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ORIGINAL ARTICLE

Phytoremediation by some Species of Aquatic Plants for as and Hg Removal (Case Study: Dezful, Iran)

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Alishir Afrous, Shahram Goudarzi, Abdolmajid Liaghat: Phytoremediation by some Species of Aquatic Plants for as and Hg Removal (Case Study: Dezful, Iran).

ABSTRACT

Tissues of aquatic plants can be accumulated for removing heavy metals and nutrients from industrial and municipal wastewaters. The capability of four aquatic plants including *Phragmites australis*, *Typha latifolia*, *Alisma plantgo* and *Scirpus (Bulrush)* evaluated to accumulate arsenic and mercury from simulated industrial wastewater in this paper. These aquatic plants were cultivated in three treatments at the concentrations of 50, 100 and 200 mg/kg As and Hg in soil under the semi-arid conditions of Dezful, Southwest Iran. Results from this study, 60 days after growth indicated that each four species were capable to uptake As and Hg from the polluted soil. Results showed significant statistical differences in accumulation of As in the below-ground tissues of four plants where the highest As accumulation (measuring 119.55 mg/kg) was observed for *Phragmites australis* in the treatment of 200 mg/kg As in soil, followed by 65.25, 47.86 and 11.76 mg/kg for *Bulrush (Scirpus)*, *Typha latifolia* and *Alisma plantgo* respectively. Maximum accumulation for Hg in below-ground tissues was observed in *Phragmites australis* measuring 6.23 mg/kg in 200 mg/kg Hg in soil treatment, followed by *Bulrush (Scirpus)*, *Typha latifolia* and *Alisma plantgo* measuring 2.23, 1.45 and 0.86 mg/kg respectively. Results indicated that the translocation factor (TF), were largely as shown by general TF values <1. The overall conclusion being that the three aquatic plants selected in this study can be used as effective catalysts for removing heavy metals from the industrial wastewater under arid and semi-arid conditions.

Key words: accumulation, arsenic, mercury and *Phragmites australis*.

Introduction

Heavy metal contamination in aquatic and soil environments is a serious environmental problem, which threatens aquatic ecosystems, agriculture, water resources and human health [19]. One approach is to treat the contaminated wastewater in order to remove their heavy metals contents and reuse the out product for agricultural production. The aquatic plants can reportedly be used as the natural catalysts to absorb and accumulate heavy metals in their tissues from wastewater [25]. Various researches have been conducted on the harmful effects of the heavy metals of the wastewater such as arsenic, nickel and mercury and the way in which these aquatic plants are able to absorb and accumulate these hazardous metals from the wastewater and therefore to mitigate their harmful consequences [16,21,24,23,17]. The metals are generally absorbed by the root and shoot systems of the plants. However, research reports [3,9] indicated various cases of heavy metals absorptions and accumulated in the tissues of the aquatic plants. The highest concentrations were reported in the root systems followed by those in the stems and leaves. Some heavy metals such as arsenic and mercury however, may exit from the wastewater bodies into environment in the gaseous form. The ability of the organs of certain aquatic plants in absorbing heavy metal elements from the soil have been investigated by [20] who also found the potential of these plants in biologically converting the absorbed metal into gaseous form that are then easily released in the environment. Metals accumulation in plants depends on various factors like its concentration in the soil, the plant species and special chemical forms of metals in soil solutions [12]. The accumulation rate of arsenic and mercury has been tested on the above and below the ground tissues of many aquatic plants species [11,28.29,10,7].

Results of field studies on plants such as *Phragmites australis* showed highest metal concentration on below the ground tissues with smaller translocation on above the ground ones 30]. The bioremoval process using aquatic plants consists of two uptake processes of biosorption and

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bioaccumulation. [31] used hybrid generation in conjunction with atomic fluorescence spectrometry (HG–AFS) to determine the total arsenic concentrations of the *bamboo* shoots. They found that, *Bamboo* (*Phyllostachys pubescens* Mazel) is one of the aquatic plants with the ability to absorb arsenic elements from the industrial wastewater. [4] investigated the ability of the *Typha angustifolia* plant in removing heavy metals such as chromium, copper and zinc from tannery sludge. Their observation showed significant absorptions of these chemical elements by the aquatic plant they investigated.

Phragmites austrulis and *Typha spp.* are among the most common aquatic plants used for wastewater treatment in constructed wetlands [25]. Various aquatic plant species thrive in arid and semi-arid regions of Khouzestan in Iran, which can be studied for their ability for their heavy metal uptake from the industrial wastewater and make it possible for the treated wastewater to be used for agricultural production [1]. Review of the literature on phytoremediation shows a limited knowledge on the behavioral characteristics of the aquatic plants under simulated metal solution conditions.

The aim of this paper was to investigate the absorption and accumulation of arsenic and mercury elements from industrial wastewater onto three aquatic plant species consisting of *Phragmites australis*, *Typha latifolia, Scirpus (Bulrush)* and *Alisma plantgo* under simulated arid and semi-arid conditions of Dezful region in southwest Iran.

Materials and Methods

Study location and plant selection:

Three aquatic plants of *Phragmites australis Bulrush (Scirpus)*, *Typha latifolia* and *Alisma plantgo* were selected because of their availability and accessibility in the study area. The samples were collected from The Dez River, Safiabad and Senjar main drains and other stream margins in Dezful, Iran during spring 2008. These were then replanted in nurtured environment of the plastic pot bed with 100 cm diameter and 60 cm height in the following June. Approximately 200 kg of river sediment materials (sands) of between 4 to 12 mm diameters were subsequently added to each pot providing a sand depth of about 0.25 m. six samples of young plant species were cultivated in the pots at 20 cm intervals. 10 days after experimental re-plantation, one herb from each pot was disposed of every ten days. All experiments were conducted at the open site adjacent to the Faculty of Agriculture, Islamic Azad University, Dezful (48° 25′ E, 32° 16′ N) under natural conditions. The minimum and maximum temperatures during the growing season ranged between 24 and 50 degrees centigrade.

Simulated Wastewater:

After filling the pots with the specified amount of sands, chemical fertilizers (N, P and K) and irrigation water were then applied prior to replanting the samples. Three level of As and Hg consisting of 50, 100 and 200 mg/kg in soils were added at three replications for each plant ten days after re-plantation. Two substances of As(NO3)3 and Hg(NO3)2 as two main sources of pollutions in the wastewater were used in this experiment to supply the As and Hg necessary for conducting the research.

Sample Analysis:

Plant samples were preliminarily dissected into the below-ground (roots and rhizomes) and above-ground (stems and leaves) in order to evaluate their different bioaccumulation capabilities. Samples of upper leaves and whole stem were considered in the experiment. The process involved washing the roots, rhizomes and stems with water prior to analyzing the data, which did not include the leaf samples. The samples were then dried at 70°C to a constant weight for approximately 48 h and grounded into ball mill. The [2] standard procedure was applied to dry the samples at 30°C in order to avoid volatilization for Hg. Inductively coupled plasma mass spectroscopy (ICPMS) was used to determine As and atomic absorption spectrophotometer to determine Hg concentration. [2] procedures were used as an analytical framework for the analyses of plant chemical accumulation.

Translocation Factor:

The translocation factor for metals within a plant was determined as the ratio of [Metal] Shoot/[Metal]Root to evaluate the extent of metal translocation from roots to shoots [8].

Statistical Analyses and Uptake Curves:

The statistical analyses were based on a totally random design using the SPSS (Version 13).

(Excel software, 2003) was used in this study to draw the accumulation of Hg and As in plant tissues.

Results and Discussion

Plants Uptake:

The metal concentration in the above and below the ground tissues of all plant samples showed increasing trend relative to time. Analysis of the As and Hg accumulations in the aquatic macrophytes in three treatments (50, 100 and 200 mg/kg of As and Hg in soil) revealed a higher accumulation rate in the

below-ground (BG) samples than the above-ground (AG) ones (figure 1, 2, 3, 4 and table 1). Results of statistical analysis showed that effect of aquatic plants on two heavy metals accumulation in plant tissues were significant at P≤0.01.

Table 1: Concentration (mg/kg dry weight ±S.D.) of heavy metals in above-ground (AG) and below-ground (BG) tissue of plants sampled in high treatment.

element	Arsenic		Mercury	
macrophytes	AG	BG	AG	BG
Phragmites australis	6.36±0.98b	119.55±7.38a	0.073±0.012b	6.23±1.34a
Typha latifolia	7.25±1.27a	47.86±4.18b	0.1±0.026a	2.23±0.64b
Bulrush (Scirpus)	1.67±0.32c	65.25±5.49b	0.046±0.0037bc	1.45±0.49b
Alisma plantgo	6.18±1.1b	11.76±1.93c	0.03±0.0026c	0.86±0.37c

Mercury:

The initial amount of mercury at first treatment in each pot was 50mg/kg in soil, which Hg accumulation in all plants increased by the end of the experiment. In this treatment, the highest accumulation of Hg was recorded in *Phragmites australis* with 3.59 mg/kg in dry matter in below-ground tissues. Afterwards *Typha latifolia, Alisma plantgo* and *Scirpus* (*Bulrush)* were with 1.26, 0.7 and 0.62 mg/kg in dry matter of below-ground tissues at sixth decade respectively. Hg accumulation in all plant above-ground tissues was lower than below-ground tissues, which Hg accumulation in *Phragmites australis*, *Typha latifolia, Alisma plantgo* and *Scirpus* (*Bulrush)* observed 0.033, 0.075, 0.041 and 0.022 mg/kg in dry matter of above-ground tissues respectively

that in *Typha latifolia* was highest at end of experiment. When plants accumulate metals, the roots and rhizomes generally show higher concentrations than the shoots [22]. In treatment of 100 and 200 mg/kg Hg in soil, results indicated this advantage with higher accumulation. In medium treatment (100 mg/kg Hg in soil), the highest accumulation of Hg was recorded in *Phragmites australis* with 4.76 mg/kg in dry matter in below-ground tissues, afterwards *Typha latifolia, Scirpus* (*Bulrush)* and *Alisma plantgo* were with 1.43, 1.12 and 0.81 mg/kg in dry matter respectively. For 200 mg/kg Hg in soil treatment, these amounts were 6.23, 2.23, 1.45 and 0.86 mg/kg in dry matter of below-ground tissues with *Phragmites australis*, *Typha latifolia, Scirpus* (*Bulrush)* and *Alisma plantgo* respectively (figure 1 and 2).

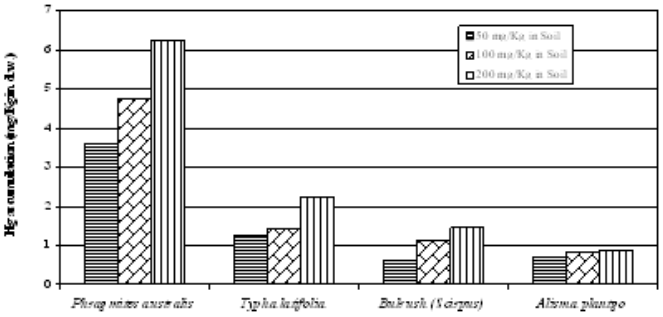

Fig. 1: Average Hg concentrations (mg/Kg in d.w.) in roots of aquatic plants at three level of polluted soil.

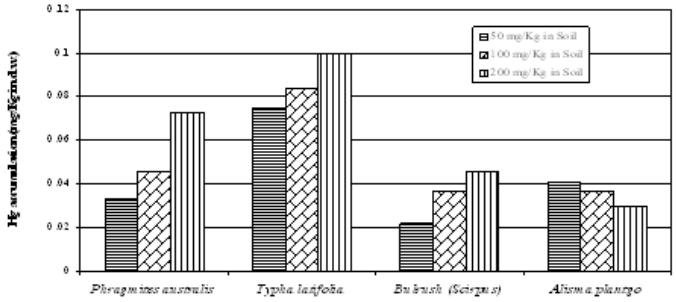

Fig. 2: Average Hg concentrations (mg/Kg in d.w.) in shoots of aquatic plants at three level of polluted soi.

Arsenic:

In treatment of 50 mg/kg As in soil, all plants absorption increased by the time of experiment. In this treatment, maximum absorption was for *Phragmites australis* with 30.18 mg/kg in dry matter in its below-ground tissues. Afterwards, *Bulrush (Scirpus), Alisma plantgo* and *Typha latifolia* were 17.56, 15.37 and 14.26 mg/kg in dry matter of below-ground tissues respectively. In other treatment (100 and 200 mg/kg As in soil), the accumulation of As by all plants increased with time and concentration in soil. As accumulation in all plant above-ground tissues increased with time, but were lower in these tissues of plants rather below-ground tissues. In above-ground tissues, the highest As accumulation was 9.46 mg/kg in dry matter for *Alisma plantgo* at 50 mg/kg As in soil, afterwards *Typha latifolia, Phragmites australis* and *Bulrush (Scirpus)* were with 3.26, 1.78 and 0.89 mg/kg respectively. With increasing in As concentration in soil, its accumulation enhanced by plants. In treatment of 100 and 200 mg/kg arsenic in soil, As accumulation in below-ground tissues of *Phragmites australis*, *Bulrush (Scirpus), Typha latifolia* and *Alisma plantgo* were 69.66, 41.23, 26.15, 12.7 and 119.55, 65.25, 47.86, 11.76 respectively. In these treatments As accumulation in above-ground tissues was lower than below-ground tissues too (figure 3 and 4).

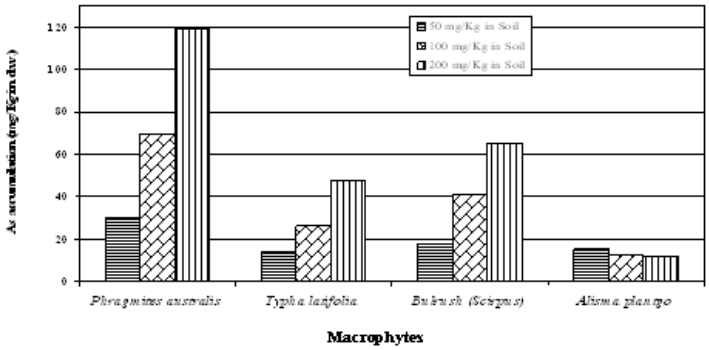

Fig. 3: Average As concentrations (mg/Kg in d.w.) in roots of aquatic plants at three level of polluted soil .

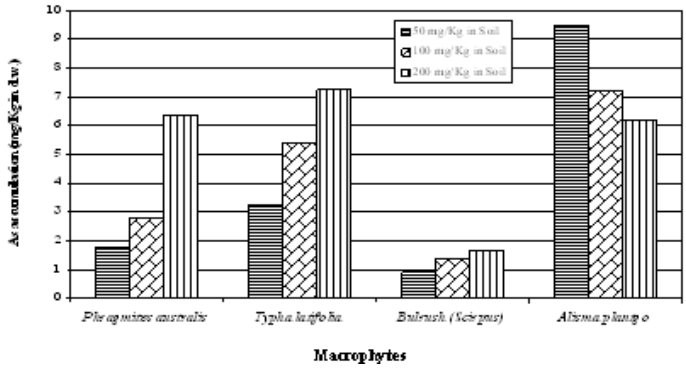

Fig. 4: Average As concentrations (mg/Kg in d.w.) in shoots of aquatic plants at three level of polluted soil.

Translocation Factor:

The translocation factor (TF), the ratio of shoot to root metals indicates internal metal transportation. Present study indicate that Hg and As accumulated by the plants growing on pots including soil polluted, were largely retained in roots as shown by general TF values <1 (Tables 2 and 3). However, in all the plants TF for As was greater than Hg and varied with in the range of 0.04 to 0.61. These amounts were 0.009 to 0.06 for the Hg.

Results showed significant statistical differences between the arsenic accumulation ratios on the above-ground to below-ground tissues of *Phragmites australis* and two other varieties. While this accumulation ratio for the Hg in *Alisma plantgo* is higher than the other varieties, which had significant difference in their accumulation ratios.

Discussion:

Phragmites australis, *Typha latifolia, Bulrush (Scirpus)* and *Alisma plantgo* were able to remove Hg and As from the studied simulated industrial wastewater, although differences in the heavy metals accumulation in their tissues were observed.

Table 2: Translocation factor (TF [Metal]Shoot/[Metal]Root) in plant species grown on pots about mercury.

Plant Species	TF		
	Low level	Medium level	High level
Phragmites australis	0.0092	0.0097	0.0117
Typha latifolia	0.0595	0.0587	0.0448
Bulrush (Scirpus)	0.0355	0.0330	0.0317
Alisma plantgo	0.0586	0.0457	0.0349

Table 3: Translocation factor (TF [Metal]Shoot/[Metal]Root) in plant species grown on pots about arsenic.

Plant Species	TF		
	Low level	Medium level	High level
Phragmites australis	0.0590	0.0401	0.0532
Typha latifolia	0.2286	0.2073	0.1515
Bulrush (Scirpus)	0.0507	0.0332	0.0256
Alisma plantgo	0.6155	0.5693	0.5255

Accumulation of Hg and As in all plants in this study was increased with initial concentration and time of start experiment. All species of plants tested, despite differences in physiology, removed much of the effective concentration of arsenic and mercury in the simulated wastewater within the 60 day test period. Most of heavy metal accumulation occurred at first 10 days in this research, as trend rate of plant absorption was slower then. The Hg and As accumulations in below-ground tissues was more than above-ground tissues in all plant species, that it showing roots and rhizomes have important role in heavy metals accumulation by aquatic plants. As accumulations in all plants were higher than Hg accumulation, the reason is the bigger mobility of As rather than Hg. The absorption of organic and inorganic mercury than some heavy metals from soil by plants is low [15] and there is probably a barrier to mercury translocation from plant roots to tops. There are some other factors which may have affected the quantities of mercury detected in the plant tissues results. It is possible that some amount of solution mercury volatilized. *Typha latifolia* showed that it could capable As and Hg accumulation in below-ground and above-ground tissues with 47.86 and 2.23 mg/kg in dry matter respectively. The results showed significant difference in Hg accumulation in *Phragmites australis* below-ground tissues than *Typha latifolia, Bulrush (Scirpus)* and *Alisma plantgo* in three treatmants. The reason for higher heavy-metals accumulation in the tissues of *Phragmites australis* is rooted in the higher intensity and numbers of the rhizomes.

Hg is a metal which, although rare in water and wastewater, can become haphazardly toxic if its bioavailability increases [5]. Unfortunately, regarding plants tested in our study literature data on Hg accumulation are poor. [13] has shown that in some aquatic plants, Hg is removed at a higher efficiency than other heavy metal contaminants, such as iron, zinc and copper. It was also founded by [13] that the removal rate of mercury was dependent on the contamination. [23] reports that the higher the concentration of mercury in the water, the higher the amount of mercury removed by the plants, specifically at the roots with water lettuce, exhibiting the largest uptake and accumulation capability overall followed by water hyacinth, taro and rush, respectively. [26] reports that the Hg accumulation in above-ground tissues and below-ground tissues of four constructed wetlands in the Czech Republic with *Phragmites australis* were 0.016 and 0.025 mg/kg, for As these amounts were 0.17 and 1.95 mg/kg. in present study the As accumulation in all plants increased, however *Phragmites australis* was highest concentrations in below-ground tissues with 119.55 mg/kg in 200 mg/kg As in soil. There were significant difference than *Typha latifolia, Bulrush (Scirpus)* and *Alisma plantgo.* [6] report that As accumulation by *Phragmites australis* in contaminated areas with 1225.6 mg/kg As in sediments was 688 mg/kg. Research by [7] has shown that belowground organs were the primary areas of metal (Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn) accumulation. Below-ground tissues through roots and rhizomes can absorb metals as well as trough their leaves and stems because the latter provide an expanded area to trap particular matter, sorb metal ions, and accumulate and sequester pollutants [27,14,7]. Our study indicated to confirm this result for *Phragmites australis*, *Typha latifolia, Bulrush (Scirpus)* and *Alisma plantgo* as ratio below-ground to above-ground for Hg and As accumulation were high particularly about *Bulrush (Scirpus)* and *Phragmites australis* respectively.

Conclusion:

In this research, the arsenic and mercury accumulation by four aquatic plants were investigated in a field during a period expanding over 60 days. There are a great numbers of small industrial plants and other sources of heavy-metals emitting source in north of Khouzestan province of Iran, which is the home to various aquatic plants species such as *Phragmites australis*, *Typha latifolia, Bulrush (Scirpus))* and *Alisma plantgo* that grow in the river margins and drains. Various behavioral aspects of the two heavy metals were critically analyzed under a simulated condition of industrial wastewater accumulated in above-ground and below-ground tissues of the three plant varieties. Results indicated the appropriateness of below-ground

tissues for metals accumulation. *Phragmites australis* and *Alisma plantgo* showed highest accumulation of As in the below-ground and above-ground tissues than other plant varieties. Highest Hg accumulation was found in below-ground tissues of *Phragmites australis*, whereas the highest Hg accumulation was found in above-ground tissues of the *Typha latifolia* variety. The overall conclusion being that the aquatic plants of the study region can be used as an effective heavy-metals removal mechanism to reduce environmental pollutions on one hand and facilitating reuse of the otherwise contaminated industrial wastewaters on the other.

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