grain were recorded in T5 and the lowest was in the check (Figs. 4a & 4c). The highest P concentration in the husk was found in the T6 and lowest in the check soil (Fig. 4b). The P concentration in straw was significantly higher in T5 (11.6 mg kg^{-1}) compared with check (3.2 mg kg^{-1}) soil. Phosphorous concentrations in husk and grain were significantly higher in all treatments over the check soil. Accumulation of phosphorous in straw (R^2 = 0.8457), husk (R^2 = 0.5243) and grain (R^2 = 0.8774) of BRRI dhan 33 and straw (R^2 = 0.622), husk (R^2 = 0.563) and grain (R^2 = 0.640) of BR 11were positively and significantly correlated with soil As (Figs. 5a and 5b). Mahimairaja et al. (2005) observed similar result in rice crop. P accumulation in rice plant parts also found by Meharg et al. (2002).

Bioconcentration and translocation factor of arsenic

Bioconcentration factor ranged from 0.55 to 1.16 and 0.42 to 1.05 in BRRI dhan 33 and BR11, respectively. In BRRI dhan 33, treatment T3 showed significantly higher BCF (1.16) followed by T2 (1.05) and T4 (1.00) and the minimum (0.55) was in T6 (Fig.6a)($P \leq 0.05$). T3 also showed significantly higher BCF value (1.05) in BR11 rice and the minimum value was observed in T6 (0.42). The bioconcentration gradually decreased with the increase of As concentrations in the media, which may imply the restriction in soil-root transfer at higher metal concentrations of soil. Similar results were found by Yoon et al. (2006). Ho et al. (2008) also observed a range of 1.92 to 3.21 BCF in Pb-treated kenaf (*Hibiscus cannabinus* L.). Translocation factor (TF) was also significantly influenced by the different levels of arsenic ($P \leq 0.05$). Translocation factor varied from 0.37 to 0.84 and 0.43 to 1.31 in BRRI dhan 33 and BR11, respectively (Fig. 6b). In BRII dhan33, the highest TF (0.84) was observed in T1 and the minimum was in T6. T1 also showed the highest TF (1.31) which was significantly higher than rest of the treatments ($P \leq 0.05$). TF of metal excluder species is <1 whereas, metal accumulator species has TF>1 (Baker, 1981). It was observed that the treatments exhibited greater BCF than TF.

Materials and methods

Experimental site

The pot experiment was conducted at net house, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. The net house area belongs to the Madhupur Tract (AEZ 28) having 24º 02´ 131" N latitude and 90º 23´ 810" E latitude, 8.4 m AMSL. It is sub-tropical humid climate and is characterized by high temperature accompanied by moderately high rainfall during *kharif* (April to September) season and low temperature in *rabi* (October to March) season.

Experimental materials and treatments

The growth media prepared with soils contaminated with different levels of As which were: T1 (3.2 ppm), T2 (11.6 ppm), T3 (18.7 ppm), T4 (38.6 ppm), T5 (57.8 ppm) and T6 (80.3 ppm).

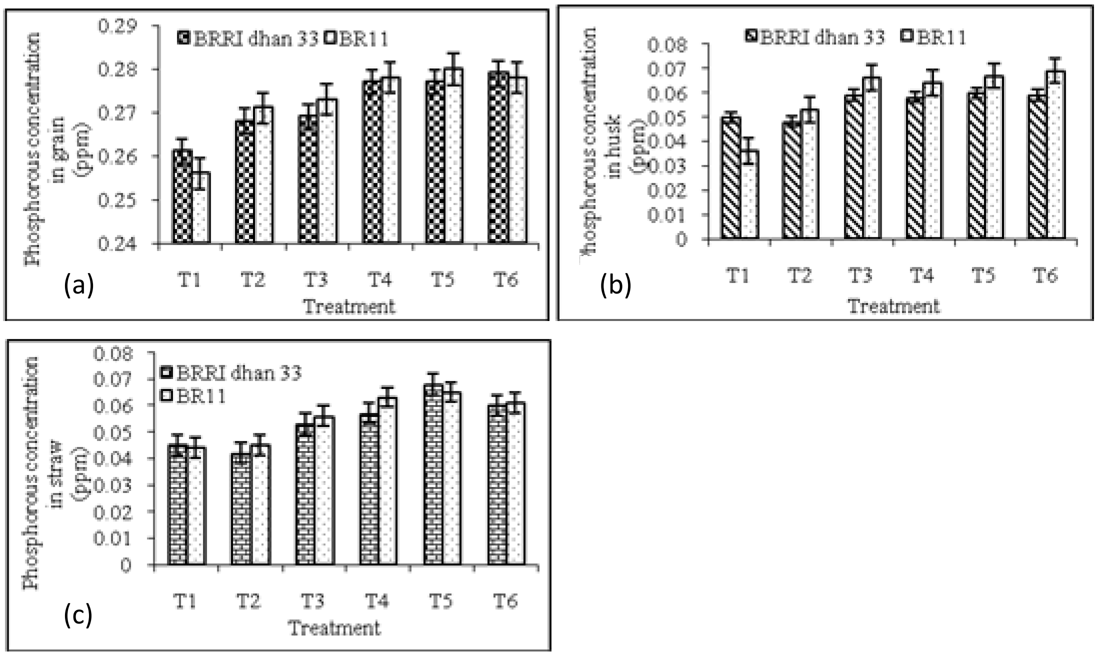

Fig 4. Phosphorous accumulation in (a) grain, (b) husk and (c) straw of rice plant as influenced by different levels of arsenic in the growth media ($P \leq 0.05$). **Note:** T1 =3.2 ppm, T2=11.6 ppm, T3=18.7 ppm, T4= 38.6 ppm, T5= 57.8 ppm and T6= 80.3 ppm. Error bars represent ± SE ($P \leq 0.05$).

Among them four elevated arsenic contaminated soils T3 (18.7 ppm), T4 (38.6 ppm), T5 (57.8 ppm) and T6 (80.3 ppm) were collected from STW irrigated area and one background soil (11.6 ppm As) collected from non-irrigated area of Komorpur, Faridpur Sadar Upazila. Another check soil (historically non-contaminated area), having soil As concentration of 3.2 mg kg^{-1}, was collected from BSMRAU campus, Gazipur. The soil was high to medium high land of Tejgaon series in Madhupur Tract. The treatments were arranged in a completely randomized design (CRD) with 6 replications.

Plant materials, Planting / sowing and fertilization

Two rice cultivars, BRRI dhan 33 and BR11, having the cropping periods of 118 and 145 days, respectively were planted in 'Aman' season. Two healthy and vigor seedlings were transplanted per hill. Accordingly, two hills were received in each pot of 30 cm deep and 12.5 cm diameter plastic pot, containing 15 kg air dried soil. Water requirement was monitored visually. Pre-tested As free (below detection limit) irrigation water was applied in each pot of the experiment almost every day as recommended. The fertilizer doses were followed AEZ 12 as per fertilizer recommendation guide. Therefore, N, P, K, S and Zn were applied at the rate of 66, 6, 12, 7 and 1 kg ha^{-1} (1.08, 0.23, 0.18, 0.29 and 0.021 g pot^{-1}) from urea, TSP, MP, gypsum and zinc sulphate fertilizer, respectively. Weeding was done manually by hand, when required. Diseases and pest infestations were monitored and necessary steps taken during the total growing period. Arsenic and P accumulation in straw, husk, and grain of Transplant Aman were recorded properly.

Plant and soil sampling and chemical analysis

Plant samples collected after harvest and soil samples were collected before and after harvest and analyzed following standard laboratory methods. Mechanical analysis was done by hydrometer method (Bouyoucos, 1926). The pH was determined by the Metrohm 691 digital pH meter (Jackson, 1962). Organic carbon was determined by Leco apparatus. Kjeldahl method was followed for the determination of total nitrogen (Page et al., 1982). Available P of Faridpur soil was extracted with 0.5 M $NaHCO_3$ extracting solution (Olsen et al., 1954) since pH value of the soil was greater than 6.5 (pH>6.5) but the Gazipur soil was extracted with 0.03M NH_4F-0.025M HCl since it was below 6.5. The ammonium acetate extraction method was followed for available potassium determination. The contents of K in the soil were directly measured by the Flame Emission Spectrophotometer (Page et al., 1982). Available S content was determined by extracting the soil with $CaCl_2$ (0.15%) solution as described by Page et al. (1982). Available Zn, Cu, Fe, and Mn contents in soil were determined by DTPA extraction method as described by Hunter (1984).

The DTPA extractable elements were measured by Atomic Absorption Spectrometer (AAS) using air-acetylene flame and matrix matched standards. The total arsenic content was determined by flow-injection hydride-generation atomic absorption spectroscopy (FI-HG-AAS), using a Perkin Elmer Analyst 200, digestion made by $HNO_3.H_2O_2$. The analytical values are presented in Table 1. Plant samples were also digested with conc. HNO_3 and 30% H_2O_2 and determined total As and P in straw, husk and grain as above methods.

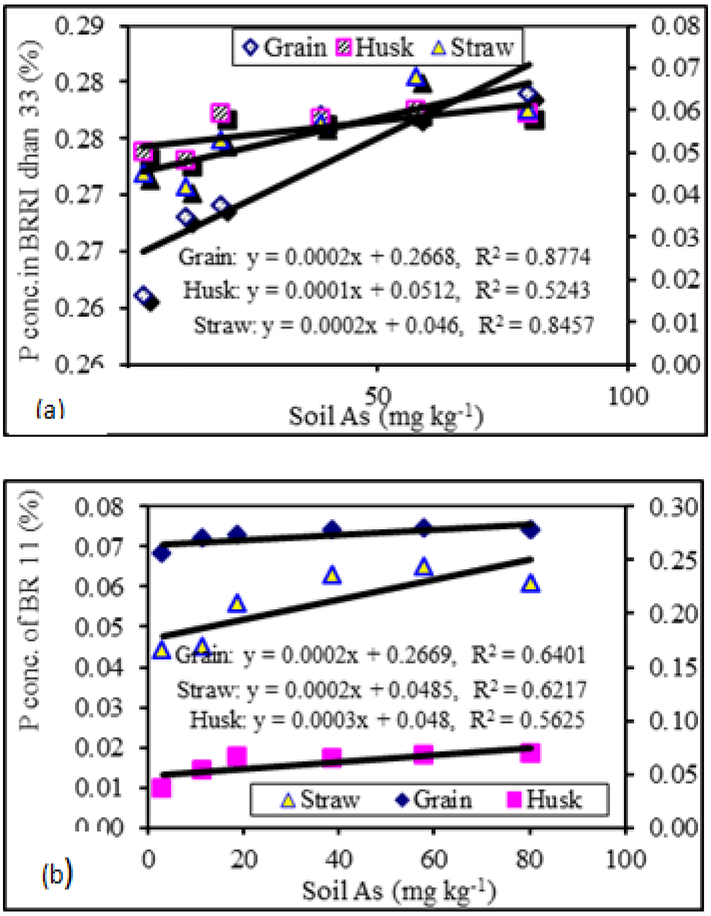

Fig 5. Relationship between soil arsenic concentration and phosphorous accumulation in (a) BRRI dhan33 and (b) BR11rice grain, husk and straw.

Bioconcentration factor (BCF) and translocation factor (TF)

The plant's ability to accumulate metals from soils and their translocation ability from roots to shoots can be estimated using the bioconcentration factor (BCF) and translocation factor (TF), respectively. The BCF and TF factors can be calculated as follows:

$$BCF = \frac{C_{root}}{C_{soil}} \ldots (1)$$

Where, C_{root} and C_{soil} are the metal concentration in the root and soil, respectively.

$$TF = \frac{C_{aerial}}{C_{root}} \ldots (2)$$

Where, C_{aerial} is the metal concentration in the aerial parts.

Statistical Analysis

Statistical analysis was done following MSTAT-C program to find out the effect of each treatment on As and P accumulation in straw, husk and grain and to compare the parameters between the treatments. The means of each treatment was compared by DMRT. Computation and preparation of graphs were done using Microsoft EXCEL 2003 program.

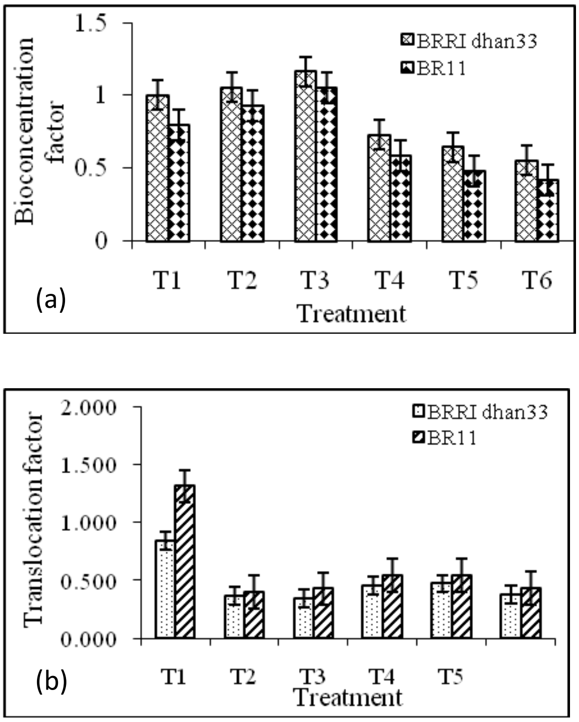

Fig 6. Bioconcentration factor (a) and translocation factor (b) of arsenic in rice plant as influenced by different levels of soil arsenic. **Note:** T1 =3.2 ppm, T2=11.6 ppm, T3=18.7 ppm, T4= 38.6 ppm, T5= 57.8 ppm and T6= 80.3 ppm. Error bars represent ± SE.

Conclusion

Elevated soil arsenic (As) influenced the As and P accumulation in rice. Arsenic contents in grain and husk of BR11 were found higher than those of grain and husk of BRRI dhan 33. The straw and root of BRRI dhan 33 showed higher concentration of As than straw and root of BR11. The decreasing trend of As concentration was found as staw> husk> grain at both varieties. Phosphorous concentration was decreased in the order of grain > husk ≥ straw. Arsenic in root, straw, husk and grain of BRRI dhan 33 and BR11 showed positive relationship with soil As concentrations. Arsenic concentration in root, straw, husk and grain increased significantly in higher soil As concentrations, which can be threat for animal and human health. If the soil is contaminated with As then BRRI dhan 33 might be recommended for rice grain production. Further studies need to be carried out for remediation of polluted soil by phytoremediation techniques.

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