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RESPONSE OF COWPEA (*VIGNA SINENSIS* L.) TO ARSENIC

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ABSTRACT

Responses of cowpea (*Vigna sinensis* L.) to different levels of spiked arsenic in pots with two soil series *viz.*, Sonargaon and Dhamrai were investigated. Arsenic (As) content in plant increased with increasing As application to soil. Arsenic treated plants of cowpea were shorter in heights and at 50 mg As/L treatment, all leaves were shed after 60 days of growth. The number and size of nodules showed gradual decrease with increasing As application. The plant N decreased while plant P increased with increasing As concentration. The nodule nitrogen content showed a decreasing tendency with increasing As accumulation in plant, thus demonstrating a negative impact on Rhizobium-legume symbiotic association.

Keywords: Arsenic, Cowpea, *Vigna sinensis*, nodulation, mineral nutrition, accumulation.

INTRODUCTION

Arsenic (As) contamination of ground water in Bangladesh has emerged as the largest water pollution event of all time where millions of people are exposed to As concentrations, the Bangladesh and WHO drinking water standards being far above. Another possible potential exposure pathway is through the food chain where As contaminated ground water is used for irrigation and there is report of increased concentrations of the element in soil and many food materials (Huq and Naidu, 2005).

Food crops such as vegetables and cereals are potential sources from which As may enter the food chain, and these can reflect the levels of As that exist in the environment in which they are grown. Leguminous plant, such as cowpea is rich in vital nutritive value protein. Any adverse effect on protein production due to use of As contaminated irrigation water may, therefore, have a negative impact on the nutritive quality of grain legume. Moreover, the presence of As at elevated levels in the growth media might adversely affect the symbiotic system, leading to a crop failure. The present study aims at understanding the risk associated with As contaminated soils for the cowpea- Rhizobium symbiotic association.

MATERIALS AND METHODS

The experiment was carried out with two soils *viz*., Sonargaon (collected from Narayanganj) and Dhamrai (collected from Dhamrai, Dhaka) soil series being spiked with different levels of As concentrations to investigate its transfer to and effect on the growth of cowpea. Arsenic levels of the Sonargaon and Dhamrai soils were 0.46 mg/kg and 0.31 mg/kg soil, respectively. These were taken as control and As at the rates of 20, 30 and 50mg /L in water were applied as solution of sodium meta arsenite ($NaAsO_2$). There were three replications for each treatment. The As solution was added to the treated soils from seed sowing to plant maturity. The total amount of solution added was recorded. Plastic pots of equal sizes (2.5L) were used. Two kg soil was put in each pot. The soil in each pot was mixed with triple super phosphate and muriate of potash according to the need of the soils as calculated from Fertilizer Recommendation Guide (BARC, 1997). Before sowing, seeds were mixed with Rhizobium biofertilizer procured from Bangladesh Institute of Nuclear Agriculture (BINA). Rhizobium biofertilizer is known to increase soil nitrogen by 50-300 kg/ha in optimum condition (BINA, 1999). The cowpea seeds were collected from BARI, Gazipur. About 10-12 cowpea seeds were sown in each of the plastic pots and then they were allowed to grow. The pots were arranged in a completely randomized way. There were a total of 24 (2×4×3) pots. In Dhamrai soil at 50 mg As/L treatment, no cowpea seeds germinated. Treatment plants received watering with As solution and control plants with tap water twice a week. Plant protection measures were taken for both sets of plants. During the whole growth period, all visible symptoms were noted. In the cowpea plants on Sonargaon soil at 50mg As/L treatment, leaves shed gradually after eight weeks of germination. The plants were harvested after 90 days of seed sowing. The harvested roots were washed with deionized distilled water several times to remove ion from the ion free space

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as well as to dislodge any adhering particles on the root surface. The numbers of plants were counted and the heights of plants were measured separately. The total number of root nodules of each plant was counted and isolated with the help of forceps. In Dhamrai soil, the nodules formed were very small in size, more particularly in the treated soils. The fresh weight of roots, shoots, leaves and nodules were taken separately. The collected plant samples were air dried and then oven dried at $70^0 \pm 5^0$C for 48 hours and the dry weights of plant samples were noted. The dried plant samples were then ground into fine powder and preserved in small plastic vials for further analysis. After harvest, the soil samples in each pot were thoroughly air dried and homogenized and were sieved to pass through a 0.5 mm sieve for chemical analysis.

For determination of As, soil was digested in aqua regia and the As in the extracts was estimated by Hydride Generated Atomic Absorption Spectrometer (HG-AAS) as described by (Portman and Riley 1964). The water soluble soil As was extracted by shaking samples of soil at a 1:5 soil-water ratios for 24 hours. Plant samples were digested with HNO_3 on a digestion block. Samples were normally predigested overnight over a temperature range of about 40-100^0 C before increasing the temperature to 140^0 C for the final dissolution of the organic material. This extract was used for the determination of As and other elements of plant (Portman and Riley, 1964). The experimental data were statistically analyzed using MINITAB Package.

The various physical, chemical and physiochemical properties of the soils were determined following procedures as described in the book by Huq and Alam (2005). Certified reference materials were carried through the digestion and analyzed as part of the quality assurance/quality control protocol. Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in the analysis of arsenic. Each batch of 20 samples was accompanied with reference standard samples to ensure strict QA/QC procedures.

Some basic properties of the selected soils are given in table 1.

RESULTS AND DISCUSSIONS

Morphological change

Symptoms due to As application were observed. The

Table 1. Some basic properties of the selected soils.

Soil	Sand (%)	Silt (%)	Clay (%)	Textural Class	pH	OM (%)	Total N (%)	Av. N (ppm)	Av. P (ppm)	As (ppm)	
										Total	Av.
Sonargaon	2.33	59.58	38.08	Silty Clay Loam	7.0	3.45	0.005	31.50	8.41	0.46	0.10
Dhamrai	9.26	49.68	41.06	Silty Clay	5.9	1.30	0.020	47.25	3.02	0.31	0.15

Table 2. Mean height and fresh weight (g/10 plants) of cowpea (*Vigna sinensis* L.) plants.

Treatment (mg As/L)	Sonargaon Soil		Dhamrai Soil	
	Height (m)	Fresh weight (g)	Height (m)	Fresh weight (g)
0	1.067	38.9	1.621	116.2
20	1.247	22.5	1.643	86.3
30	1.016	30.2	1.328	56.1
50	0.889	44.6	-	-

LSD at 1% level, 1.075 and 0.814 for height and 4.51 and 1.83 for fresh weight in Sonargaon and Dhamrai soil, respectively.

Table 3. Number, fresh and dry weights of root nodules.

Treatment (mg As/L)	Sonargaon Soil			Dhamrai Soil		
	Number per plant	F.W.	D.W.	Number per plants	F.W.	D.W.
		(mg/plant)			(mg/plant)	
0	5.5	12	5	1.2	6	2
20	2.1	6	2	0.6	4	1
30	1.0	4	1	0.4	2	1
50	0.6	2	1	-	-	-

LSD at 1% level, 0.38 and 0.036 for number and 0.03 and 0.04 for fresh weight of root nodule in Sonargaon and Dhamrai soil, respectively.

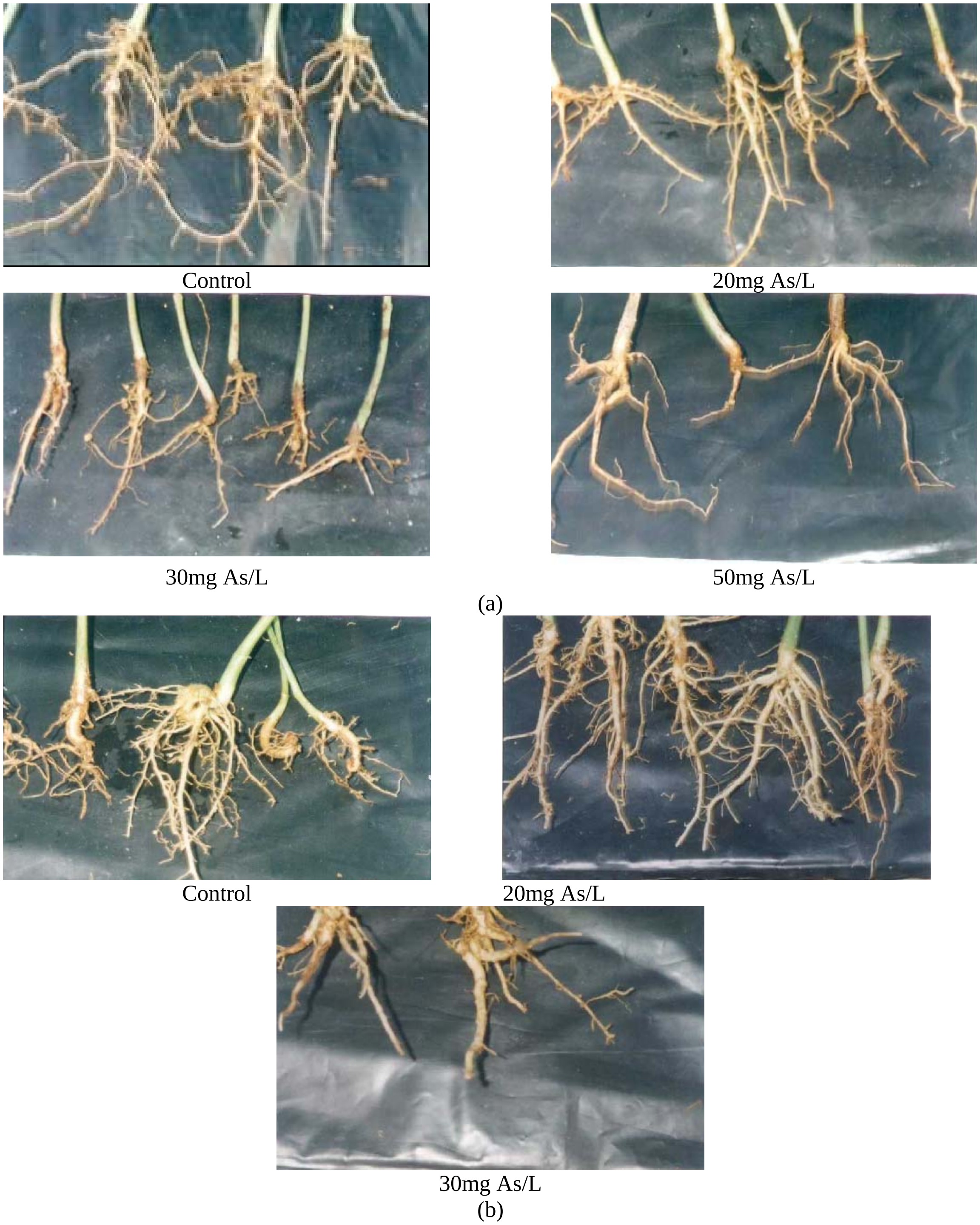

Fig. 1. Root nodules of cowpea: (a) Sonargaon soil; (b) Dhamrai soil.

seeds did not germinate on Dhamrai soil at 50mg As/L treatment. The plants showed severe symptoms at higher As concentration and the symptoms became pronounced with time of exposure to arsenic stress. The symptoms were shading of leaves, reduced plant growth, and brown necrotic spots on the leaves. The brown necrotic spots were observed on old leaves at 30 and 50mg As/L treatment. Red-brown necrotic spots on old leaves, tips and margins due to arsenic toxicity have also been reported by Aller *et al*. (1990) and Marin *et al*. (1992). Reduced chlorophyll content due to As toxicity and resulting chlorosis were reported in garden pea by Paivoke (1983). The plants grew vigorously up to approximately 45 days and then growth of treated plants declined and caused a reduction in plant heights over control plants. The mean values of heights (m) and fresh weights (g/10 plants) of the plants were recorded after harvest of the plants at 90 days and are presented in Table 2. The plants on both soils at 20mg As/L treatment grew taller than control plants and had the maximum height (1.247 m and 1.643 m for Sonargaon and Dhamrai soil, respectively). It could be so because of the fact that the presence of As in soil might have induced the release of phosphorus and P requirement of legumes is high. The height of the plants for other treatments decreased significantly with increasing As treatment in both the soils ($p = 0.000$ for both the soils). Fresh weight of plants also decreased with increasing As treatment in both the soils; however, in Sonargaon soil, the trend of decrease was not uniform; a significantly higher fresh weight was observed for 30 and 50mg As/L As treatment over 20mg As/L treatment (Table 2). The effect of As on fresh weight was statistically significant ($p = 0.000$ for both the soils). Sizes (Fig. 1) and numbers and weights (Table 3) of the root nodules were reduced with increasing As treatment ($p = 0.000$ for Sonargaon soil and $p = 0.005$ and 0.025 for number and weight respectively for Dhamrai soil).

Arsenic accumulation in plants

The mean values of arsenic concentration (mg/kg) in root, shoot, and leaf of Sonargaon and Dhamrai soils are presented in figure 2. Corresponding soil As concentrations (mg/kg) after harvest are also presented in figure 2. It is important to note that in control plants As concentrations increased slightly through the uptake of water extractable As present (Table 1) in both the soils.

Arsenic concentrations in root, shoot and leaf increased with increasing arsenic treatment more in the Sonargaon than in the Dhamrai soil. The reasons could be attributed to the differences in clay contents of the two soils and soil reaction. Dhamrai soil contained higher clay and the pH was lower than the Sonargaon soil. Higher clay content in soil is known to retain As more tenaciously and make it less phytoavailable (Joardar *et al*., 2005) while higher pH make the As more phytoavailable (Huq and Naidu, 2003). Arsenic treatment in soil had a significant ($p = 0.000$ in all the cases and in both the soils) increase in As accumulation in all parts of cowpea. Arsenic accumulation was found to be higher in root followed by shoot and leaf.

The consequence might be due to the fact that the upward transport of As from roots was inhibited by its high toxicity to the membranes of radicle (Wauchope, 1983; Barrachina *et al*., 1995). Higher accumulations of arsenic in roots in many plants have also been reported by many investigators *e.g.* in arum (Parvin *et al*., 2006), in marigold and ornamental arum (Huq *et al*., 2005), in rice (Huq and Naidu, 2005; Odanka *et al*., 1985) in lodgepole pines (Frans *et al*., 1988; Yamare, 1989), in alfalfa (Maclauchlan *et al*., 1988), and in lettuce and wheat (Kapustka *et al*., 1995). Accumulation of arsenic in some aquatic plants like duckweed (*Spirodela polyrhiza* Dh 116) and water fern (*Azolla pinnata* var. pinnata Dh 113) is also in report (Aziz, 2002). After harvest, the soil As was also found higher in Dhamrai soil than in Sonargaon soil and it might be due to the fact that the high clay content in Dhamrai soil retained high amount of As (Huq *et al*., 2006).

A mass balance of arsenic was calculated between the amount of As added to soil and the amount of As in plant and in soil. A substantial fraction of the total As applied remained unaccounted for. This fraction, however, varied between the two soils. The unaccounted As was higher except at 50 mg As/L treatment in Sonargoan soil. The uncounted fraction was 39%, 27% and 25% in Sonargaon soil for 20, 30 and 50mg As/L treatment respectively and it was 36.4% and 28.3% in Dhamrai soil at 20 and 30mg As/L treatment respectively. It needs to be mentioned again that in Dhamrai soil the seeds treated with 50mg

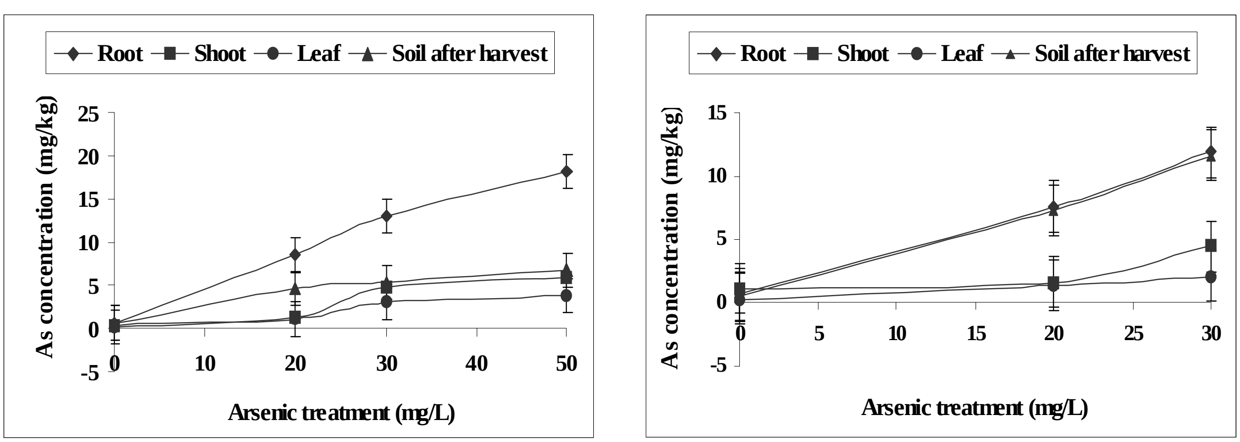

Fig. 2. Arsenic concentrations in different parts of cowpea: (a) Sonargaon soil (b) Dhamrai soil.

Table 4. Nitrogen and Phosphorus contents in plant parts and in soils after harvest.

Soil	Treatment (mg As/L)	Root (%)		Shoot (%)		Leaf (%)		Soil (%)	
		N	P	N	P	N	P	N	P (total)
Sonargaon	0	7.7	0.1	1.7	0.2	3.9	0.2	0.08	0.06
	20	5.0	0.2	2.1	0.2	4.8	0.4	0.11	0.08
	30	4.5	0.5	2.0	0.2	3.5	0.4	0.07	0.08
	50	4.3	0.4	2.2	0.2	3.7	0.4	0.05	0.09
Dhamrai	0	7.9	0.3	2.8	0.2	3.4	0.2	0.10	0.08
	20	4.7	0.3	2.7	0.2	3.4	0.4	0.12	0.08
	30	4.9	0.4	2.7	0.2	3.5	0.4	0.10	0.07

As/L did cause no germination. The possible reason for the unaccounted fraction might be that the method used for extraction was not able to bring most of the As into solution from soil, or there was a loss of arsenic through volatilization by biomethylation process active in soil.

Mineral nutrition as affected by arsenic toxicity

Increasing levels of As had a pronounced effect on the nutrient uptake as well as its transport in plants. To evaluate the effect of As on N and P concentrations in root, shoot and leaf both N and P were analyzed.

Nitrogen content

Mean values of the nitrogen content in soil after harvest and in different parts of cowpea are presented in table 4. Nitrogen content significantly decreased in plant roots with increased As concentration ($p = 0.000$ for both the soils) whereas there has been an increase in the shoot N content of cowpea grown on Sonargaon soil ($p = 0.043$). There was no significant reduction of leaf nitrogen. Decreased nitrogen content in different plant parts might be due to reduced growth and reduced number of root nodules.

It needs to be mentioned here that the nodules formed on Dhamrai soil were not sufficient enough to be analyzed separately. These were analyzed with the roots. The poor growth of nodules, even in the control plants on Dhmarai soil could be due to the fact that the Dhamrai soil does not contain indigenous cowpea Rhizobium; the nodulation that occurred could have been due to inoculation with biofertilizer. In Sonargaon soil, however, the nodule nitrogen concentrations were 6.1%, 3.6%, 3.1% and 2.8%, in 0, 20, 30 and 50 mg As/L treated plants respectively. The corresponding nodule As concentrations were 0.5, 5.6, 7.9, and 10.3 mg/kg respectively. This shows that with increasing As accumulation in nodules, the nodule nitrogen also decreased. From this observation, it is realized that *Rhizobium*-legume symbiotic association is affected by high level of As in the growth medium. It has been observed that high concentration of As has a depressing effect on the *in vitro* growth of *Rhizobium* (data not shown in this paper) Similar effects might have occurred *in vivo* too.

Root nodules of control plants in Sonargaon soil contained the highest amount of nitrogen (6.1%) among all other plant parts. Nodule nitrogen concentrations significantly differed among control and all treated plants and decreased with increased As concentrations. Paivoke (1983) reported that the nodulation and nitrogen fixation were depressed and plant nitrogen content decreased at As treatments in garden pea. The antagonistic effects between As and nitrogen have also been reported in rice (Yamare, 1989) and in silver beet (Merry *et al*., 1986).

Phosphorus content

Phosphorus content in different plant parts are shown in Table 4. Phosphorus content increased with As treatment compared to the control in roots but there was no difference among the treatments. Leaves contained comparatively higher amount of phosphorus than root and shoot. But the increased phosphorous content in root and leaf was highly significant ($p = 0.000$ for root and leaf in both the soils) whereas in shoot it was insignificant in both the soils. Increased uptake of P in presence of As might be due to the fact that arsenate and phosphate are taken up by the same plasma membrane transport system (Meharg and Macnair, 1990) in plants. Increased P accumulation in presence of As in the growth medium has also been observed in hydroponically grown rice by Shaibur *et al*. (2006).

CONCLUSION

It is apparent from the present study that As in the growth medium of leguminous plants could adversely affect the symbiotic system and cause a negative effect on the nutritive quality of the legumes. Further studies with more comestible grain legumes are emphasized.

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