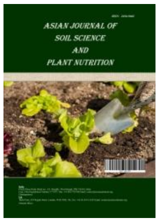

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Influence of Soil Arsenic Levels on Biomass Production and Relationship the Concentration of Arsenic between Rice Straw and Grain

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Authors' contributions

This work was carried out in collaboration among all authors. Author MI designed the study, executed the field study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GKMMR and GMP managed the analyses of the study and the literature searches. Author HK managed the laboratory analyses. Author JCB edited draft manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Arsenic (As) contamination is widespread in Bangladesh. It can cause health hazards depending on consumption of foods grown on As contaminated soil. Two pot experiments were conducted at net house, Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur to study the effect of As on above ground biomass of different rice genotypes and to determine the relationship of As concentration between rice grains and straw. Sixteen rice genotypes were grown in pots soils having 0, 20, 40 and 60 mg/kg As both in winter and wet seasons. Soil As levels reduced above ground biomass of rice by 8-65%. Above ground biomass reduction was the least in BRRI dhan47 with variable soil As levels. Total As

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concentrations in straw and grains increased with increasing soil As levels. Moreover, the concentration of As in rice grain was also increased with greater As concentration in straw. Grain to straw ratio of As concentration was lower at higher As concentration in straw. It is indicated that reduced movement of As from rice straw to grain take place when straw As concentration was high.

Keywords: Biomass production; pot experiment; arsenic concentration; grain to straw ratio.

1. INTRODUCTION

Bangladesh is currently facing the challenge of high arsenic (As) contamination in groundwater, which is widely used for growing rice crops. Shallow tube wells (STWs) are utilized to use sub-surface water for irrigating about 70% of total arable land in Bangladesh [1]. This As contaminated irrigation water is blamed for increasing As levels in paddy soils and soil solutions [2-4] and thus enter into food chain through rice consumption, which has been considered a major threat to human health in Bangladesh.

Naturally and artificially elevated levels of As in irrigation water or soil can reduce growth and productivity of rice [5-8] because of its toxic effect. Arsenic impairs metabolic processes and thus reduces plant growth and development [9]. When plants are exposed to excess soil As, its height decreases [10-13], reduces tillering ability [14-15], lessen shoot growth [16-17], lower fruit and grain yield [10,14,18], and sometimes leads to death [19-20].

The pathway of As translocation from roots to shoots and from shoots to grain is less well understood, although the sharp declining gradient in the concentration of As from roots to stems, leaves and grain. Liu, et al. [21] and Zheng, et al. [22] suggests a limited mobility of As in rice. Several authors [15,18,23] reported elevated As content in rice tissues when grown in presence of its higher concentrations. Onken and Hossner [24] also reported that plants grown in As treated soil had higher rate of its uptake compared to untreated soil. In principle, As concentration in rice straw increases significantly with its increasing concentration in soil. Generally, As concentrations in rice tissues follow the trend of root>straw> husk>grain [5,15,19,25].

Recently, some reports are available on As uptake by rice straw and grain at greenhouse conditions [11,23]. However, limited literatures are available on the influence of As on above ground biomass reduction of widely cultivated rice varieties in winter (irrigated Boro rice) and wet (rainfed T. Aman rice) seasons. So, the present study was undertaken to determine the effect of As on above ground biomass reduction of different rice genotypes in Boro and T. Aman season and to establish relationship of total As concentration in straw and grain of various rice genotypes.

2. MATERIALS AND METHODS

2.1 Description of Experimental Site

The pot experiment was conducted at net house, Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. This experiment site enjoys good sunshine throughout the day. The climate of this area is sub-tropical, characterized by high temperatures during April–August, high rainfall during the monsoon season (June-August) and low temperatures during winter (November–February). Though the experiment was conducted in net house, normal environmental conditions were maintained inside the net house.

2.2 Soil Collection and Pot Preparation

Soils were collected from BSMRAU Research field at a depth of 0-15 cm. Soils were stack in net house and air dried. Prior to potting, soils was ground and mixed thoroughly. For determining initial soil As content, physical and chemical properties, 20 samples were made from different site of soil stack. These dried soil samples were further ground to pass through 2-mm sieve for analysis of physical and chemical properties of soil as per standard protocols and pass through 100 µm sieve for initial As content determination. Soil pH, organic carbon, total N, available P, exchangeable K, available S and Available Zn were analyzed by the methods given in Table 1.

Moisture content of selected soil samples were determined by gravimetric method. Initially, each pot was filled with 10 kg dry soil followed by addition of soil test based fertilizer. Pot soil was flooded with water and kept it standing over night. In the following day, Sodium Arsenate ($Na_2HAsO_4.7H_2O$) at 0, 20, 40 and 60 mg/ kg (oven dry soil basis) was mixed. After mixing of sodium arsenate with soil, pots were kept standing for three days without irrigation prior to transplanting. As free tap water was used to soften the pot soils prior to transplanting. Forty days-old Boro and 30 days-old T. Aman seedlings were used at two seedlings/pot. About 3–4 cm water level was maintained above pot soil from transplanting to physiological maturity with As free tap water. Top dressing and other intercultural operations were done during growing season.

2.3 Treatments and Design

Sixteen rice varieties including Bangladeshi and exotic (Chinese, IRRI and USA) were used as test varieties (eight genotypes in each season). Four levels (0, 20, 40 and 60 mg/kg) of As concentration were spiked using sodium arsenate in present research. Randomized Complete Block Design (RCBD) with three replications was followed.

2.4 Harvesting and Sample Preparation

All plants grown each pot were removed separately for biomass, straw and grain yield. Grain and straw of all rice varieties were air dried. Straw yield was recorded after oven drying at 70ºC for 72 hours. Grain yield per pot at 14% moisture content were also recorded. Straw were then chopped into smaller sizes (5-7 cm). All chopped plant samples were oven-dried, ground and homogenized with Vibrating Sample Mill, HEIKO TI-200 and kept for total As measurement using HG-AAS machine.

2.5 Sample Digestion

Straw and rice flour samples were digested by modified nitric (HNO_3)-perchloric ($HClO_4$) acid digestion [26] with block digester (Model-VELP). The block digester consists of 24 blocks of which one for standard reference materials (SRM), one for blank, one for duplicate sample and 21 were used for sample digestion. Prior to digestion, over-night pre-digestion was followed with nitric acid for total As analysis. After pre-digestion, samples were heated for one hour followed by addition of perchloric acid and digestion continued up to four hours. After cooling, the digest was diluted to 50 ml in volumetric flask. Samples were filtered through Whatman 42 filter paper prior to total As analysis. In rice flour digestion, the SRM was used to compare the certified value of 0.29 ± 0.03 mg/kg. In case of straw digestion, the secondary reference material (SeRM) was used. Recovery percentage of digestion of SRM and SeRM at 90% and above was considered As accepted range.

2.6 Total as Analysis by Atomic Absorption Spectrophotometer

Straw and rice flour digests were treated with 5% potassium iodide and ascorbic acid [27] to reduce As (V) to As (III) for determination of As by hydride generation atomic absorption spectrophotometer (HG-AAS), Buck Scientific 210 VGP continuous flow hydride generation system (HG-AAS). Prior to pre-reduction, samples were acidified by concentrated hydrochloric acid (HCl). Sodium borohydride ($NaBH_4$, 0.5%) and 3M HCl was used at sample injection time to generate hydride of As (arsine, AsH_3). The acidified sample and the reductant ($NaBH_4$) solutions were taken in suitable mixed

Table 1. Physical and chemical properties of initial soil of the pot experiment, Bsmrau, Gazipur

Properties	Value	References
Soil texture	Silty Clay Loam	Hydrometer method [28]
pH (1:2.5)	5.98	Glass Electrode pH meter method with soil water ratio 1: 2.5. [29]
Organic carbon	1.10 (low)	Walkley and Black’s wet oxidation method as described by Jackson [30]
Total N (%)	0.092 (low)	Kjeldhal systems. [31]
Available P (mg/kg)	20 (optimum)	Olsen Method. [32]
Exchangeable K (meq 100 g/soil)	0.12 (low)	Ammonium acetate extraction method. [33]
Available S (mg/kg)	20.15 (medium)	Turbidimetric method [28]
Available Zn (mg/kg)	4 (high)	DTPA Extraction method [28]

in a reaction coil, and the gaseous hydride heated silica tube aligned in the optical path of the spectrophotometer. The wavelength used for As analysis was 193.7 nm. The standards were prepared following same analytical matrix of sample preparation and used for generation of standard calibration curve. The overall reactions at the time of As determination are as follows

$$\text{Sample} + KI + \text{Ascorbic acid} + HCl \longrightarrow KCl + HI$$

$$2HI \longrightarrow 2H^{+} + 2I^{-} + 2e^{-}$$

$$As^{5+} + 2e^{-} \longrightarrow As^{3+}$$

$$NaBH_4 + HCl \longrightarrow NaCl + NaBCl + H_2$$

$$2As^{3+} + 3H_2 \longrightarrow 2AsH_3\ (\text{Arsine})$$

2.7 Calculations and Statistical Analysis

Statistical analyses were performed using statistix 10 software. Regression analysis was done to establish relationship between As concentration in straw and rice grain. Relationship of as concentration in straw and grain with straw and grain yield reduction percentages were also established. Four levels (0, 20, 40 and 60 mg/kg) of soil arsenic concentration were used for calculating reduction percentages of straw and grain yield using the following formula:

$$R\ (\%) = \{(Y_1 - Y_2)/Y_1\} * 100$$

Where: R (%) represent reduction percentage; Y_1 represents yield at 0 mg/kg soil As level; Y_2 stand for yield at 20, 40 and 60 mg/kg soil As levels separately.

3. RESULTS AND DISCUSSION

3.1 Above Ground Biomass (g/pot) and its Reduction Percentages

In Boro and T. Aman season, above ground biomass of rice genotypes progressively decreased because of soil As gradient from 0 to 60 mg/kg (Tables 2 and 3). Previously, Panaullah, et al. [34] acknowledged progressively decreased of rough rice yield from about 7 to 2 t/ha in 2006 and 9 to 3 t/ha in 2007 across the soil As gradient. Several authors [23,35-36] also reported yield reduction as a result of higher levels of As load. Significant varietal effect of gradient soil As levels were also observed among all tested rice genotypes in two consecutive rice growing season. At 0 mg/kg soil

separated and swept by argon (Ar) gas into a As level, BRRI dhan29 in Boro and BRRI dhan53 in T. Aman season produced the highest aboveground biomass (120 g/pot and 103 g/pot, respectively) but when As was added @ 20, 40 and 60 mg/kg, high reduction in biomass production in BRRI dhan29 (55-63%) and BRRI dhan49 (34-55%) were recorded. High reduction percentages due to As load were also observed in BRRI dhan45, BRRI dhan28, IR44595 (13-66%), IR76895, IR71676 (28-51%). The variety BRRI dhan47, which had found significantly reduced above ground biomass with the increase of soil arsenic levels in both seasons but the reduction in biomass production were comparatively low (8-52%) among all tested Boro and T. Aman rice genotypes. Similar reduction percentages (11-48%) were also observed in BRRI dhan28, Jefferson, BINA dhan8 and BRRI dhan53.

3.2 Arsenic Concentration

There was a significant variation of As concentration in rice straw and grain of different rice genotypes in both the seasons. The concentration of as in rice straw and grain was in increasing trend in both the growing seasons depending on soil As levels (Tables 4 and 5). Straw and grain As concentration as a function of soil As concentration closely compared to respective data from earlier studies like as [34,37-38]. Rahman, et al. [23] reported significant differences in the accumulations of As in straw and grain based on gradient concentrations of As in pot soil. At the maximum levels of As in soils (40 and 60 mg/kg), BRRI dhan47 in both season and BRRI dhan28 in Boro, BINA dhan8 in T. Aman season accumulated lower As compared to other tested genotypes. BRRI dhan45 and IR44595 in Boro season and BRRI dhan49 in T. Aman season accumulated higher as in contrast to other genotypes. Very high concentration of As on rice straw indicates that feeding cattle with such contaminated straw would be a direct threat to their health and also indirectly, to human health via ingesting As contaminated meat and drinking milk [5].

Among the eight genotypes in Boro season, the as concentrations in straw and grain ranged from 0.42 to 41.97 mg/kg with an average of 17.55 mg/kg and 0.18 to 0.88 mg/kg with an average of 0.53 mg/kg respectively. Moreover in T. Aman season among eight genotypes, straw and grain As concentration ranged from 0.32 to 46.01

mg/kg and 0.12 to 0.86 mg/kg with an average of 14.51 mg/kg and 0.35 mg/kg, respectively. There was a significant genotypic effect on straw and grain As (P < 0.001). A positive quadratic correlation was observed between as in straw and grain with genotypes in Boro and T. Aman season. There was a significant genotype effect in straw and grain As concentration depending on genotypic variations. But poor correlation between the concentration of as within grain and straw at harvest (Fig 1), was observed in T. Aman season. This suggests that there was genetic regulation for both grain and straw As but that the two are only closely related when there was a low concentration of straw As. It is worth noting that the lowest average grain As had the highest grain to straw ratio, that is, the genotypes had a higher proportion of As in the grain compared to the As concentration in the straw. The second lowest grain As also had the second highest grain to shoot ratio. This trend is in agreement with previous studies where the grain to shoot ratio across multiple environments was observed as a hyperbolic relationship, with a decrease in translocation efficiency alongside increasing straw As accumulation [39-41]. In other words, the higher the straw As concentration, the lower the proportion (but not the amount) of As that reaches the grain (Fig. 2).

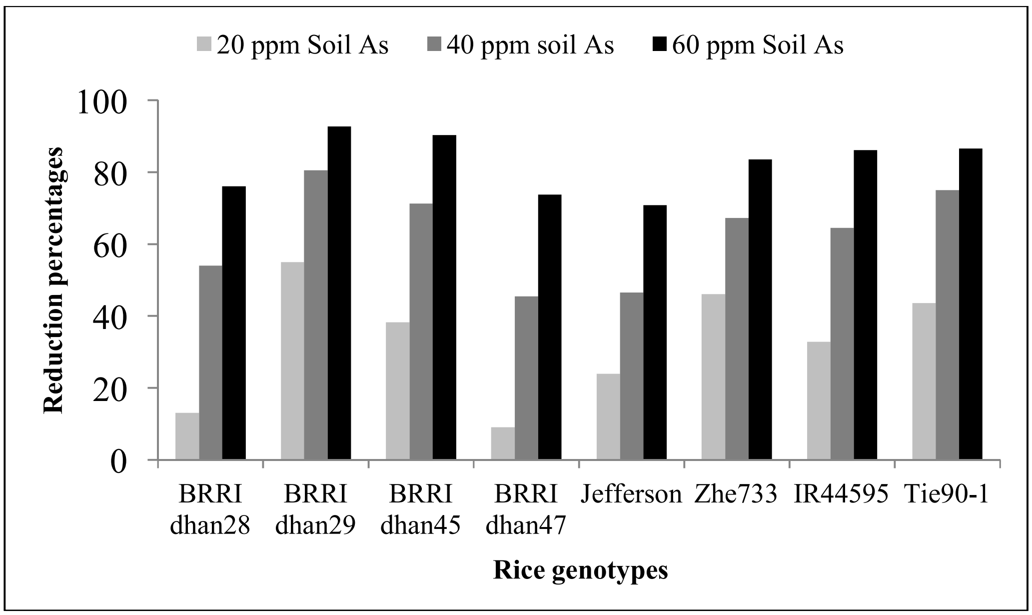

Fig. 1. Above ground biomass reduction percentages of different rice genotypes as influenced by various soil Arsenic doses, Boro season

Table 2. Above ground biomass (g/pot) of different rice genotypes as influenced by various soil Arsenic doses, Boro season

Arsenic dose (mg kg^{-1})	**Rice genotypes**								
	BRRI dhan28	**BRRI dhan29**	**BRRI dhan45**	**BRRI dhan47**	**Jefferson**	**Zhe733**	**IR44595**	**Tie90-1**	**Mean**
0	79.0	120.0	67.2	78.3	23.0	46.7	90.2	53.2	69.7
20	68.7	54.0	41.5	71.2	17.5	25.2	60.6	30.0	46.1
40	36.3	23.4	19.3	42.7	12.3	15.3	32.0	13.3	24.3
60	18.9	8.7	6.5	20.5	6.7	7.7	12.5	7.1	11.1
Mean	50.7	51.5	33.6	53.2	14.9	23.7	48.8	25.9	
CV	12.9								
$LSD_{0.05}$	**Arsenic dose = 2.8 Variety = 5.4 Arsenic dose X Variety = 8.6**								

Table 3. Above ground biomass (g/pot) of different rice genotypes as influenced by various soil Arsenic doses, T. Aman season

Arsenic dose (mg kg^{-1})	Rice genotypes								
	BRRI dhan47	BINA dhan8	BRRI dhan53	BRRI dhan49	IR76895	IR71676	Starbonnet	Aijionante	Mean
0	76.1	86.0	102.8	101.1	55.2	68.3	66.7	63.6	77.5
20	69.4	73.6	91.4	67.0	37.7	48.6	45.8	45.6	59.9
40	58.1	44.5	75.6	37.1	17.3	23.4	25.4	24.5	38.2
60	34.7	27.6	40.6	16.9	11.3	11.4	15.3	18.0	22.0
Mean	59.6	57.9	77.6	55.5	30.4	37.9	38.3	37.9	
CV	9.5								
$LSD_{0.05}$	**Arsenic dose = 2.7 Variety = 4.7 Arsenic dose X Variety = 8.1**								

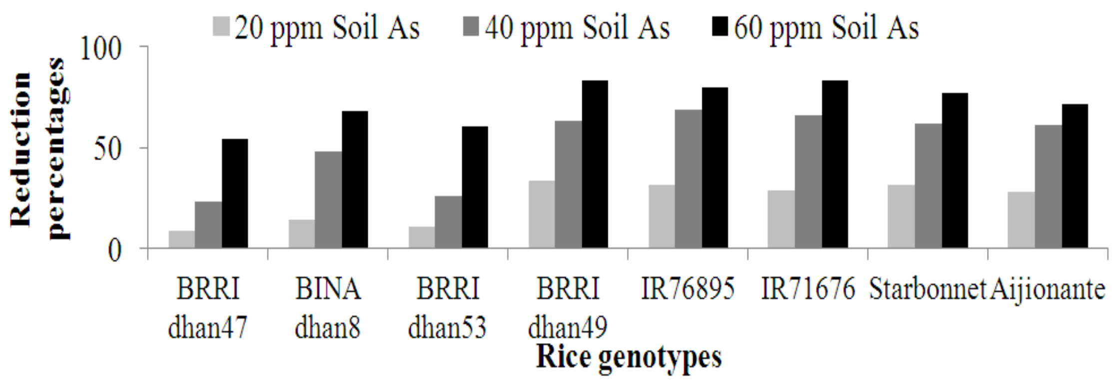

Fig. 2. Above ground biomass reduction percentages of different rice genotypes as influenced by various soil Arsenic doses, T. Aman season

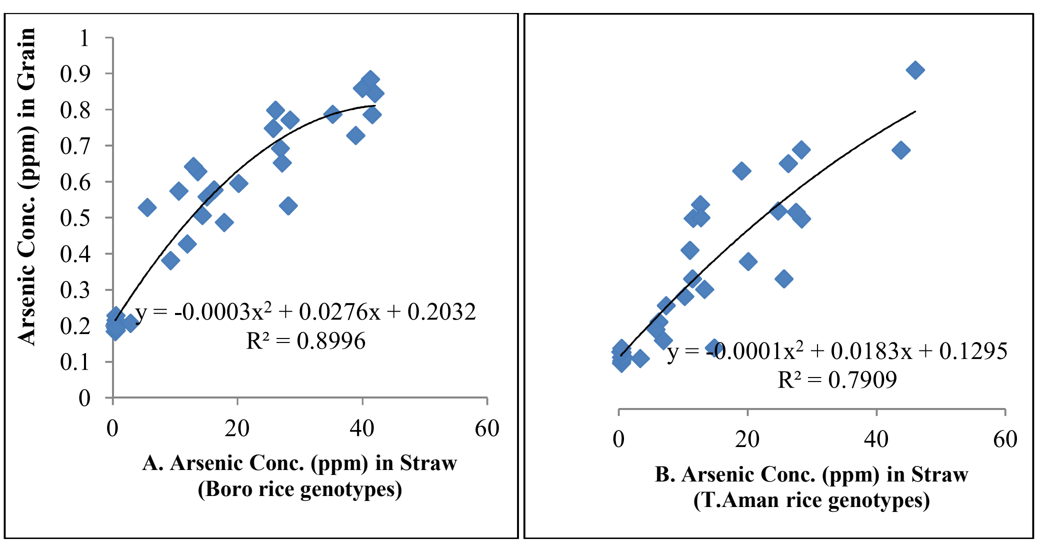

Fig. 3. Concentration of as in grain and straw at harvest for the 16 genotypes in common in Boro and T. Aman season

Table 4. Arsenic concentration (mg/ kg) of straw and grain of different rice genotypes after harvest in Boro season

Soil As levels	As conc.	BRRI dhan28	BRRI dhan29	BRRI dhan45	BRRI dhan47	Jefferson	Zhe733	IR44595	Tie90-1	Mean
0	Straw	0.53	0.62	0.53	0.42	0.44	0.55	0.44	0.57	0.51
	Grain	0.23	0.20	0.22	0.18	0.20	0.19	0.21	0.19	0.20
20	Straw	5.54	10.59	15.10	8.86	9.27	13.62	12.93	14.36	10.53
	Grain	0.53	0.57	0.56	0.22	0.65	0.63	0.64	0.51	0.54
40	Straw	16.23	25.71	28.40	11.95	20.15	26.85	26.06	27.12	22.81
	Grain	0.58	0.75	0.77	0.42	0.60	0.69	0.80	0.65	0.66
60	Straw	28.12	38.90	41.56	17.85	35.23	39.97	41.25	41.97	35.61
	Grain	0.53	0.73	0.79	0.49	0.79	0.86	0.88	0.85	0.74
Mean	Straw	12.60	18.96	21.40	16.27	9.77	20.25	20.17	21.01	
	Grain	0.47	0.56	0.58	0.33	0.56	0.59	0.63	0.55	
$LSD_{0.05}$	Straw	Arsenic dose =0.91 Variety = 1.68 Arsenic dose X Variety = 3.27								
	Grain	Arsenic dose =0.01 Variety = 0.02 Arsenic dose X Variety = 0.03								

Table 5. Arsenic concentration (mg/ kg) of straw and grain of different rice genotypes after harvest in T.Aman season

Soil As levels	As conc.	BRRI dhan47	BINA dhan8	BRRI dhan53	BRRI dhan49	IR 76895	IR 71676	Starbonnet	Aijionante	Mean
0	Straw	0.43	0.45	0.43	0.76	0.49	0.53	0.32	0.46	0.48
	Grain	0.12	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.14
20	Straw	3.33	5.15	6.23	11.44	11.05	5.77	7.36	10.24	7.57
	Grain	0.13	0.17	0.22	0.33	0.40	0.20	0.26	0.29	0.25
40	Straw	14.84	19.65	12.67	28.33	26.31	11.56	12.75	19.08	18.15
	Grain	0.15	0.30	0.52	0.66	0.63	0.49	0.49	0.61	0.48
60	Straw	25.64	20.09	24.73	46.01	43.8	28.38	27.52	38.43	31.83
	Grain	0.33	0.38	0.50	0.86	0.66	0.48	0.50	0.69	0.55
Mean	Straw	11.06	11.34	11.01	21.64	20.41	11.56	11.99	17.05	
	Grain	0.18	0.24	0.34	0.50	0.46	0.33	0.35	0.43	
$LSD_{0.05}$	Straw	Arsenic dose =1.17 Variety = 1.12 Arsenic dose X Variety = 2.40								
	Grain	Arsenic dose =0.01 Variety = 0.02 Arsenic dose X Variety = 0.04								

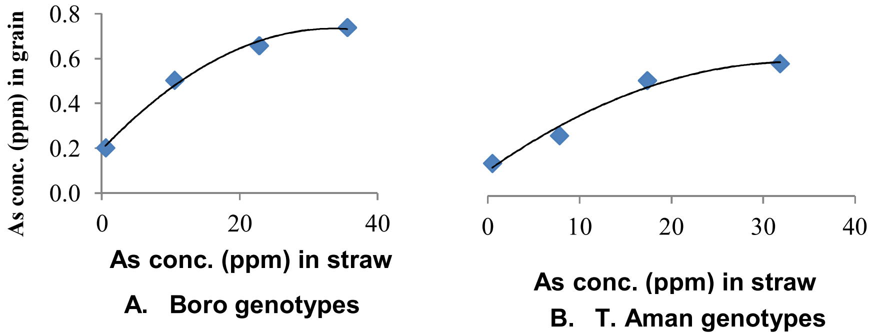

Fig. 4. Hyperbolic relationship of as concentration in rice grain and straw in Boro and T. Aman season

4. CONCLUSION

In the light of above results and discussion it is concluded that BRRI dhan47, BINA dhan8 and BRRI dhan53 can sustained their growth at presence of 20 mg/kg soil As but beyond this As level above ground biomass decreases significantly. BRRI dhan29, BRRI dhan45 and BRRI dhan49 are more sensitive to soil As. Moreover, high reduction percentages due to As addition were observed in BRRI dhan28, BRRI dhan29, BRRI dhan49 and IR71676 in Boro and T. Aman season, respectively. It is also found significant variations in as concentration with rice straw and grain of different rice genotypes. BRRI dhan45 and IR44595 in Boro season and BRRI dhan49 in T. Aman season accumulated higher As in contrast to other genotypes. A positive quadratic correlation was observed between As in the straw and grains with genotypes of both the seasons. Finally, we have found higher concentration of straw As in rice genotypes but only lower concentration that can reaches in grains.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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