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Plant Growth as Affected by Concomitant Movement of Arsenic and Sulphur in Saline Soils

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Abstract

A study was carried out to assess the interaction of Arsenic with excess Sulphur present in saline soil and their impact on plant growth. Two different types of saline soils S_1 (2.0 dS/m) and S_2 (5.061 dS/m) were collected from the southwestern part of Bangladesh. The experiment was conducted in two parts: *in vitro* incubation study and pot experiment. Arsenic treatments at the rates of 0, 0.05 and 1.0 mg/L were applied with water. The incubated soils were sequentially extracted with three different extractants, *viz*, distilled water, 0.01 M $CaCl_2$, and 1 M HCl. 1 M HCl was found to extract the maximum amounts of soluble salts as well as arsenic from the saline soil. Rice was selected as the test plant for pot experiment. An improved variety of rice (BRRI 41) was grown on the experimental soils. Sulphur in saline soil was found to reduce the accumulation of Arsenic by rice plant.

Keywords

Sulphur, Arsenic, Saline Soil, Rice

1. Introduction

Arsenic (As) is a toxic metalloid element in ground and surface waters [1]. Groundwater contamination by As has been reported in 20 countries of the world, encompassing all the continents [2], but the extent of groundwater contamination in Bangladesh is by far the most severe, as it covers almost 80% of the country. It has been proved beyond doubt that the origin of arsenic in groundwater in Bangladesh is geogenic [3]. The development of strongly reducing condition is believed to be responsible for the release of naturally occuring arsenic from the

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sediment into the ground water. Rice is the staple crop of 3 billion people worldwide and Asian countries account for 90% of its production as well as consumption [4]. Unlike other cereal crops, rice is particularly efficient in As (III) accumulation and for that impaired cellular functions through strapping of sulfhydryl groups of enzymes and proteins occur [1]. In addition, inhibition of SH containing enzymes by As alters cellular redox state and finally leads to cytotoxicity [5].

A large part of the soils of Bangladesh in the southern coastal areas are afflicted with the problem of salinity. The impact of climate change has aggravated the severity of salinity in soils. During the last 40 years it has been increased by 26% [6]. Sulphate (SO_4^{2-}) is one of the principal soluble anions in saline soil and sulphur (S) is an essential nutrient element for plant. It is one of the components of sulphur amino acids (cysteine and methionine) and many other compounds, e.g. glutathione or ferredoxin, which play important physiological functions [7]. But excessive S in soil, which is associated with salinity, might have impact on plant function. With the increase in Sulphate ion accumulation in the nutrient solution, their uptake by plants increases. Having reached a certain level, varying with plant species, further increase of concentration does not affect the uptake any longer. However, high Sulphate concentrations may affect plant development and crop yield [8]. High content of S in soil causes soil contamination and acidification too. Besides, it is indirectly responsible for mobilization of phytotoxic chemicals, such as aluminum and some trace elements [9].

During the dry season ground water moves upward by capillary action and leaves As in the soil. In addition, dry season aggravate salinity condition too. Though S has some functions of notable meditative effect to both plants' environmental stress and heavy metal pollution [10], our knowledge on interaction of As with S in saline soil is limited. Therefore, this research attempt was initiated to investigate the interactions of As and S under *in vitro* incubation as well as *in vivo* pot culture experiment.

2. Materials and Methods

2.1. Soil Sampling

Soil samples were collected from four sampling sites of Khulna, a district situated in the southern part of the country. The soils belong to two representative soil series namely Dumuria and Bajoa series. According to USDA Soil Taxonomy all the series belong to Typic Endoaquepts subgroup. The georeferences of the sampling sites are presented in **Table 1**. Both the soil series belong to "Calcareous Grey" Soils of "Ganges Tidal Floodplain" physiography.

The soil samples were collected following the standard procedures [11]. The collected samples were air dried, debris were removed and larger aggregates were ground by gently crushing with a wooden hammer. Then the ground samples were sieved by passing through a 0.5 mm and 5 mm stainless steel sieve for *in-vitro* incubation experiment and pot experiments respectively.

2.2. Experimental Set-Up

2.2.1. Incubation Experiment

The incubation experiment was done using two types of saline soils. Arsenic treatments at the rates of 0, 0.05 and 1.0 mg/L were applied while, the incubation periods were 0, 30 and 60 days. A combination of sodium meta arsenite ($NaAsO_2$) and sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) at a proportion of 80:20 were used to prepare the

Table 1. GPS location of the collected soil samples.

	Soils for incubation experiment		Soils for pot experiment	
	S_1 (2 - 4 dS/m)	S_2 (4 - 8 dS/m)	S_1 (2 - 4 dS/m)	S_2 (4 - 8 dS/m)
GPS location	22˚48'50" and 89˚29'32"	22˚47'45" and 89˚26'55"	22˚47'39.1" and 89˚27'30.3"	22˚46'17.7" and 89˚28'10.3"
District	Khulna	Khulna	Khulna	Khulna
Soil series	Dumuria	Bajoa	Bajoa	Bajoa
Determined EC	2.0	5.1	2.1	5.2

treatment water. Plastic pots (250 g in size) were used and each pot was filled with 100 g soils. Then the pots were subjected to incubation with required doses of arsenic treatments at field moisture condition for the required time of incubation. At the end of each incubation period, the soils in each of the pots were mixed thoroughly for ensuring uniform sampling. Then the soil samples from each of the pots were collected randomly for further analysis. The pH and EC were measured following the standard procedures described in Imam and Didar [12].

The extractability of S and Asof the soils were determined by sequential extraction process. Three different extractants *viz*, H_2O, 0.01 M $CaCl_2$ [13] and 1 M HCl [14] were used for extraction of the elements from the soils sequentially in the order as mentioned above [15]. Content of Sulphur and arsenic in the above mentioned samples were determined following turbidimetric method [12] and HG-AAS technique [16] respectively.

2.2.2. Pot Experiment

The pot experiment was carried out in a net house using air dried soil in earthen pots. The pots (with 4 kg soil) were arranged following completely randomized design and they were regularly shuffled for proper light allocation. An improved variety of rice (BRRI 41) was used as the test variety and the seeds were sown directly on the soil. BRRI 41 rice failed to grow on S_2 soil. Fertilizer (N, P and K) requirement was calculated following the fertilizer recommendation guide [17]. Seedling emerged after 7 days of sowing. The same arsenic solution which was used for incubation experiment was also used for pot experiment. All treatments were repeated three times and total number of pots was 12. Pots were watered daily with arsenic laden irrigation water to keep them water logged. The plants were harvested manually after 110 days of seed sowing. After harvesting, the plants were cleaned by washing with tap water followed by deionized distilled water for three times and soaked with paper towel and then fresh weight was taken. The plant samples were separated into three parts-roots, stems and grains and then kept in an oven at a temperature of 70˚C ± 5˚C for 72 hours. Dry weights as well as 1000 grain weights of the samples were recorded after that. The plant samples were crushed and sifted through a 0.2 mm sieve and stored in pots for further chemical analyses. Samples of rice root, stem and grain were digested with conc. nitric acid in block digester [16] [18]. This extract was used for the determination of S and As content of the plant samples. The quality control/quality assurance (QC/QA) of the analyses was maintained following the standard procedure. Statistical analysis was done by using Microsoft Excel (2010) version.

3. Results and Discussions

3.1. Initial Characteristics of the Soil

Some common physical and chemical properties were analyzed **Table 2** before the experimental setup, in order to know the initial status of the soil. In soils used for incubation experiment, both S and As contents were higher in S_2 soil than S_1 soil while, in the soils for pot experiment, S_1 soil contained more As and less S than S_2.

3.2. Interaction of S with As in Soil

The incubation experiment showed that **Figure 1**, without arsenic treatment both distilled water (H_2O) and 0.01

Table 2. Initial characteristics of soil.

Soil properties	Soils for incubation experiment		Soils for pot experiment	
	S_1 Soil	S_2 Soil	S_1 Soil	S_2 Soil
pH	6.4	7.3	6.3	7.3
EC (dS/m)	2.0	5.06	2.1	5.2
Available N (%)	0.16	0.12	0.22	0.13
Available P (mg/kg)	2.32	15.51	7.67	9.64
Available K (me/100 g)	0.02	0.04	0.03	0.1
Available S (mg/kg)	337.4	558.4	100.3	253.8
Total As (mg/kg)	0.32	0.76	3.03	2.8

M $CaCl_2$ extracted the minimum amount of As as well as S. Besides, among the three incubation periods, release of S was increased initially and then reduced. However, 1 M HCl extracted much higher amounts of S and As than either 0.01 M $CaCl_2$ or H_2O.

Treatment of As at a rate of 0.05 mg/L showed that **Figure 2**, extractability of As by 0.01 M $CaCl_2$ for As decreased with increased amount of extracted S in any of the three incubation periods. While sequential extraction with H_2O and 1 M HCl showed that extractability of As increased with the increase in incubation period. However, S extraction showed an initial decrease after 30 days of incubation and then a steep increase after 60 days of incubation.

Incubation with the highest concentration of As *i.e*, 1 mg As/L **Figure 3** showed that 0.01 M $CaCl_2$ extracted similar amounts of S and As as for 0.05 mg As/L treatment. On the other hand, both H_2O and 1 M HCl extracted higher amounts of S and As from the soil. Extractability by 1 M HCl for S and As was found to be the best followed by H_2O for S_1 soil. There was no significant correlation between extracted As and S except for 1 M HCl extracted ones(r value = 0.5873, $p < 0.05$). Moreover, the extracted amount of S was always found to be lower than the initial S content of the S_1 soil *i.e*, 337.37 mg/kg.

In the S_2 soil **Figure 4**, without arsenic treatment, the three extractants failed to extract As irrespective of the incubation periods. The test soil contained some As and the extraction of arsenic was very little either by H_2O or 1 M HCl upto 30 days of incubation while no arsenic was found at the end. It needs to be mentioned here that extraction of As by $CaCl_2$ was always lower. However, in all the cases, concentration of S was much greater and was in an increasing trend with the decreasing concentration of As for the three incubation periods.

Treatment of S_2 soil with arsenic at a concentration of 0.05 mg As/L **Figure 5** and 1 mg As/L **Figure 6** showed a similar result like the control. Extractability of the three extractants followed a declining trend to extract As with the increase of incubation period. Extractability of S was found to be much less than the control with any of the extractants and the extracted amount of S was lower than the background S content of the S_2 soil *i.e*, 558.42 mg/kg. Moreover, the correlation analysis between the extracted S and As in S_2 soil showed that there was no significant interaction between As and S.

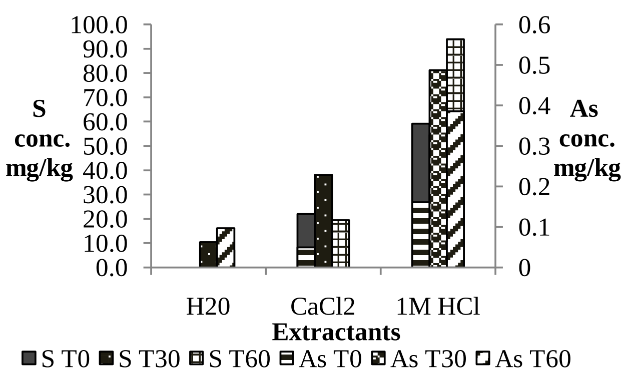

Figure 1. S-As interaction in treatment As_0 in S_1 soil.

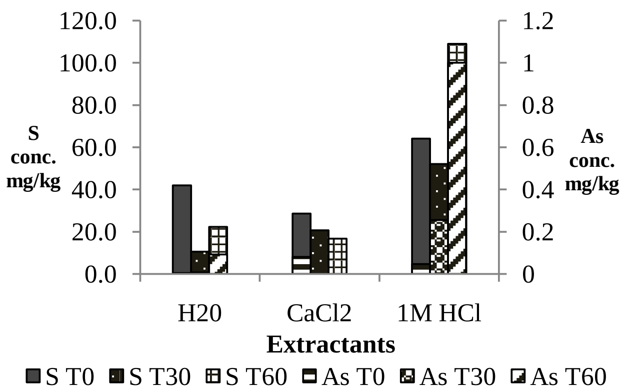

Figure 2. S-As interaction in treatment $As_{0.05}$ in S_1 soil.

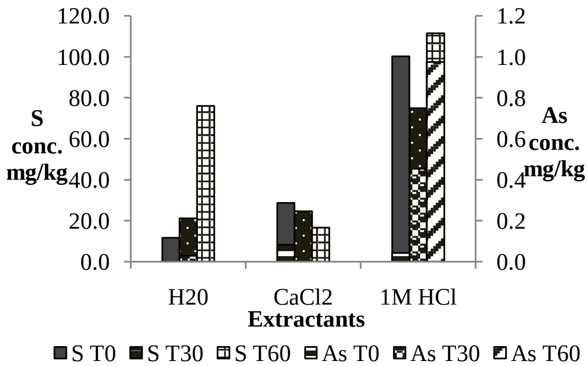

Figure 3. S-As interaction in treatment in As_1 in S_1 soil.

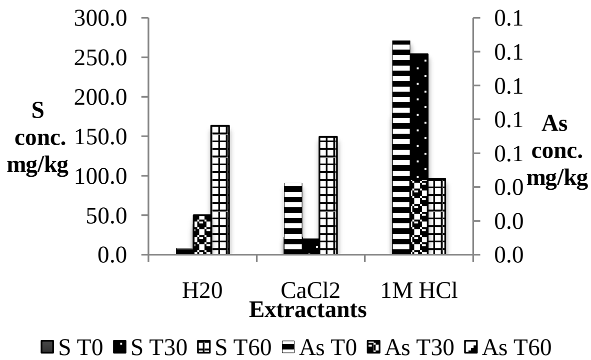

Figure 4. S-As interaction in treatment As_0 in S_2 soil.

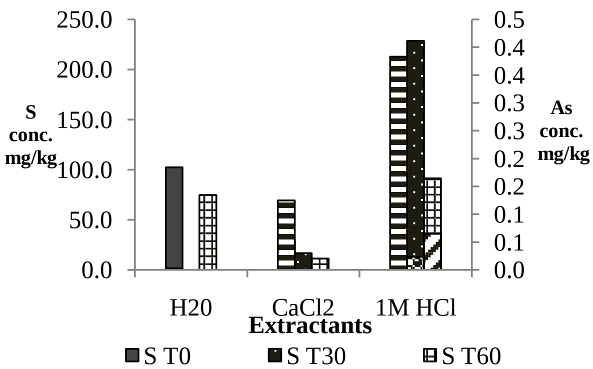

Figure 5. S-As interaction in treatment $As_{0.05}$ in S_2 soil.

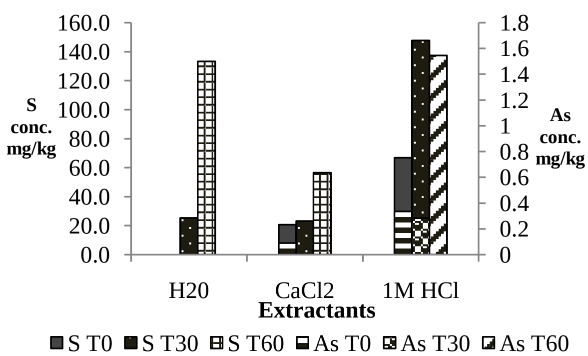

Figure 6. S-As interaction in treatment As_1 in S_2 soil.

It was found that higher the As treatment, lesser was the S concentration obtained with the extraction. It might be due to the fact that S undergoes chemical reactions with As. It was observed in the study that when there was more As, lesser amount of S could be extracted. Previous research showed that, microorganisms play a very important role in the biogeochemical cycles of As [19] and S [20] and consequently, in the mobilization and immobilization processes of As in the environment. If a concurrent microbial reduction of As and Sulphur takes place, the biomineralization of As will occur and as a result, As-S complexes will be formed [21].

3.3. Interaction of S and As and Plant Growth

The germination, growth and visual appearance of the rice plants were observed. A reddish color along with stunted growth of root was found and roots felt slippery to touch because of As toxicity. The intensity of reddish color was increased with increasing As treatment. This might be due to the formation of Fe precipitates or because of the Fe plaque formation on the root surface of the rice [22]. Besides, microbial attack was observed through physical appearance during growth of the plant.

3.3.1. Accumulation of As in Rice

Arsenic finds its way into soils used for rice (*Oryza sativa*) cultivation through As contaminated ground water irrigation. Uptake of arsenic by BRRI 41 is presented in **Table 3**. Uptake is calculated by multiplying total concentration with dry matter production of plant.

It is observed from **Table 3** that As concentration was greater in root with increasing doses of As. Similar result was found byShaibur *et al*, [23] and Yamane [24]. Generally, anions are strongly adsorbed to the membrane surface of the roots. The As anions (arsenite and arsenate) may rapidly be adsorbed onto the root surface, leading to the intense high As concentration [25]. However, only in case of the highest As treatment, stem of rice accumulated more As than the root.

3.3.2. Accumulation of Sulphur (S) in Rice

The concentration and uptake of S by rice plant is presented in **Table 4**.

It is observed from **Table 4** that, concentrations of S in root decreased gradually with increasing concentration of As treatments. The opposite result was found in stem, where concentration of S was increased with increased As treatments. Arsenic treatment at a rate of 0.05 $mg \cdot L^{-1}$ resulted in the maximum accumulation of S in root, stem and grain rather than the treatment of 1 $mg \cdot L^{-1}$.

The interactions between S with As in different parts of plants are represented in **Figures 7-9**. There was no significant relation between S and As. It is clear that plant stem contained much more S than As. So far about the concentration of As, rice roots contained more than the stem while the opposite was true for S. The result is supported by similar observation made with rapeseed (*Brassica napus* L.) by Zhong *et al.*, [26]. Thus the

Table 3. Concentration and uptake of As in rice.

As treatment	Concentration of As (mg/kg)				Uptake of As (µg/100 plant)
	Root	Stem	Grain	Total plant	
As_0	2.5	0.67	0.64	3.9	113.8
$As_{0.05}$	3.0	1.3	1.7	6.0	982.4
As_1	5.3	8.0	0.66	13.8	871.7

Table 4. Concentration and uptake of S in rice plant.

As treatment	Concentration of S (mg/kg)				Uptake of S (µg/100 plant)
	Root	Stem	Grain	Total Plant	
As_0	30696	12923	28719	72338	2136882
$As_{0.05}$	28732	13294	33484	75510	12353592
As_1	24700	14037	24169	62907	3965038

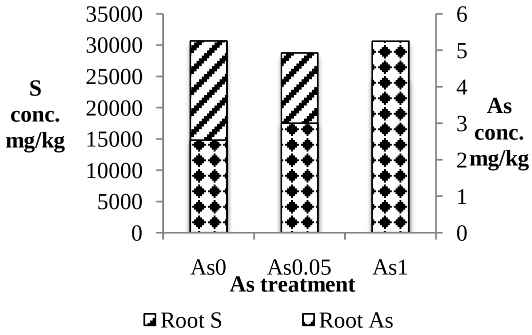

Figure 7. S-As concomitant movement in root of rice.

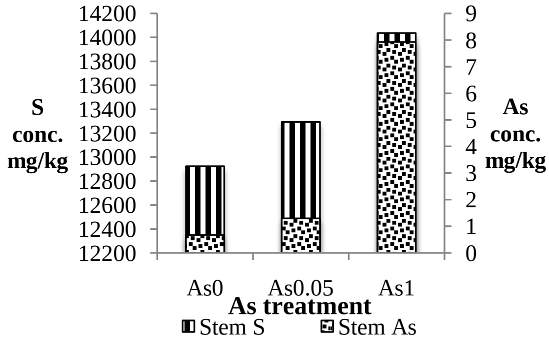

Figure 8. S-As concomitant movement in stem of rice.

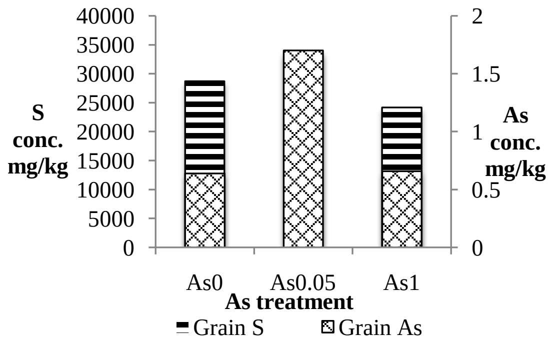

Figure 9. S-As concomitant movement in grain of rice.

presence of excess amounts of S in soil medium might be taken up by plants and it might also reduce the content of As in stem and leaves. This is because of the fact that, Arsenic has a high affinity with sulfur, it can exert its toxicity to plants after reduction to arsenite (As III), through interaction with thiol (-SH) groups of proteins, amino acid and enzymes [27] [28]. Moreover, excessive S supply increased the formation of iron plaque (found in visual observation) on the root surface under As stress. Iron plaque may be a barrier or a buffer to the uptake of As [29]. Again, excessive S supply may decrease As availability by forming indiscerptible As_2S_3 or FeAsS. Bacterium, such as *Desulfotomaculum auripigmentum*, was reported to induce precipitation of As_2S_3 by its reduction of As (V) to As (III) and S (VI) to S (−II) under anoxic conditions with excessive S [30]. As a result of these mechanisms individually or combined, excessive S present in soil could significantly alleviate As uptake and accumulation in rice exposed to As treatment.

4. Conclusion

The present study found an antagonistic relationship between As and S present in saline soil. Excessive S was not found to be detrimental to plant growth rather it reduced the amount of As in plant by different mechanism.

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