ASSESSMENTS OF PLANTS FOR PHYTOREMEDIATION OF ARSENIC-CONTAMINATED WATER AND SOIL

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Phytoremediation is an innovative technology that uses plants in order to remediate polluted water and soil. A 10 week study in flower pots was performed in order to determine the arsenic (As) removal potential of *Shoenoplectus americanus*, *Eleocharis macrostachya* and *Baccharis salicifolia* and also to evaluate their tolerance capacity to increasing doses of As. The experiment involved five different treatments with distinct As concentrations (1, 2, 3, 4 and 5 mg L^{-1}) and a control (tap water) to determine the acclimatization capacity of the species to the different concentrations. The number of plants and their height were determined during the experiment. The values for the factors of translocation, accumulation and enrichment were obtained at the end of the experiment; the maximum values for these factors were, respectively, 1.86, 92.13 and 1.63 for *E. macrostachya*, 1.73, 59.74 and 0.56 for *S. americanus* and 8.96, 27.94 and 6.72 for *B. salicifolia*. The maximum growth value belonged to the *S americanus*. The maximum concentration of As in water tolerated by *E. macrostachya* and *B. salicifolia* were 2 and 3 mg L^{-1} respectively. *B. Salicifolia*, has no tolerance for environments with high concentrations of arsenic. *S. americanus* showed the highest As accumulation capacity and the greatest tolerance in the tested concentrations. *E. macrostachya* proved to be translocator plants and *S. americanus* was confirmed to be a stabilizer plant with a high potential for phytostabilization and rhizofiltration techniques.

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INTRODUCTION

As removal of water sources for human consumption is one of the great challenges in the world. Millions of people have been or are being exposed to excessive levels of As through drinking water[1,-3]. The health effects attributed to the consumption of water with high concentrations of As include: skin diseases, cardiovascular, neurological, hematological, renal and respiratory and liver cancer, kidney and prostate [4]. Therefore minimizing the metalloid concentrations in soils and water contaminated through various physical, chemical and biological, processes is one of the current challenges. Phytoremediation has been proposed as an alternative technology eco-friendly and cost-effective to remediate soils contaminated with As.[5,6] This technique has been applied to the removal of different contaminants (including metals and metalloids) of soil, surface water, ground water and municipal and industrial wastewaters.[7] In Mexico there are several areas affected by high concentrations of As in groundwater. In recent decades, studies conducted at different sources of drinking water in the state of Chihuahua, have shown the presence of arsenic in concentrations exceeding the maximum permissible limits of NOM-127-SSA1-1994,[8] for human consumption, with an average concentration of 0.152 mg L^{-1} and a maximum concentration of 0,517 mg L^{-1} . In addition, the population growth and overexploitation of the different aquifers have caused an increase in the concentrations of this metalloid, which is found naturally in subsurface geological formations. E. *macrostachya*, *S. americanus and B. salicifolia* are three species of native plants from the state of Chihuahua that have been reported as As tolerant with potential application in phytoremediation techniques [9,10]). Thus, the aim of this study was to determine the translocation, accumulation, enrichment and tolerance factors of these plants species, for potential use in the phytoremediation of arsenic in water and soil.

EXPERIMENTAL

The experiment was performed in pots, for a period of 10 weeks, with 3 suspected As tolerant species: *E. macrostachya*, *S. americanus* (Cyperaceaes family) and *B. salicifolia* (Asteraceae family). The plants were subjected to increasing doses of As prepared with sodium arsenite (1, 2, 3, 4 and 5 mg L^{-1}) using tap water as a control. The experiment was performed by duplicate in two different media: one with soil (loamy sand) composed of 91% sand and 9% of fine particles. The other was a hydroponic medium prepared with tap water and Arsenic.

Collection and acclimation of plants

E. macrostachya, S. americanus, and *Bacharis salicifolia* were collected in the state of Chihuahua, Mexico (28 ° 35 '53.55 "N, 105 ° 33' 41.93"). Plant species were removed completely (leaf, stem and root) from their native place and transplanted into plastic pots. The soil was composed of silty sand. Plants were kept under flooded conditions with tap water, adding nutrients once a week for an acclimatization period of three months prior to the start of the experiment. Before placing the plants in pots, their size were measured, the wet weights determined, and the number of plants per pot was counted. These data were considered as the initial values of the experiment. The plants in hydroponic medium were placed in plastic bottles (2 L).

Plant monitoring

Throughout the period of experiment, the number of plants per pot were counted weekly, thereby determining the reproduction of plants subjected to various doses of As. To determine the growth, three individuals per pot were randomly selected, and their growth was measured weekly. At the end of the experiment, all plants were weighed to determine the biomass produced by each species.

Chemical analysis of plants

At the end of the experiment all the plants were extracted from their pots, washed with water, separated in different sections (roots, stems and leaf) and placed in plastic bags previously identified. The samples were dried at 60 ° C for 24h in an oven, and subsequently digested in a CEM microwave equipment Model MarsX. A certificated standard of tomato leafs was used as an analytical control.

The determination of As was carried out by atomic absorption spectrophotometry with hydride generator, GBC brand, model Avanta Σ, and an inductively coupled plasma (ICP-OES) Thermo Jarrell Ash brand, model AP-Duo Iris. To ensure the quality of analyzes, duplicate samples and controls were considered, as well as the measuring of certified standards.

Determination of translocation, accumulation and enrichment coefficient

The translocation factor (TF) was calculated according to the criteria established by Fitz and Wenzel[11], by dividing the concentration of As in shoot biomass (mg kg^{-1}) by the concentration of As in the root biomass (mg kg^{-1}). The accumulation factor (AF) was calculated by the ratio of As in the stem of the plant and the concentration of As in the stalk. Finally the enrichment coefficient (EC) was calculated by the ratio of As concentration in the treated plants, and the concentration of arsenic in soil[12]).

RESULTS AND DISCUSSION

The highest concentration of As was found in the roots of the analyzed plants. *E. macrostachya and S. americanus* had the highest accumulation of arsenic in the hydroponic medium (Table 1), while *B. salicifolia* in soil (Table 2).

E.macrostachya presents a proportional accumulation of As with the concentration increase of arsenic in the feed water. At lower As concentrations (>2 mg L^{-1}), the plant behaves as a tolerant, absorbing and holding As in the root. Whereas at higher As concentrations (≤4 mg L^{-1}) the plant allows to accumulate greater amounts of As in the shoot also.

S. americanus tolerated arsenic concentrations up to 4 mg L^{-1} in hydroponic medium for a period of 10 weeks. At concentrations of 5 mg L^{-1} , the plant decreased its capacity of arsenic accumulation; this may be due to a blocking defense or to prevent damage from the element in their tissues[13]). The plant accumulated 30 times more arsenic from soil, than from the hydroponic media, behaving as a tolerant during all tests.

B. salicifolia behaves poorly under high arsenic concentrations in the media. Showing a possible saturation or activation in defense mechanisms to inhibit the toxic effects of arsenic. The plant behaves as traslocator in hydroponic media.

The biomass of *E. macrostachya as S. americanus*, had increased proportionally with the increase of As concentration in water. In contrast *B. salicifolia* produced more biomass at low As concentrations in water. At the end of the experiment it was observed that *E. macrostachya and S. americanus*, roots developed rapidly at high As concentrations, while the size of stem decreased.

S. americanus showed positive reproduction at all As concentrations in both media (soil, hydroponic). The maximum increase was of 73 new plants. *E. macrostachya* plants showed an increase in the number of plants under concentrations of As in the range of 1-3 mg L^{-1} . Under these concentrations, reproduction was the largest (76 new plants). *B. salicifolia* reproduction didn't occur in any of the As concentrations.

With regard to size, all plants showed positive values in both mediums, but the plants developed better in flooded soil. *E. macrostachya* showed the higher size with a maximum value of 40 cm in hydroponic media and 104 cm. in soil.

Plant growth is a major parameter used to assess survival and adaptation of a given species to the environment. Plants responded to the excess of As supply in water and soil mediums by developing visible symptoms of toxicity, besides decreasing stem biomass[14].

B. salicifolia accumulated Arsenic in the root (11 to 329 mg kg^{-1}) and in the stem from 29 to 141 mg kg^{-1}, in the loamy sand media (soil), irrigated with arsenic concentrations between 0.02 and 4 mg L^{-1} . Although the plant could accumulate arsenic, its reproductive capacity was inhibited, and the plant damage was evident for all arsenic concentrations tested in this study. Therefore, the plant is not suitable for phytoremediation purposes. The translocation factor was 4.79, which is about six times higher than the value reported for plants belonging to the same family as *B. sarothroides* (TF = 0.80) (Haque et al., 2008[15]). High translocation of arsenic by *B. salicifolia* may be the cause of its damage.

E. macrostachya showed a severe clorosis damage in the stem by the forth week of experimentation, for arsenic concentrations greater than 2 mg L^{-1} in the hydroponic media.

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Table 1. Biosorption of arsenic by plants (mean ± standard deviation), translocation, and biomass accumulation using a hydroponic medium.

	Arsenic, mg kg^{-1}					
(mgAs L^{-1})	Root	Stalk	leaves	TF	AF	Biomass (g)
Test Solutions			*E. macrostachya*			
Control	42.4 ± 2.2	1.8 ± 0.4	-	0.0	-	20.0
1	93.9 ± 4.1	35.6 ± 1.2	-	0.4	18.9	51.2
2	54.9 ± 3.4	73.4 ± 3.7	-	1.3	38.9	29.0
3	91.9 ± 6.0	156.1 ± 6.5	-	1.7	82.6	34.8
4	124.7 ± 6.3	162.7 ± 10.9	-	1.3	86.1	33.8
5	183.6 ± 8.3	174.1 ± 10.2	-	1.0	92.1	71.8
Test Solutions			*S. americanus*			
Control	39.2 ± 2.2	2.9 ± 0.1	-	0.1	-	60.6
1	62.8 ± 3.8	31.4 ± 1.2	-	0.5	10.5	125.5
2	239.4 ± 9.8	34.1 ± 2.2	-	0.1	11.4	108.2
3	357.7 ± 22.7	52.7 ± 3.9	-	0.1	17.6	101.3
4	616.5 ± 26.6	178.6 ± 11.4	-	0.3	59.7	149.6
5	419.5 ± 24.9	130.6 ± 6.8	-	0.3	43.7	166.6
			Hydroponic media			
Control	1.0 ± 0.1	2.6 ± 0.1	3.2 ± 0.2	5.5	-	12.4
1	1.4 ± 0.1	4.5 ± 0.2	8.0 ± 0.5	9.0	2.1	8.3
2	26.3 ± 1.4	32.5 ± 2.0	20.7 ± 1.2	2.0	9.1	2.7
3	51.8 ± 3.3	74.0 ± 5.7	60.5 ± 4.7	2.6	22.9	7.9
4	56.9 ± 3.2	65.0 ± 3.9	5.7 ± 0.4	1.2	12.0	9.3
5	94.1 ± 8.2	57.6 ± 3.4	109.2 ± 6.9	1.8	28.4	6.5

Table 2. Biosorption of As by plants (mean ± SD), translocation factors, accumulation and enrichment coefficient, using soil medium (silty sand).

	Arsenic, mg kg^{-1}						
(mgAs L^{-1})	Root	Stalk	leaves	TF	AF	EC	Biomass (g)
Test Solutions			*E. macrostachya*				
Control	4.3 ± 0.3	2.5 ± 0.1	-	0.6	-	-	66.0
1	12.1 ± 0.7	4.8 ± 0.2	-	0.4	1.9	0.5	58.8
2	11.4 ± 0.7	18.7 ± 1.0	-	1.6	7.6	1.1	33.0
3	16.4 ± 0.7	30.5 ± 2.0	-	1.9	12.3	1.1	95.7
4	40.4 ±1.7	32.1 ± 2.3	-	0.8	13.0	0.9	66.0
5	72.9 ± 3.9	71.9 ± 6.1	-	1.0	29.1	1.6	84.2
Test Solutions			*S. americanus*				
Control	6.7 ± 0.3	2.2 ± 0.1	-	0.3	-	-	86.3
1	3.7 ± 0.1	5.0 ± 0.3	-	1.3	2.3	0.6	158.6
2	29.1 ± 1.3	2.0 ± 0.1	-	0.1	0.9	0.1	110.2
3	48.1 ± 2.0	4.8 ± 0.3	-	0.1	2.2	0.2	155.8
4	24.9 ± 1.9	11.7 ± 0.7	-	0.5	5.4	0.3	149.1
5	14.1 ± 1.0	24.4 ± 1.4	-	1.7	11.2	0.5	191.3
Test Solutions			*B. salicifolia*				
Control	11.3 ± 0.7	28.8 ± 1.3	2.5 ± 0.1	0.2	-	-	17.6
1	18.5 ± 1.0	57.9 ± 3.02	3.4 ± 0.2	3.3	2.0	6.6	19.6
2	25.7 ± 1.3	118.3 ± 9.1	4.6 ± 0.2	4.8	3.9	6.7	27.0
3	329.6 ± 19.9	64.6 ± 3.1	3.3 ± 0.2	0.2	2.2	2.4	18.0
4	321.5 ± 12.0	140.8 ± 10.2	33.2 ± 1.9	0.5	5.6	4.0	15.2
5	40.5 ± 3.0	79.2 ± 4.0	3.7 ± 0.1	2.0	2.6	1.8	33.1

These physical changes were consistent with the high translocation factors (TF) obtained by the chemical analysis of the plants at the end of the experimentation (1.3, 1.7, 1.3 and 1) for As concentrations of 2, 3, 4 and 5 mg L^{-1} respectively. Indicating that, when the plant is under high As concentrations, the metaloid is translocated to the shoot, causing damage to the cell membrane. This can be attributed to the possible interaction of arsenic with sulfhydryl groups of proteins in the plant [16]). For As concentrations lower than 3. The root of the plant suffered no apparent damage, but its size increased proportionally to the increase in As concentration in water (Figure 1)

Figure 1. Root and stem size in E. macrostachya, after undergoing As concentrations of 0,016 mg L^{-1} and 5 mg L^{-1} for a period of 10 weeks.

Accumulation factors (AF) calculated for *E. macrostachya* in soil (Table 2) shown that at higher concentrations of arsenic in the irrigated water, the plant also accumulated more arsenic in root and stem. The higher values correspond to the higher As concentration of 5 mg L^{-1} . These values are approximately 7 times higher than those reported in previous studies of the plant when it was subjected to arsenic concentrations in irrigation water, between 3 and 9 mg L^{-1} , for a period of four months (Nuñez et al., 2007). However, translocation factors obtained in both the studies are a coincident, confirming that the plant is tolerant to arsenic concentrations lower than 2 mg L^{-1} whereas above this value, the damage is visible, ceasing to be tolerant when the As concentration increased to 3 mg L^{-1} in the irrigated water.

S. americanus in soil, irrigated with 3 mg L^{-1} of arsenic, was able to accumulate 48 mg/kg of arsenic in their roots and 4.8 mg/kg in their stems (Table 2). In previous studies (Flores et al., 2003) reported that for soils irrigated with concentrations of 9 mg L^{-1} of arsenic in water, the arsenic accumulation in the plant roots was approximately 6 times less than the results obtained in the present study (7.6 mg kg^{-1}). However, the amounts of arsenic in the stalk are a coincident (4.7 mg kg^{-1}). This behaviour is attributable to the fact that the plant decreases arsenic absorption at concentrations greater than 3 mg L^{-1} and it behaves as exception to arsenic, possibly to restrict the adverse effects on the vegetal structure (Castillo-Michel et al., 2009[17]).

Studies with plants associated with desert environments shows that other plants like *Prosopis sp. and Chilopsis linearis* accumulate As up to 445 mg kg^{-1} in their roots[18,15]) *S. americanus* accumulated As up to 616 mg kg^{-1} in hydroponic medium (Table 1). Due to this characteristic feature researchers are suggesting its potential use in phytoremediation of contaminated water and soil. *E. macrostachya* can also be considered in phytoestabilization techniques for sites with concentrations up to 2 mg L^{-1} . Considering that the characteristic of extracting metals from water is known as ‘rhizofiltration’, *E. macrostachya* and *S. americanus* could, therefore, also be considered as rhizofilterers of As, due to its capability of grow in flooded environments[19].

This research shows that, *B. Salicifolia*, has least tolerance for environments with high concentrations of arsenic. *E. macrostachya* and *S. americanus* are tolerant to high concentrations of arsenic in soil and water. The higher concentrations of As, the greater the As accumulated in the roots of plants. The maximum tolerated concentration of arsenic in hydroponic medium for *E. macrostachya* was 2 mg L^{-1}, and for soil (silty sand) it was 4 mg L^{-1}.

S. americanus showed the highest As accumulation capacity and greater tolerance for As concentrations up to 3 mg L^{-1} , showing no evidence of physical damage to the cell or tissue limitation in their reproduction and growth. Both species *E. macrostachya and S. americanus* behave as tolerating and excluding with a high potential for use in phytoremediation strategies.

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