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REGULAR ARTICLE

IDENTIFICATION OF ARSENIC HYPERACCUMULATING PLANTS FOR THE DEVELOPMENT OF PHYTOMITIGATION TECHNOLOGY

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SUMMARY

Out of 35 plant samples collected from different districts of Bangladesh, only 14 species were arsenic (As) hyper accumulating. Species identified as hyperaccumulating were barnyard grass, water cress, cockle bur, Azolla, rice, water lettuce, water taro, taro, fern, water hyacinth and alligator weed. Maximum accumulation of As was in barnyard grass at 61.3 ppm, 67.9 ppm and 67.8 ppm in root, shoot and grain, respectively. From the chemical analysis of 35 naturally grown plants barnyard grass can mitigate soil As contamination. Water hyacinth, water cress, water lettuce and Azolla were able to absorb As from contaminated stagnant water. From this study an idea for phytoremediation of As by naturally grown plants has been found.

Keywords: Arsenic Pollution, Hyperaccumulation, Phytoremediation, Plants, Weeds.
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1. Introduction

Arsenic is of great concern due to extensive contamination and carcinogenicity. Arsenic contamination of ground water has been reported from 20 countries, including Bangladesh. At present 59 districts across the country are affected by As poisoning. Consequently, 80 million people are exposed to As poisoning and 10,000 people have shown the symptoms of arsenicosis [1].

Arsenic contamination of irrigation water has a detrimental effect on food quality and a devastating long-term effect on the soil and is a constant threat to agricultural sustainability. Few plant species accumulate As at high concentrations, and it is a hazard to human and animal health. Phytotoxicity usually occurs before high concentrations are reached [2]. Humans may be affected directly, if plants such as watercress and mint are consumed, or indirectly when humans consume species that have high As levels due to contamination in the food-chain [3]. The most common As minerals are arsenopyrites, mispickes (FeSAs), orpiment (As_2S_3) and realgar (AsS). Arsenic is associated with many types of mineral deposits especially those which include sulfide mineralization.

Certain plants concentrate essential and non-essential heavy metals in their roots and shoots to levels exceeding those in the soil. The mechanisms of metal accumulation, which involve extracellular and intracellular metal chelation, precipitation, compartmentalization and translocation in the vascular system, are poorly understood and it is called biosynthesis and bioaccumulation of a specific element by a specific plant. Interest in these mechanisms has led to the development of phytoremediation, a new technology, which is uses plants to clean up soil and water contaminated with heavy metals without residual effects.

This cost effective plant based approach to remediation, takes advantage of the remarkable ability of plants to accumulate As from the environment and metabolize it in their tissues. Recently, knowledge of the physiological and molecular mechanisms of phytoremediation has begun to emerge, together with biological and engineering strategies designed to optimize and improve phytoremediation. Several field trials have confirmed the feasibility of using plants to remove As from the soil [4].

In the field of phytoremediation, the utilization of plants to transport and concentrate metals from the soil into harvestable parts of roots and above round shoots is called phytoextraction. Phytomitigation will be able to remediate water and soil contaminated with As and will be economically feasible especially in Bangladesh. Arsenic-contaminated sites that are leaching it into water could be cleaned up or contained using phytoremediation. This technology uses plants to either, stabilize As *in situ,* or extract it from the soil so it can be stored in an area where it is not an environmental risk [5].

Our research emphasis is on pollution free soil and water for Bangladesh and it is a great challenge for 21st century. A special management practices should be developed to maintain soil and water quality. To overcome adverse soil conditions caused by As phytomitigation technology needs to be applied which will be easily take up by farmers to eliminate As build up in the soil. If this technology was implemented on contaminated soil and water, it would be economical and socially acceptable in Bangladesh and other countries.

The work reported here seeks to identify naturally grown As hyper accumulating plants from As contaminated soil and water and to asses As the accumulating pattern in different plant parts.

2. Materials and Methods

Thirty five naturally grown plant samples were collected from As contaminated rice fields and around As contaminated irrigation water channels from three districts (Chapainowabganj, Rajbari and Faridpur) in Bangladesh, with an aim of identifying As hyperaccumulating plants. From the contaminated area, whole plant samples were collected and bagged in individual marked and tagged polythene bags. Samples were taken to the laboratory after removing dust and other debris. Information on the different plant species is presented in Table 1.

Sample digestion

After harvesting the plants of each pot were dried and weighed. They were stored in a close chamber. Samples of 0.5 g of ground plant were put into 125 ml conical flasks. Five ml of concentrated nitric acid was then added to each flask. The contents of the flasks were mixed and flasks were left overnight covered with aluminum foil. Flasks were then placed on a hot plate at 180-200^{o}C. After 3 hours, 2 ml of perchloric acid was added to each flask. Sample digestion takes 1 hour. After cooling the digest with distilled water it was transferred to a 10 ml volumetric flask, was made up to volume with distilled water. The digests were cooled and filtered through Whatman No. 42 filter paper into dry plastic bottle, mixed and filtered. Individual filtrates were stored in plastic bottles.

Arsenic chemical analysis

First, all samples were screened through an As kit test. After completing the qualitative test probable samples were processed for qualitative chemical analysis. The As content of samples was analyzed calorimetrically. For further confirmation of As status samples were analyzed in an Atomic absorption spectrophotometer (AAS) (Perkin-Elmer Model 3110) following Peterson [6]. Preparation of standard and blank solutions was done using a micro pipette where 10 ml of standard solution was transferred to a beaker with 3 ml 27% and 1 ml 10% KI solution and mixed thoroughly.

3. Results and Discussion

Naturally grown plants were collected from As contaminated rice fields at the different sites. The principle behind plant selection was that a rice field irrigated with As contaminated water where rice growth had either ceased or most rice plants had died but some naturally grown plants showed an optimum growth level. The idea was that As contaminated water was toxic to rice plants but was favorable for growth of the other plants. Thirty five weeds, of different plant species, were collected from the banks of irrigation channels or nearby water bodies contaminated with the same water. The As status of irrigation water of the selected As affected areas ranged between 0.5 to 2.0 mg As l^{-1}. Soil As status was 12 to 78.4 mg kg^{-1} soil (Table 2). Zagury *et al.* (2003) showed that soil contaminant levels were more strongly correlated with soil type rather than soil age. They found that more leaching occurred in soils with a low-organic matter and clay content.

Results from Chapainawabganj District

Sixteen different plant species were collected from different locations in the Chapainawabganj District. Estimated As concentration in root, shoot and grain varied in the range 23.2 -67.8 ppm. Water cress, usually found on the water surface had 46.6 ppm As in its root. Robinson *et al*. [8] observed remarkable As uptake in water cress in New Zealand. Barn yard grass, generally found in upland rice fields, had 61.3 ppm As in roots and 67.8 ppm As in both shoots and grain separately. Cockle bur had 40.7 ppm As in roots and 23.2 ppm As in shoots. The other 13 plant samples did not respond to the As kit test and were not subjected to the colorimetric and atomic absorption methods of As analysis (Table 3). This result corroborated by Onken and Hossner [9] and Longhurst *et al*. [10].

Results from Rajbari District

In this District, among 12 plants sampled, 7 species responded to the As test including roots and shoots (Table 4). Taro accumulated the highest amount of As (42.1 ppm) in roots. This was followed by water taro (40.8 ppm). In shoots, the highest As was in barnyard (67.9 ppm) and Bermuda grass (67.8 ppm). A high As concentration in Bermuda grass was reported by Jonnalagadda and Nenzon [11]. The rice variety BR 29 accumulated 12.1 ppm As in roots and 9.4 ppm As in the shoot. Xie and Huang [12] reported similar findings. Water lettuce which usually grown on a stagnant water surface accumulated 12.0 and 33.7 ppm As in roots and shoots, respectively. These results are in agreement with those of Pitten *et al*. [13].

Results from Faridpur District

Arsenic affected rice fields in the Faridpur District were included in the sampled area and 7 different plant species including fern, common sedge, water hyacinth and alligator weed contained arsenic (Table 5).

Table 1. Information on the different naturally grown plants sampled

Sample Number	Common Name	Botanical name	Plant family
Chapainawabganj District			
1	Yellow nut sedge	*Cyperus difformis*	Cyperaceae
2	Water taro	*Monochoria hastata*	Pontederiaceae
3	Water cress	*Jussieua repens*	Compositae
4	Water clover	*Marsilia quadrifolia*	Marseliaceae
5	Barnyard grass	*Echinochola cruss-galli*	Gramineae
6	White eclipta	*Eclipta prostrata*	Compositae
7	Creeping water primrose	*Ludwigia repens*	Onagraceae
8	Clammy ground cherry	*Physalis hetrophylla*	Solanaceae
9	White verticilla	*Leucus linifolia*	Labiatae
10	*Wahlenbergia*	*Wahlenbergia marginata*	Campanulaceae
11	Goat weed	*Asertum conyzodies*	Compositae
12	Cockle bur	*Xanthium italicum*	Compositae
13	Spotted catsear	*Hypocareris radiata*	Compositae
14	Lambs quarter	*Chenopodium album*	Chenopodiaceae
15	Vetch	*Vicia sativa*	Leguminosae
16	Canada thistle	*Cirsium arvense*	Compositae
Rajbari District			
17	Joint grass	*Pasalum scrobiculatum*	Gramineae
18	Bermuda grass	*Cynodon dactylon*	Gramineae
19	Sessile Amaranth	*Alternathera sessilis*	Amaranthaceae
20	Azolla	*Azolla* sp.	Azollacae
21	Rice	*Oryza sativa*	Gramineae
22	Water lettuce	*Pistia stratiotes*	Araceae
23	Barn yard grass	*Echinochola cruss-galli*	Gramineae
24	Water taro	*Monochoria hastata*	Pontederiaceae
25	Taro	*Colocasia esculanta*	Araceae
26	Green nutsedge	*Cyperus difformis*	Cyperaceae
27	Crab grass	*Eleusine indica*	Gramineae
28	Algae	-	-
Faridpur District			
29	Fern	*Pteris longifolia*	Polipodiaceae
30	Common sedge	*Cyperus rotundus*	Cyperaceae
31	Umbrella sedge	*Cyperus irria*	Cyperaceae
32	Water hyacinth	*Eichhornia crassipes*	Pontederiaceae
33	Water clover	*Marsilia quadrifolia*	Marseliaceae
34	Alligator weed	*Alternanthera philoxeroides*	Amaranthaceae
35	Duck weed	*Lemna polyrrhiza*	Gramineae

Water hyacinth roots were an As sink with 67.8 ppm As. Arsenic accumulated more in roots than shoots as reported by Zhu *et al*. [14]. Alligator weed accumulated 67.3 ppm As. This was followed by fern (34.5 ppm in roots and 32.1 ppm in shoots). Common sedge, a wide spread weed accumulated 32.5 ppm As in its shoot and 11.1 ppm As in the root. The other three plant species did not respond to the As test.

Hyperaccumulating plant parts and uptake pattern of samples collected from arsenic contaminated areas

Barnyard grass from Chapainawabganj accumulated 61.3 ppm As roots and 67.8 ppm As in shoots and grain (Table 6). The same plant species, when collected from Rajbari, accumulated 67.9 ppm As in its root and 8.2 ppm in the shoot. Arsenic accumulation pattern differed in different region.

Table 2. Information on sampling sites and arsenic status of irrigation water

Sample Number	Common name	As mg l^{-1} water	Well depth-m	Installation year	Sampling distance -m	Land type
Chapainawabganj District (Arsenic contaminated area)						
1	Yellow nutsedge	0.1	85	1991	10	LL
2	Water taro	0.05	80	1991	100	LL
3	Water cress	0.1	85	1997	100	LL
4	Water clover	0.1-0.5	80	1997	10	LL
5	Barnyard grass	0.1	110	1998	10	MHL
6	White eclipta	0.1-0.5	80-85	1999	100	MHL
7	Creeping water primrose	0.1-0.5	80-85	1999	100	MHL
8	Clammy ground cherry	0.1-0.5	80-85	1996	10	HL
9	White verticilla	0.1-0.5	80-85	1998	10-100	HL
10	Yellow nutsedge	0.1-0.5	95	2001	10	MHL
11	Goat weed	0.1-1.7	90	1999	10-100	MHL
12	Cockle bur	0.1-1.7	80-85	1995	10-100	LL
13	Spotted catsear	0.1-1.7	80-85	1995	10-100	LL
14	Lambs quarter	0.1-1.7	80-85	1982	10-100	MHL
15	Vetch	0.1-1.7	80-85	1998	10-100	MHL
16	Canada thistle	0.1-0.5	95-110	2001	10	MHL
Rajbari District (Arsenic contaminated area)						
17	Joint grass	1.7	85	1999	10	MHL
18	Bermuda grass	1.7	85	1999	10	MHL
19	Sessile Amaranth	1.7	85	1999	10	MHL
20	Azolla	0.5-1.7	120	1999	10	MHL
21	Rice	0.5-1.7	80-85	1999	10	MHL
22	Water lettuce	0.5-1.7	80-85	1999	10	MHL
23	Barn yard grass	0.5-1.7	80-85	1999	10	MHL
24	Water taro	0.5-1.7	80-85	1999	10	MHL
25	Taro	0.5-1.7	80-85	1999	10	MHL
26	Green nutsedge	0.1-0.5	85	1998	100	MHL
27	Crab grass	0.1	85	1996	100	MHL
28	Algae	-	-	1996	Adjacent	MHL
Faridpur District (Arsenic contaminated area)						
29	Fern	0.3	105	1995	Adjacent	MHL
30	Common sedge	0.3	105	1994	Adjacent	MHL
31	Umbrella sedge	0.2	135	1996	Adjacent	MHL
32	Water hyacinth	0.5	105	2000	100	MHL
33	Water clover	0.5	175	2000	Adjacent	MHL
34	Alligator weed	0.1-0.5	105	2000	Adjacent	MHL
35	Duck weed	0.3	105	1995	Adjacent	MHL

LL=Low land, MHL= Medium high land, HL= High land

Table 3. Concentration of As in different plant parts of naturally grown plants from the Chapinawabganj district

Sample Number	Plantname	As (ppm) Root	Shoot	Grain
1	Yellow nutsedge	ND	ND	ND
2	Water taro	ND	ND	-
3	Water cress	46.6	ND	-
4	Water clover	-	ND	-
5	Barnyard grass	61.3	67.8	67.8
6	White eclipta	ND	ND	-
7	Creeping water primrose	ND	ND	-
8	Clammy ground cherry	ND	ND	-
9	White verticilla	ND	ND	-
10	Yellow nut sedge	ND	ND	-
11	Goat weed	ND	ND	-
12	Cockle bur	40.7	23.2	-
13	Spotted catsear	ND	ND	-
14	Lambs quarter	ND	ND	-
15	Vetch	ND	ND	-
16	Canada thistle	ND	ND	-
Range		46.7-61.3	23.2-67.8	

ND= not detected

Table 4. Concentration of As in different plant parts of naturally grown plants from the Rajbari district

Sample Number.	Plantname	As (ppm) Root	Shoot
17	Joint grass	ND	ND
18	Bermuda grass	ND	67.8
19	Sessile Amaranth	ND	-
20	*Azolla*	-	27.7
21	Rice	12.1	9.4
22	Water lettuce	12.0	33.7
23	Barnyard grass	8.2	67.9
24	Water taro	40.8	-
25	Taro	42.1	ND
26	Green nut sedge	ND	ND
27	Crab grass	ND	ND
28	Algae	ND	ND
Range		8.2-42.1	9.4-67.9

ND= not detected

Except Burbyard grass, water lettuce and common sedge all other plants showed the accumulation pappern of root > shoot. In case of these three plants it was in the order of shoot > root. In both the region (Table 6). Average As concentration was 65.6 ppm in barnyard grass. Shaheen *et al*. [15] observed that in 45 days barnyard grass removed 36.0 μg As from treated soil. Whole water cress plants accumulated As in the range 46.6 to 59.4 ppm. Cockle bur root accumulated 40.7 ppm As and shoots, of same plant, 23.2 ppm As. This plant was collected in the Chapainawabganj District.

Table 5. Concentration of As in different plant parts of naturally grown plants from the Faridpur district

Sample number	Plantname	As (ppm) Root	Shoot
29	Fern	34.5	32.1
30	Common sedge	11.1	32.5
31	Umbrella sedge	ND	ND
32	Water hyacinth	67.8	ND
33	Water clover	ND	ND
34	Alligator weed	67.3	ND
35	Duck weed	ND	ND
Range		11.1-67.8	32.1-32.5

ND= not detected

The uptake pattern of this plant was root > shoot. These findings are supported by Gulz and Gupta [16]. *Azolla* accumulated 27.7 ppm As when collected in the Rajbari District. Accumulated As in rice roots and shoots was in the order of root > shoot (Table 6). Doyle and Otte [17] and Zaman *et al*. [1] reported a similar trend. Shaheen *et al*. [15] also observed As accumulation was higher in shoots than roots. Accumulated As in shoots and roots of water taro was 40.8 ppm. A wild taro variety accumulated 42.1 ppm As roots in the Rajbari District. In Faridpur there were higher amounts of As in fern root than shoots. Water hyacinth roost accumulated 67.8 ppm As in Faridpur. Whole alligator weed plants accumulated 67.3 ppm As in a plant from Gopalganj (Table 6). However, before any conclusions can be drawn in this area an investigation plant biomass production and As uptake in terrestrial as well as in aquatic environments.

Table 6. Identified naturally grown arsenic hyper accumulating plants with arsenic concentration in different plant parts

Sample Number	Plantname	Quantity of As (ppm)			Accumulation pattern	Sample site
		Root	Shoot	Grain		
3	Water cress	-	46.5	-	Whole plant	Chapinowabganj
5	Barnyard grass-1	61.3	67.8	67.8	Shoot > Grain > Root	Chapinowabganj
12	Cockle bur	40.7	23.2	-	Root > Shoot	Chapinowabganj
18	Bermuda grass	-	67.8	-	Whole plant	Rajbari
20	Azolla	-	27.7	-	Whole plant	Rajbari
21	Rice	12.1	9.4	-	Root > Shoot	Rajbari
22	Water lettuce	12.0	33.7	-	Shoot > Root	Rajbari
23	Barnyard grass-2	8.2	67.9	-	Shoot > Root	Rajbari
24	Water taro	40.8	-	-	Root > Shoot	Rajbari
25	Taro	42.1	-	-	Root	Rajbari
30	Fern	34.5	32.1	-	Root > Shoot	Faridpur
31	Common sedge	11.1	32.5	-	Shoot > Root	Faridpur
33	Water hyacinth	67.8	-	-	-	Faridpur
35	Alligator weed-1	-	67.3	-	-	Faridpur

Conclusions

Out of 35 plant samples collected from rice field of 3 districts of Bangladesh only 14 plant species were found to be As hyper accumulating. Between two samples of barnyard grass, one had seed. The other 13 plants were only root and shoot. Plants identified as hyper accumulating plants were barnyard grass, water cress, cockle bur, *Azolla*, rice, water lettuce, water taro, taro, fern, water hyacinth and alligator weed. Maximum As accumulation was in barnyard grass at 61.3 ppm, 67.9 ppm and 67.8 ppm in root, shoot and grain, respectively. uptake pattern of root shoot grain . Rice grain did not accumulate As. The uptake pattern in the sequence of shoot > root in barnyard grass and water lettuce. The results of chemical analysis of 35 naturally grown plant species reflected the fact that barnyard grass is can be used to mitigate As contaminated rice fields. Thus this plant can be recommended for As mitigation. On the other hand water hyacinth, water cress, water lettuce and *Azolla* can absorb As from As contaminated stagnant water. These plant species can be recommended for mitigation of As in water.

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