

## ASSESSMENT OF ARSENIC ACCUMULATION EFFICIENCY BY SELECTED NATURALLY GROWN WEEDS

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### ABSTRACT

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A pot experiment was conducted at the Bangladesh Agricultural University on the year 2003 for studying the effect of different levels of added arsenic on growth of and arsenic accumulation by nine naturally grown weeds for the possible development of phytoremediation technology. The experiment comprised seven arsenic doses viz. 0, 10, 20, 30, 50, 70 and 100 ppm as added as NaAsO<sub>2</sub> in solution. Out of nine selected weed species, Water cress (*Enhydra fluctuans*) absorbed highest amount (42.76 µg pot<sup>-1</sup> of arsenic followed by Joina (*Fimbristylis miliacea*) (36.73 µg pot<sup>-1</sup>), Barnyard grass (*Echinochloa crus-galli*) (36.04 µg pot<sup>-1</sup>) and Malancha (*Alternanthera philoxeroides*) (29.80 µg pot<sup>-1</sup>). Arsenic absorption was in the order of root > shoot. Joina accumulated higher amount of arsenic in root (32.37 ppm) than in shoot (1.80 ppm). Barnyard grass absorbed 30.25 ppm As in root and 1.90 ppm As in shoot. This sequence of As absorption would presumably naturally safeguard our domestic herbivorous animals as well as human from As-poisoning. Water cress was the best arsenic accumulator. But considering the intensity of infestation rate in rice field, Joina and Barn yard grass are more appropriate for mitigating arsenic contaminated rice fields. This two plant species may be proposed for detoxification of arsenic contaminated soil. On the other hand, Water cress and Malancha may be proposed for the remediation of arsenic from contaminated stagnant water.

**Key Words: Arsenic Accumulation, Phytoremediation, Plant biomass**

### INTRODUCTION

Arsenic (As) is one of the environmental hazardous elements because of its toxicity and carcinogenicity. Arsenic contamination in Bangladesh is probably geological in nature, originating from the fine alluvial sediments of the Ganges Delta. Consequently 80 million people are now exposed to the threat of arsenic poisoning and 10,000 people have shown the symptom of arsenicosis (Zaman *et al.*, 2001). The contamination poses a great cause of concern to people and agricultural sustainability. The magnitude of arsenic problem can be compared with the great scourge, we encountered in the past like cholera, small pox, cyclones, floods.

Ground water contamination by arsenic (As) in Bangladesh appears to be the largest mass poisoning in the world. Arsenic contaminated ground water is randomly used in Bangladesh for irrigation, which in turn enhances the levels of soil arsenic. Long term use of arsenic contaminated ground water to irrigate crops, especially paddy rice (*Oryza sativa*), has resulted in elevated soil arsenic levels in Bangladesh.

Arsenic concentration in Bangladesh soils ranges from 4-8 µg g<sup>-1</sup>, but in irrigated areas soil arsenic level can reach up to 83 µg g<sup>-1</sup> (Ullah, 1998). For example, Durba (*Cynodon dactylon*) accumulate 67.82 ppm arsenic in the roots. Barn yard grass (*Echinochloa crus-galli*) accumulates 67.91 and 8.20 ppm arsenic in shoot and root respectively (Molla, 2002).

Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize and destroy contaminants in the soil and ground water. The idea of using rare plants which hyperaccumulate metals to selectively remove and recycle excessive soil metals was introduced in 1983 (Chaney, 1983). This practice gained public exposure in 1990 (Anonymous, 1990) and has increasingly been examined as a potential for more practical and more cost effective technology than soil replacement, solidification or washing strategies presently used (Salt *et al.*, 1996).

Due to continuous irrigation with arsenic contaminated water, the level of arsenic in soil is increasing day by day. From this viewpoint, the phytoremediation technology may become very effective for remediation of arsenic contaminated soils of Bangladesh. The main advantage of this green technology is that it leaves no residual effect in soil, water and on biodiversity. Keeping this fact in mind, a study has been conducted for the remediation of arsenic contaminated soils by naturally grown weeds for a) assessment of arsenic accumulation by selected naturally grown weeds and b) observation of the effect of different levels of added arsenic on the growth of these weeds.

## MATERIALS AND METHODS

### Collection and Preparation of Soils

The soil was collected from the village of Vabokhali Sadar Thana, Mymensingh. The initial soil (virgin soil) was collected at 0-15 cm depth. The virgin soil was collected because; these soils were not treated by any fertilizer. The unwanted materials like stones, gravels, pebbles, plant roots, etc. were removed from the bulk soil. Then the soil was dried in the sun for 4 days and the clods were broken. The initial soil contains 2.56 ppm arsenic.

### Pot Preparation

An amount of 5 kg soil was taken in a series of plastic pots. Considering the field condition, the pots were maintained in the natural condition. Proper spacing was maintained among pots for convenience of cultural operations.

### Selected weed species and treatments

The experiment was conducted in the net house of Agricultural Chemistry Department, BAU, during July to November 2003. The criteria used for selecting plants for phytoremediation were high arsenic tolerance, high bioaccumulation factor, short life cycle, high propagation rate, wide distribution and large shoot biomass. The selected nine weed species were Barnyard grass (*Echinochloa crus-galli*), Joina (*Fimbristylis miliacea*), Mutha (*Cyperus rotundus*), Water cress (*Enhydra fluctuans*), Malancha (*Alternanthera philoxeroides*), Panikachu (*Monochoria hastata*), Panilong (*Ludwigia hyssopifolia*), Chisra (*Scirpus juncoideus*) and Topapana (*Pistia stratiotes*).

There were seven treatments with arsenic viz. 0, 10, 20, 30, 50, 70 and 100 ppm arsenic (soil basis) from NaAsO<sub>2</sub> (Na-arsenite). Urea, TSP, MP and Gypsum were added @135 kg ha<sup>-1</sup>, 100 kg ha<sup>-1</sup>, 70 kg ha<sup>-1</sup> and 60 kg ha<sup>-1</sup> respectively to each pot.

### Data collection

Plant height (cm) was measured from the ground level to the top of the plants from each pot and number of leaves for each plant was recorded. After harvesting the plants of each pot were dried and weighed and averaged. The result was expressed as g pot<sup>-1</sup>

### Digestion of the samples

A representative sample weighing 0.5g for plant and 1 g for soil was transferred in to a dry clean 125 ml conical flask. 5 ml concentrated nitric acid was added to each of the flasks. The content of the flasks was mixed and the flasks were left overnight covering with aluminum foil. The following day, the flasks were placed on a heating block and heated at a temperature slowly raised to 120°C for two hours. After heating, the vessels were allowed to cool and 3 ml of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added. Again, the flasks were heated 120°C for an hour and volume reduced to 3-4 ml. The digests were cooled, diluted to 50 ml with deionized water and filtered through Whatman No. 42 filter paper into dry plastic bottle. Some authors suggest that root should be washed out with diluted acid before subjected to digestion.

### Total soil and plant arsenic

Total arsenic content of soil and plant was determined from the digest by flow injection hydride generator atomic absorption spectrophotometer with Unicam 969 and with a hydride generator assembly using matrix-matched standards (Welsch *et al.*, 1990)

### Statistical Analysis

The analysis of variance for the different parameters were completed and tabulated in proper form for statistical analysis. Analysis of variance was done following Completely Randomized Design (CRD) and the mean results of significant F value were adjusted by the Duncan's Multiple Range Test (DMRT) with the help of Computer package MSTATC.

## RESULTS AND DISCUSSION

### Plant height

Addition of As to soil markedly reduced the plant height of the weeds (Table 1). Plant height was recorded at 45 days after transplanting and was found to vary significantly. The plant height decreased as the doses of arsenic increased from 10 to 100 ppm. In case of Barnyard grass, Joina, Malancha, Panikachu, and Topapana ; the tallest plants were found in the control pots that received 0 ppm arsenic and were statistically identical with those from the pots receiving 10 ppm As. Mutha, Water cress, Panilong, Chisra also showed highest plant height on T<sub>0</sub> (control). The shortest plant was observed in the pots that received 100 mg As kg<sup>-1</sup>. The possible reasons of stunted growth of weeds due to sequentially increasing doses of As might be that excess levels of As might have retarded the division of meristematic tissue of weeds. Hossain (1997) conducted a pot culture experiment with different arsenic treatments and found that higher concentrations of arsenic treatment reduced plant height. The present results were at par with the above report.

### Number of Leaves

The number of leaves per plant significantly decreased with As addition and in most cases the effect was prominent at 30 ppm or above doses except for Topapana. Topapana showed maximum number of leaves in the plant that received 50 ppm As but did not survive at 70 and 100 ppm As doses (Table 2). In case of Joina, Water cress, Malancha and Panilong the highest number of leaves per plant was observed in control (T<sub>0</sub>), which was statistically identical with those found in T<sub>10</sub> (10 mg As kg<sup>-1</sup>) treatment. The number of leaves per plant for different As doses varied from 5-20, 22-36, 18-67, 96-189, 30-60, 9-32, 32-115, 30-45 and 36- 124 in respective weeds (Table 2). The lowest and the highest number of leaves per plant were observed in 100 and 0 ppm As treatments respectively, except Topapana. The highest levels of As might have retarded the cell division of those weeds. But in case of Topapana it is difficult to explain.

### Plant biomass

The biomass production of nine weeds was affected significantly by As application (Table 3). In case of Barn yard grass, Joina, Mutha, Water cress, Malanelma, Panilong, Chisra and Topapana biomass production due to 0-100 ppm As treatments was found to vary from 2.01-17.13, 3.76-10.82, 2.18-7.99, 33.21-51.28, 5.62-21.93, 11.04-30.15, 0.25-4.67, 6.25-15.85 and 2.56-3.86 g pot<sup>-1</sup> respectively. In case of Topapana no biomass could be recorded at 70 and 100 ppm As treated plants, because the weeds did not survive. The highest biomass was found at the control treatment. The lowest biomass was found at the 100 ppm As treatment.

In most cases biomass was remarkably reduced as compared to control. The possible reasons would be that the higher doses of As interfered with the division of cell as well as normal plant physiology. Similar results were observed by Marin *et al.*, (1993) who reported that significant reduction of dry biomass (root and shoot) was recorded with increasing concentration of arsenic.

### Concentration of arsenic in weeds under different treatments

Accumulation of As was found higher in root than in shoot. The arsenic concentration in root was significantly increased with increasing levels of As application (Table 4). The highest concentration of arsenic (32.375 ppm) in root was observed in the 100 ppm As treated Joina and lowest (0.175 ppm) was found in the root of control Panilong. In case of shoot, highest concentration was found in Malancha (2.775 ppm) with 100 ppm As treatment and the lowest in Topapana (0 ppm) under control condition. The accumulation difference of As among the roots and shoots was very wide. In case of Barnyard grass root accumulated 30.25 ppm As whereas shoot contained 1.90 ppm As. Arsenic concentration was gradually increased with increasing rate of arsenic both for root and shoot. As accumulation in roots of Barnyard grass, Joina, Mutha, Water cress, Malancha, Panikachu, Panilong, Chisra, ranged from 1.35-30.25, 0.30-32.375, 0.85-10.05, 0.55-7.45, 0.45-7.875, 0.210-4.225, 0.175-6.50 and 0.60-7.80 ppm respectively (Table 4). Similar trend of As accumulation was found for shoots of the all the weeds under test. In case of Topapana accumulation could not be measured at 70 and 100 ppm As treatments due to the fact that plants did not survive. Though As ion is characteristically mobile, the maximum accumulation of As was detected in roots. The possible reason might be that there were some heavy metals accumulating sinks in roots of the weeds or some other physiological reasons were responsible for this phenomenon. Tlustos *et al.*, (1998) also expressed similar views that As content was higher in roots than in shoots. The present results were at par with that report.

### Apparent arsenic recovery

An attempt was made to compute the amount of As uptake by weeds. It appears that total As uptake ranged from 10.52-36.04, 1.88-36.73, 2.40-12.60, 13.26-42.76, 3.61-29.80, 4.71-17.74, 0.41-1.715, 4.25-19.98 and 0.172-3.01 µg pot<sup>-1</sup> from different levels of As (Table 5) in Barnyard grass, Joina, Mutha, Water cress, Malancha, Panikachu, Panilong, Chisra and Topapana within 45 days after transplanting respectively. Results in the Table 5 showed significant difference under different treatments of Arsenic on the uptake of As in weeds. Highest uptake was found in Water cress (42.76 µg pot<sup>-1</sup>) and lowest was found in Topapana (0.172 µg pot<sup>-1</sup>). The amount of As retained in soil after 45 days varied between 0.60-6.88, 0.88-8.19, 1.44-8.50, 0.63-5.63, 1.06-10.56, 0.88-9.56, 1.06-7.09, 1.31-7.19 and 0.88-7.44 ppm As (Table 6) under different treatments. Difference between As uptake and As retention showed that a large amount of As added to the soil was not recovered. It was assumed that a considerable amount of As might have lost from the soil due to methylation (transformation of inorganic As to organic As by methanogenic bacteria), even flow of water from pots due to unprecedented rain that was out of control as reported by Smith *et al.*, (1998).

Addition of arsenic to soil significantly affected the plant height, number of leaves plant<sup>-1</sup> and total biomass. Generally the values of the parameters decreased as the doses of arsenic increased. On the contrary, in case of Topapana number of leaves was increased with increasing rate of arsenic but plant did not survive at 70 and 100 ppm As. The reasons of increase of number of leaves, decrease of leaf area and reduction of plant height are very difficult to explain.

Among the nine species, higher concentration of As was found in Joina followed by Barn yard grass and Mutha. But due to higher biomass, Water cress absorbed highest amount of arsenic from the treated soil. Since the infestation rate of water cress in rice fields is relatively less as compared to Join; Barn yard grass and Mutha, this may not be applicable for phytoremediation of As contaminated soil. These nine species of weed sample showed that the concentration of arsenic in root was very much higher than in shoot. It is a very significant finding that shoots of the different weeds accumulate very less amount of arsenic as compared to root. Since herbivorous domestic animals such as cow, goat, buffalo, etc. consume above ground portion of the weeds and if these domestic animals feed on weeds grown on As contaminated soils, they may not be victim to As-poisoning, because minimum accumulation of As was detected in the shoots of the weeds.

The experimental findings revealed that Joina and Barnyard grass were the best arsenic accumulator for the phytoremediation technology. Within 45 days Joina and Barnyard grass removed 36.73 and 36.04 µg arsenic from the treated soil respectively. Until today, there have been only a few examples of successful applications in small scale. A full remediation of As from As contaminated soils may require a couple of years. But this is the only eco-friendly arsenic remediation technology. Transgenic plant should be developed, which hyperaccumulate the toxic metal from the contaminated soil within short period.

**Table 1. Effect of arsenic on plant height (cm) of different naturally grown weeds**

Treatments	Barnyard grass	Joina	Mutha	Water cress	Malancha	Pani Kachu	Panilong	Chisra	Topapana
T <sub>0</sub>	75a	33a	44a	70a	60a	50a	72a	55a	4.00a
T <sub>10</sub>	75a	30ab	38b	65b	60a	44ab	60b	51b	4.00a
T <sub>20</sub>	72ab	28b	34bc	60c	54ab	44ab	53c	50b	3.50ab
T <sub>30</sub>	70b	28b	30cd	60bc	52b	40bc	51c	46c	3.20d
T <sub>50</sub>	65c	27b	27d	59c	45c	38bc	48c	46c	1.5c
T <sub>70</sub>	65c	27b	27d	58c	35d	35c	30d	45c	Θ
T <sub>100</sub>	60d	23c	27d	52d	30d	23d	21e	41d	Θ
Level of significance	**	**	**	**	**	**	**	**	**
CV(%)	4.75	7.01	8.08	4.98	7.67	10.13	6.32	4.86	11.49

\*\* Significant at 1% level of probability

Note: In a column figures with dissimilar letters differ significantly according to DMRT.

Θ Plant did not survive

**Table 2. Effect of arsenic on number of leaves of different naturally grown weeds**

Treatments	Barnyard grass	Joina	Mutha	Water cress	Malancha	Pani Kachu	Panilong	Chisra	Toipapana
T <sub>0</sub>	20a	36a	67a	189a	60a	32a	115a	45a	36a
T <sub>10</sub>	12b	35ab	63b	176a	55ab	29b	113a	36b	35b
T <sub>20</sub>	12b	34ab	57c	128b	54ab	24c	93b	33c	48b
T <sub>30</sub>	8c	33a-c	41d	120bc	52b	24c	82b	32cd	61b
T <sub>50</sub>	7c	32bc	40d	109c-d	45c	15d	62c	31cd	124a
T <sub>70</sub>	5d	30c	24c	107cd	35d	12e	48d	30d	Ø
T <sub>100</sub>	5d	22d	18d	96d	30d	9f	32e	30d	Ø
Level of significance	**	**	**	**	**	**	**	**	**
CV(%)	8.57	6.84	4.60	7.91	8.99	6.32	9.71	4.81	10.72

\*\* Significant at 1% level of probability

Note: In a column figures with dissimilar letters differ significantly according to DMRT.

Ø Plant did not survive

**Table 3. Effect of Biomass of different naturally grown weeds (g pot<sup>-1</sup> on oven dry basis)**

Treatments	Barnyard grass	Joina	Mutha	Water cress	Malancha	Pani Kachu	Panilong	Chisra	Toipapana
T <sub>0</sub>	17.13 a	10.82 a	7.99 a	51.28 a	21.93 a	30.15 a	4.67 a	15.85 a	3.86 a
T <sub>10</sub>	16.35 a	9.67 b	6.83 b	44.39 b	20.63 ab	27.87 b	2.85 b	15.25 a	3.22 b
T <sub>20</sub>	9.80 b	8.66 c	6.62 b	42.45 bc	18.49 b	22.08 c	2.30 bc	14.45 a	3.21 b
T <sub>30</sub>	7.82 c	8.23 c	6.54 b	39.92 c	15.32 c	18.44 d	2.04 c	10.90 b	2.99 be
T <sub>50</sub>	7.37 c	6.70 d	4.42 c	39.82 c	12.82 d	17.03 de	1.59 c	8.66 c	2.56 c
T <sub>70</sub>	4.60 d	3.99 e	2.33 d	33.99 d	12.72 d	15.20 e	0.61 d	8.39 c	No biomass
T <sub>100</sub>	2.01 e	3.76 e	2.18 d	33.21 d	5.62 e	11.04 f	0.25 d	6.25 d	No biomass
Level of significance	**	**	**	**	**	**	**	**	**
CV (%)	9.09	5.95	7.06	4.05	8.16	5.28	19.91	7.42	13.36

\*\* Significant at 1% level of probability

Note: In a column figures with dissimilar letters differ significantly according to DMRT.

**Table 4. Arsenic concentration (ppm) in different naturally grown weeds**

Treatments	Barnyard grass	Joina	Mutha	Water cress	Malancha	Pani Kachu	Panilong	Chisra	Topapana
T <sub>0</sub>	10.52d	1.88e	2.40d	13.26f	3.61f	4.71e	0.46c	4.25e	0.17e
T <sub>10</sub>	23.35b	10.69d	5.95c	23.33e	13.08e	5.89d	0.41c	10.17d	0.72d
T <sub>20</sub>	16.71c	11.59d	10.11b	27.68d	16.13d	4.97e	1.26b	15.05c	1.08c
T <sub>30</sub>	25.76b	36.18a	12.60a	32.01c	21.63c	7.72c	1.72a	14.48c	2.73b
T <sub>50</sub>	24.96b	31.41b	11.53a	42.76a	23.47c	13.00b	1.65a	13.68c	3.01a
T <sub>70</sub>	36.04a	19.75c	9.70b	36.21b	29.80a	16.94a	1.12b	21.57a	-
T <sub>100</sub>	24.51b	36.73a	9.03b	38.11b	26.47b	17.74a	0.61c	19.98b	-
Level of significance	**	**	**	**	**	**	**	**	**
CV(%)	5.90	3.64	7.67	4.47	6.02	4.55	12.89	5.67	11.96

**Table 5. Total uptake of arsenic (µg As pot<sup>-1</sup>) by different naturally grown weeds**

Treatments	Barnyard grass		Joina		Mutha		Water cress		Malancha		Panikachu		Panilong		Chisra		Topapana		
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	
T <sub>0</sub>	0.32f	1.35f	0.12f	0.30e	0.10d	0.85f	0.15e	0.55f	0.07e	0.45f	0.17d	0.21e	0.07d	0.17e	0.10e	0.60f	0e	0.2e	
T <sub>10</sub>	0.55e	3.90e	0.25e	3.35d	0.25d	2.35e	0.25d	2.10e	0.20de	1.95e	0.19d	0.27e	0.12d	0.20e	0.15e	1.95e	0.15d	0.45d	
T <sub>20</sub>	0.65d	5.35d	0.27e	3.75d	0.95c	2.85d	0.30c	2.65d	0.42d	2.05de	0.20d	0.30e	0.30c	1.32d	0.40d	1.65d	0.25c	0.60c	
T <sub>30</sub>	1.10c	9.35c	0.45d	12.75c	1.45b	3.00d	0.30c	3.75c	1.02c	2.25d	0.25d	0.92d	0.42c	2.12c	0.45d	3.30c	0.60b	1.90b	
T <sub>50</sub>	1.13c	9.57c	0.67c	14.25c	1.60b	4.05c	0.35b	5.75b	1.15bc	3.37c	0.32c	1.97c	0.47c	2.50c	0.65c	3.40c	0.70a	2.60a	
T <sub>70</sub>	1.60b	8.27b	0.75b	7.67b	1.70b	9.35b	0.35b	5.66b	1.30b	4.80b	0.35b	3.15b	0.92b	3.55b	0.80b	6.05b	-	-	
T <sub>100</sub>	1.90a	30.25a	1.80a	32.37a	2.00a	10.05a	0.50a	7.45a	2.77a	7.87a	0.62a	4.22a	1.15a	6.50a	0.95a	7.80a	-	-	
Level of significance	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
CV (%)	2.85	3.22	6.36	9.94	13.25	4.07	6.01	2.64	7.12	4.76	13.12	3.77	20.15	16.16	6.28	4.24	9.87	6.96	

\*\* Significant at 1% level of probability

Note : In a column figures with dissimilar letters differ significantly according to DMRT.

**Table 6. Concentration of arsenic (ppm) in post harvest soil**

Treatments	Barnyard grass	Joina	Mutha	Water cress	Malancha	Pani Kachu	Panilong	Chisra	Topapana
T <sub>0</sub>	0.60g	0.88f	1.44d	0.63e	1.06f	0.88f	1.06e	1.31e	0.88g
T <sub>10</sub>	1.19f	1.94e	1.69d	1.44de	2.00e	2.75e	2.19d	2.13d	1.88f
T <sub>20</sub>	2.00e	2.69d	2.63c	2.06cd	2.69d	3.63d	2.44d	3.94c	2.25e
T <sub>30</sub>	3.69c	3.25c	3.00c	2.87c	4.31c	4.38c	4.19c	3.88c	3.25d
T <sub>50</sub>	2.63d	6.50b	3.44c	4.19b	5.57b	4.50c	4.69d	4.13c	4.75c
T <sub>70</sub>	5.56b	6.75b	5.38b	5.63a	5.44b	7.81b	7.09a	5.06b	5.88b
T <sub>100</sub>	6.88a	8.19a	8.50a	5.00ab	10.56a	9.56a	6.93a	7.19a	7.44a
Level of significance	**	**	**	**	**	**	**	**	**
CV(%)	4.03	3.51	6.21	6.04	4.18	7.27	5.26	4.28	4.25

\*\*Significant at 1% level of probability.

In a column figures with dissimilar letters differ significantly according to DMRT.

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