# EFFECT OF ARSENIC ON THE NUTRIENT UPTAKE PATTERN OF AMARANTHUS

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### **Abstract**

A macrocosm study was carried out to assess the transfer of arsenic (As) from irrigation water and sludge of arsenic removal water filter to two varieties of *Amaranthus* and their effects on phosphorus (P), potassium (K), sulfur (S), calcium (Ca) and magnesium (Mg) uptake. P and K uptake in shoot and root were negatively affected by As that were not significant. However, a significant negative relationship was observed between As and S in shoot. A positive and significant relationship existed between As and Ca of green *Amaranthus* shoot but it was negative and insignificant in case of red *Amaranthus*. Mg content of shoot and root was positively affected by As in green *Amaranthus*. On the other hand, in the red *Amaranthus* the relation between As and Mg was positive in the shoot but negative in the root.

#### Introduction

Arsenic is a highly toxic element and its presence in food composites is a matter of concern to the well-being of both humans and animals. Recent reports have shown that some of our foodstuffs are contaminated with arsenic<sup>(1-2)</sup>. It might be due to a high concentration of As in the soil where these foodstuffs are grown. Bangladesh economy is primarily based on agriculture. So, widespread uses of ground water for irrigation suggested that ingestion of irrigated crops and vegetables could be a major exposure route for As<sup>(3)</sup>. The uptake and accumulation of As by cereals, pulses and vegetable crops resulting in considerable reduction in the yields has been very well documented. Arsenic significantly decreases the biomass of winter wheat shoots and roots and significantly increases the concentrations of total Mg and Ca in shoots and enhances the transport of Mg and Ca from roots to shoots but decreases K, N and P concentrations in both shoots and roots<sup>(4)</sup>.

Bangladeshi people consume mainly rice and vegetables. A wide variety of vegetables are available in Bangladesh. Most of the vegetables that need supplemental irrigation, and if the irrigation water is arsenic contaminated, it is likely that the vegetables will contain high amount of arsenic<sup>(5)</sup>. The serious As contamination in ground water leads to an accumulation of As in top soil which ultimately leads to As

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uptake by crops. At present, the disposal of the sludges from As filter media has become a great concern for the environmental, as well as plant and soil scientists. Once the sludges are disposed of into the soil, it would come into chemical reaction in soil at a certain time and there is a possibility of soil contamination with the toxic elements and it is likely that the element will end up in the growing plants<sup>(6)</sup>. Any adverse effect in the uptake of nutrients by plants due to As from contaminated ground water and disposed sludge is, therefore, important to ascertain.

#### Materials and Methods

The experiment was conducted in a macrocosm in the Department of Soil, Water and Environment, University of Dhaka.

Soil samples were collected from Dhamrai (Thana - Dhamrai, Village - Basna, Mouja - Sanora, District - Dhaka) which belongs to Dhamrai soil series. The geographic location of the sampling site is 23°56.75N and 90°07.746E.

The bulk of soil samples representing 0 - 15 cm depth from the surface were collected by composite soil sampling method as suggested by the Soil Survey Staff of the USDA (7). The collected soil samples were dried in air for 5 days (~ 30°C). Visible roots and debris were removed from the soil samples and discarded. The larger and massive aggregates were broken down by gently crushing them using a wooden hammer and mixed thoroughly for making the composite. The bulk soil samples collected for pot experiment were screened through a 5 mm sieve. Two mm sieved soil samples were used for various physical analyses and 0.5 mm sieved soils were used for chemical and physicochemical analyses.

For the pot culture experiment, two leafy-vegetables commonly known as green *Amaranthus* (Data shak), and red *Amaranthus* (Lal shak) were used. All pot cultures were carried out in the three categories of soils *viz.*, control, soil spiked with arsenic filter sludge (two sludges *viz.*, granular ferric hydroxide and activated alumina were used as a source of As. A mixture of 50% granular ferric hydroxide and 50% activated alumina at a rate of 1 ton/ha was used for this purpose), and irrigated soil with As salt (As was applied with irrigation water by dissolving As salt. The sources of As salt were sodium arsenate and sodium arsenite. A mixture of 80% sodium asenite and 20% sodium arsenate was used for this purpose. In this case As was applied at a rate of 1 mg/l).

Pots of 5 kg sizes were taken having no holes in the bottom and marked in accordance with soil, plant and replication number. Then 4 kg of 5 mm sieved soil samples were taken in each of the earthen pots. The fertilizer requirement was assessed following Fertilizer Recommendation Guide by BARC<sup>(8)</sup>. The soil in each pot was mixed with the required amounts of urea, muriate of potash (MP) and triple super phosphate (TSP). The pots were arranged in a completely randomized design and were set in the net

house (the macrocosm) of the department of Soil Water and Environment, University of Dhaka.

Seeds of green and red *Amaranthus* were collected from local market. Seeds (20 - 25) were sown in each of the pots on February 10, 2010 and allowed to germinate. After germination, 10 seedlings were kept in each pot.

Plants received watering every day. Tap water was used for control and arsenic spiked pots. In case of arsenic containing irrigated pots, a measured amount of water containing a definite concentration of As was applied. The amount of irrigation water was noted. From the total amount of water added, the content of total As applied was calculated. Intercultural operations were carried out whenever necessary.

The plants were harvested manually by uprooting them after 35 days of emergence. The roots of the harvested plants were washed first with tap water and then again with deionized distilled water. The plant samples were separated into two parts - roots and edible one. The fresh weights of the collected plant samples were taken. The samples were first air-dried and then oven-dried at  $70 \pm 5$ °C for 48 hrs and the dry weight of the plant samples were used for chemical analyses. The samples were ground with an electrical grinder and sieved through a 0.2 mm sieve.

Various physical, chemical and physico-chemical properties of the soil samples were analyzed in the laboratory as described in Huq and Alam<sup>(9)</sup>. For the determination of total As, P, K, S, Ca and Mg the soil samples were digested with aqua regia (HNO<sub>3</sub>: HCl = 1:3). The plant samples were digested with HNO<sub>3</sub>. The total nitrogen in soil and plant samples was determined by Kjeldahl's method following H<sub>2</sub>SO<sub>4</sub> digestion. The total P was determined colorimetrically using a spectrophotometer at 490 nm by developing yellow color with vanadomolybdate. The available phosphorus of the soil was determined calorimetrically following the blue color method using ascorbic acid at wavelength of 880 nm after extracting by using the Bray & Kurtz solution. The available and total K was determined by flame analyzer. The available potassium of the soils was extracted with 1N ammonium acetate at pH 7. The total S was determined by turbidimetry using Tween-80. The total arsenic in soil and plant was determined by a hydride generation-atomic absorption spectrometer (HG-AAS)<sup>(10)</sup>. All elements of plant samples were calculated on oven dry basis.

All data in the present experiment were statistically analyzed by using Microsoft Excel and MINITAB (version 13) Packages.

## **Results and Discussion**

The collected soil samples were analyzed in the laboratory before setting up of the experiment to find out the nutrient status of the soil. Some important chemical properties of sludge were also determined. The contents of available N, P and K were found to be

very low. Total N, P, K and S of the soil were also determined. The concentration of As in soil was not much high but sludge contained much higher concentration of As compared to soil. The experimental soil was loam in texture, acidic in reaction (pH 5.4). The soil contained less than 0.591% organic matter. Important chemical properties are furnished as organic carbon 0.46%, total N 0.102%, total P-0.089%, total K 0.346%, total S 0.090%, available N 61.11 mg/kg, available P 4.187 mg/kg, available K 11.76 mg/kg, aqua regia extractable As 2.79 mg/kg. Sludge contained 311.75 mg/kg As and 13.11% Fe.

The mean height and amount of fresh matter of green and red *Amaranthus* are given in Table 1. Plant height of red *Amaranthus* was found to be higher than green *Amaranthus*. A significant difference was found in plant height between two varieties giving p value of 0.038. In case of green *Amaranthus*, the treatment effects did not vary but in case of red *Amaranthus*, a significant difference (p value 0.007) was found among treatments in relation to plant height. A highly significant difference was found among treatments in relation to fresh matter of both green *Amaranthus* (p value 0.00) and red *Amaranthus* (p value 0.00). Similar findings were reported where fresh yield of red *Amaranthus* decreased with the increase of As level<sup>(11)</sup>.

Table 1. Height (cm) and fresh matter (gm) of shoot of green and red Amaranthus.

Treatments	Green Ama	ranthus	Red Amaranthus			
	Height	Height Fresh weight		Fresh weight		
Control	14	26.02a	18ª	$37.98^{a}$		
Spiked	15	27.42a	19ª	39.3ª		
Irrigated	12	20.63b	15 <sup>b</sup>	31.53 <sup>b</sup>		

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

The mean values of arsenic content in shoot and root of green and red *Amaranthus* plants are presented in Table 2. Arsenic accumulation increased in arsenic treated plant samples compared to control. The total uptake (calculated by multiplying the concentration with the total dry matter produced) of arsenic by green *Amaranthus* was found to be 0.45, 0.57 and 6.38 mg/kg in control, As spiked and irrigated plant samples, respectively. The total uptake of As by red *Amaranthus* was 0.90, 2.7 and 8.63 mg/kg in control, As spiked and irrigated plant samples, respectively. As concentrations were higher in roots than in shoots.

Concentration and accumulation of As increased significantly both in shoot and root due to As treatments as compared to the control indicating that the bioavailability of arsenic depends on the concentration of As in the rooting medium and the higher the concentration the higher is the bioavailability. There was a trend that As concentration in roots was higher than that of shoots. The roots of vegetables and rice plants were found

to accumulate highest amount of As compared to other parts of plants<sup>(6)</sup>. Abedin *et al.*<sup>(12)</sup> also reported higher concentrations of As in roots of rice plants than the other parts.

Table 2. Concentration of Arsenic (mg/kg) in shoot and root of green and red Amaranthus.

Treatments	Green A	Amaranthus	Red A	Red Amaranthus			
	Shoot	Root	Shoot	Root			
Control	0.056 <sup>b</sup>	0.396 <sup>b</sup>	0.115 <sup>b</sup>	0.788 <sup>c</sup>			
Spiked	$0.113^{b}$	$0.458^{b}$	$0.18^{b}$	2.523 <sup>b</sup>			
Irrigated	$0.784^{\mathrm{a}}$	5.594a	0.803a	7.832a			

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

The contents of N, P, K, S, Ca and Mg in shoot and root of green and red *Amaranthus* are shown in Tables 3 and 4. The contents of P and K in shoot and root of both green and red *Amaranthus* were found to be highest in As spiked plant samples. In green *Amaranthus*, the maximum accumulation of S was found in shoot of As spiked plants, whereas in red *Amaranthus*, the maximum accumulation was found in shoot and root of control plant samples. Ca and Mg contents in shoot of green *Amaranthus* were found to be highest in irrigated plant samples. In case of red *Amaranthus*, control plant samples showed maximum accumulation of Ca and irrigated plant samples showed maximum accumulation of Mg though a little difference was found between control and irrigated plant samples.

Table 3. Concentration of N, P, K, S, Ca and Mg in shoot and root of green Amaranthus.

Treatments -	N (%)	P (%)		K (%)		S (%)		Ca (%)		Mg (%)	
	Shoot	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	3.27	0.3	0.25	4.46	3.75	$0.86^{ab}$	$0.85^{a}$	$1.45^{b}$	$0.72^{a}$	1.9	0.31
Spiked	3.52	0.4	0.34	4.72	3.94	$1.05^{a}$	$0.81^{a}$	$1.43^{b}$	$0.56^{ab}$	1.6	0.27
Irrigated	3.82	0.3	0.31	4.21	3.21	$0.60^{b}$	$0.59^{b}$	$1.85^{a}$	$0.45^{b}$	2.08	0.4

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

Table 4. Concentration of N, P, K, S, Ca and Mg in shoot and root of red Amaranthus.

Treatments	N (%)	P (%)		K (%)		S (%)		Ca (%)		Mg (%)	
				Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	3.58	0.39 <sup>b</sup>	0.24	5.64ab	4.77	0.76a	0.9	1.07	0.7	1.56	0.60a
Spiked	3.8	$0.48^{a}$	0.29	$6.03^{a}$	5.14	$0.54^{b}$	0.7	0.87	0.68	1.3	$0.80^{a}$
Irrigated	3.49	$0.41^{b}$	0.26	5.12 <sup>b</sup>	4.46	$0.45^{b}$	0.74	0.93	0.49	1.58	$0.25^{b}$

Means followed by the same letter(s) in a column do not differ significantly from each other at 5% level of significance.

The effect of As on P content is presented in the Fig. 1. The relationship between As and P in shoot was found to be negative both for green and red *Amaranthus* the values being insignificant (p value 0.608 and 0.671, respectively). The root arsenic and root P of red *Amaranthus* also showed insignificant negative correlation the p value being 0.987. Reduction of P concentrations may be due to the fact that the arsenate is taken up by the phosphate transport system<sup>(13)</sup> and decreasing both P concentration and P accumulation. However, the relationship between As and P in the roots of green *Amaranthus* was found to be positive but insignificant (p value 0.641).

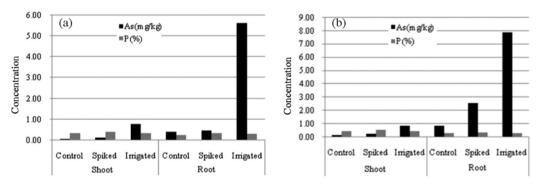


Fig. 1. Effect of As (mg/kg) on P (%) content in shoot and root of (a) green *Amaranthus* and (b) red *Amaranthus*.

The effect of As on K content is shown in the Fig. 2. As had a negative and insignificant effect on the uptake of K in shoot (p value 0.545) and root (p value 0.305) of green *Amaranthus*. In case of red *Amaranthus*, the relationship between As and K was also found to be negative and insignificant both for shoot (p value 0.079) and root (p value 0.409).

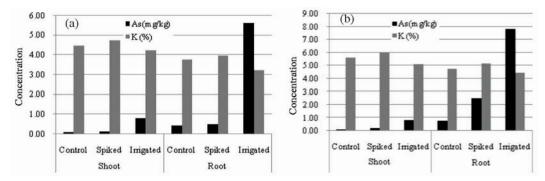


Fig. 2. Effect of As (mg/kg) on K (%) content in shoot and root of (a) green *Amaranthus* and (b) red *Amaranthus*.

Arsenic decreased the accumulation of K both in shoots and roots which is in agreement with the result of Wallace *et al.*<sup>(14)</sup>, who showed that depression of K concentration in roots of bush bean plants was due to arsenic in the nutrient solution.

A significant negative relationship was found between As and S in shoot of both green *Amaranthus* (p value 0.049) and red *Amaranthus* (p value 0.05). Although As had significant negative effect on the concentration of S in root (P value 0.012) of green *Amaranthus*, the effect of As on S in root of red *Amaranthus* was found to be negative but insignificant (p value 0.625). Merry *et al.*<sup>(15)</sup> reported the interaction between sulfur and As. They pointed out that S and As exists in soil solution in similar ionic forms (e.g. sulfate and arsenate) and there should be a competition between these two ions. The effect of As on the content of S is shown in the Fig. 3.

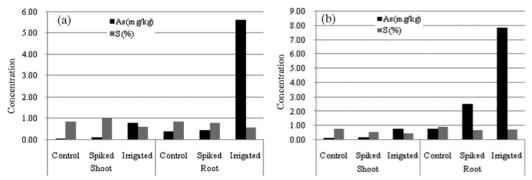


Fig. 3. Effect of As (mg/kg) on S (%) content in shoot and root of (a) green *Amaranthus* and (b) red *Amaranthus*.

The effect of As on Ca content is shown in Fig. 4. In case of green *Amaranthus*, a positive correlation was found between arsenic and calcium in shoot that was significant (p value 0.002). But the relationship between As and Ca was negative and significant (p value 0.026) for root. A negative but insignificant relation between As and Ca was found both for shoot (p value 0.668) and root (p value 0.234) of red *Amaranthus*.

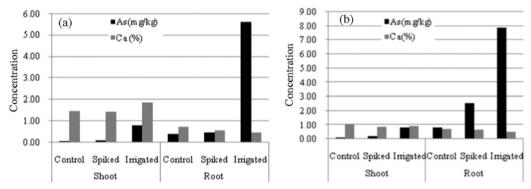


Fig. 4. Effect of As (mg/kg) on Ca (%) content in shoot and root of (a) green Amaranthus and (b) red Amaranthus.

The decrease in Ca content in As treated plants is in agreement with the result of Wallace *et al.*<sup>(14)</sup> who showed the depression of Ca concentration in leaves and roots of bush bean plants was due to As-toxicity. However, the significant increase in the

concentration of Ca in shoot of green *Amaranthus* could be due to some metabolic aberrations where Ca-pectate formation has been increased because of higher arsenic toxicity on green *Amaranthus*.

The effect of As on the content of Mg is shown in Fig. 5. A positive relationship was found between As and Mg in the shoots of both green *Amaranthus* (p value 0.186) and red *Amaranthus* (p value 0.946). The relationship between As and Mg in root of green *Amaranthus* was also positive and insignificant (p value 0.178). Carbonell-Barrachina *et al.*<sup>(16)</sup> have shown similar result in stems of bean plants at harvesting stage when sodium arsenite was applied at 5 mg As/l. But the relationship between As and Mg in root of red *Amaranthus* was found negative. Shaibur<sup>(17)</sup> found a significant negative effect on the concentration of Mg both in shoots and roots of barley plants due to As treatments.

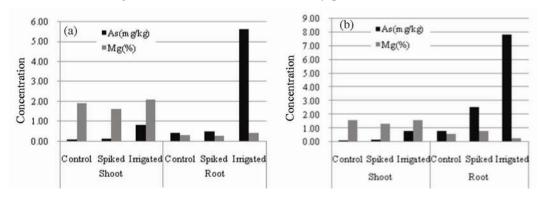


Fig. 5. Effect of As (mg/kg) on Mg (%) content in shoot and root of (a) green *Amaranthus* and (b) red *Amaranthus*.

It could be concluded from the present observations that As in the growth medium of *Amaranthus*, a very common and popular leafy vegetable, could differently affect the mineral nutrition of the plant. However, the varietal difference of the vegetable has influence on the effect of As in the mineral nutrition.

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