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Full Length Research Paper

Mercury and arsenic accumulation by three species of aquatic plants in Dezful, Iran

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Heavy metals can be absorbed by living organisms, such as aquatic plants or non-living biomass. Aquatic plants can be used for removing heavy metals and nutrients from industrial and municipal wastewaters. This paper investigates the capability of *Phragmites australis*, *Typha latifolia* and *Scirpus (Bulrush)* to uptake arsenic and mercury from industrial wastewater. The accumulation capacities of these aquatic plants in three treatments consisting of 50, 100 and 200 mg/kg As and Hg in soil were studied under the semi-arid conditions of Dezful, Southwest Iran. Data observed from the treatments 60 days after growth indicated that each three species were capable to uptake As and Hg from the solution. Results showed significant statistical differences in accumulation of As in the below-ground tissues of three plants where the highest As accumulation (measuring 119.55 mg/kg) was observed for *P. australis* in the treatment of 200 mg/kg As in soil, followed by 65.25 and 47.86 mg/kg for *Bulrush (Scirpus)* and *T. latifolia,* respectively. Maximum accumulation for Hg in below-ground tissues was observed in *P. australis* measuring 6.23 mg/kg in 200 mg/kg Hg in soil treatment, followed by *Bulrush (Scirpus)* and *T. latifolia* measuring 2.23 and 1.45 mg/kg, respectively. The results indicated that As and Hg accumulations in below-ground tissues were higher than those for the above-ground tissues for all plants. The results also indicated highest below-ground to above-ground tissues rations (BG/AG) for As and Hg in *P. australis* and *Bulrush (Scirpus)* ranging 85.3 to 108.8 and 19.7 to 39, respectively. Data obtained from this research conformed well to the exponential association model. 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

Contamination of water and wastewater with heavy metals is emerging as a global environmental challenge caused by this phenomenon. One approach is to treat the contaminated wastewater in order to remove their heavy metals contents and reuse the out product for agricultural that has attracted the attention of the researchers and decision-makers on methods to overcome the problems production. The aquatic plants can reportedly be used as the natural catalysts to absorb and accumulate heavy metals in their tissues from wastewater (Vymazal, 2008). 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 (Ma et al., 2001; Robinson et al., 2003; Taggrat et al., 2005; Skinner et al.,

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Abbreviations: BG, Below-ground; **AG,** above-ground; **ICPMS,** inductively coupled plasma mass spectroscopy.

2007; Mishra and Tripathi, 2008, 2009).

The metals are generally absorbed by the root and shoot systems of the plants. However, research reports (Baldantoni et al., 2004; Gothberg and Greger, 2006) 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 Padmavathiamma and Li (2007) 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 (Kabata-Pendias and Pendias, 1992). The accumulation rate of arsenic and mercury has been tested on the above and below the ground tissues of many aquatic plants species (Hozhina et al., 2001; Weis et al., 2003, 2004; Greger et al., 2005; Bonanno and Giudice, 2010). The 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 (Windham et al., 2003).

However, The bioremoval process using aquatic plants consists of two uptake processes of biosorption and bioaccumulation. Further, Zhao et al. (2006) 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. Bareen and Khilji (2008) investigated the ability of the *T. 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. *P. austrulis* and *Typha spp.* are among the most common aquatic plants used for wastewater treatment in constructed wetlands (Vymazal, 2008). Various aquatic plant species thrive in arid and semi-arid regions of Khouzestan in Iran, which can be studied for their ability of heavy metal uptake from the industrial wastewater and make it possible for the treated wastewater to be used for agricultural production. 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 is to investigate the absorption and accumulation of arsenic and mercury elements from industrial wastewater onto three aquatic plant species consisting of *P. australis*, *Typha latifolia* and *Scirpus (Bulrush)* 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 *P. australis*, *T. latifolia* and *Scirpus* (*Bulrush)* 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°C.

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 $As(NO_3)_3$ and $Hg(NO_3)_2$ 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 APHA (1999) 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 concentration and atomic absorption spectrophotometer to determine Hg concentration. APHA (1999) procedures were used as an analytical framework for the analyses of plant chemical accumulation.

Statistical analyses and uptake curves

The statistical analyses were based on a totally random design using the Statistical Package for the Social Sciences (SPSS) (Version 13). Curveexpet software (version 1.3) was used in this study to draw the accumulation of Hg and As in plant tissues relative to time.

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 low treatment.

Element	Arsenic		Mercury	
Macrophyte	**AG**	**BG**	**AG**	**BG**
Phragmites australis	1.78 ± 0.34b	30.18 ± 3.28a	0.033 ± 0.007b	3.59 ± 0.89a
Typha latifolia	3.26 ± 0.53a	14.26 ± 2.17b	0.075 ± 0.012a	1.26 ± 0.35b
Bulrush (Scirpus)	0.89 ± 0.11b	17.56 ± 2.33b	0.022 ± 0.002b	0.62 ± 0.18c

Table 2. Concentration (mg/kg dry weight ± S.D.) of heavy metals in above-ground (AG) and below-ground (BG) tissue of plants sampled in medium treatment.

Element	Arsenic		Mercury	
Macrophyte	**AG**	**BG**	**AG**	**BG**
Phragmites australis	2.79 ± 0.53b	69.66 ± 5.33a	0.046 ± 0.012b	4.76 ± 1.03a
Typha latifolia	5.42 ± 0.76a	26.15 ± 3.78c	0.084 ± 0.023a	1.43 ± 0.42b
Bulrush (Scirpus)	1.37 ± 0.16b	41.23 ± 4.08b	0.037 ± 0.0031b	1.12 ± 0.37b

Table 3. 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	
Macrophyte	**AG**	**BG**	**AG**	**BG**
Phragmites australis	6.36 ± 0.98a	119.55 ± 7.38a	0.073 ± 0.012a	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 ± 0.32b	65.25 ± 5.49b	0.046 ± 0.0037b	1.45 ± 0.49b

RESULTS

Plants uptake

The metal concentration in 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 (Tables 1, 2 and 3).

Mercury

The initial amount of mercury at first treatment in each pot was 50 mg/kg in soil, with Hg accumulation in all plants increased by the end of the experiment. In this treatment, the highest accumulation of Hg was recorded in *P. australis* with 3.59 mg/kg in dry matter in below-ground tissues. Afterwards *T. latifolia* and *Scirpus* (*Bulrush)* were with 1.26 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, with Hg accumulation in *P. australis*, *T. latifolia* and *Scirpus* (*Bulrush)* observed (0.033, 0.075 and 0.022 mg/kg) in dry matter of above-ground tissues, respectively with *T. latifolia* being the highest at the end of experiment (Table 1). When plants accumulate metals, the roots and rhizomes generally show higher concentrations than the shoots (Sinicrope et al., 1992). In treatment of 100 and 200 mg/kg Hg in soil, results indicated this advantage with higher accumulation (Tables 2 and 3). In medium treatment (100 mg/kg Hg in soil), the highest accumulation of Hg was recorded in *P. australis* with 4.76 mg/kg in dry matter in below-ground tissues, afterwards *T. latifolia* and *Scirpus* (*Bulrush)* were with 1.43 and 1.12 mg/kg in dry matter, respectively. For 200 mg/kg Hg in soil treatment, these amounts were 6.23, 2.23 and 1.45 mg/kg in dry matter of below-ground tissues with *P. australis*, *T. latifolia* and *Scirpus* (*Bulrush)*, respectively.

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 *P. australis* with 30.18 mg/kg in dry matter in its below-ground tissues. Afterwards,

Table 4. Below-ground to above-ground tissues ratios for As and Hg accumulation in plants studied.

Element	As	Hg
Macrophyte	**BG/AG**	**BG/AG**
Phragmites australis	85.3 - 108.8a	16.9 - 25a
Typha latifolia	16.8 - 22.3b	4.4 - 6.6b
Bulrush (Scirpus)	28.2 - 31.5b	19.7 - 39a

Table 5. A comparison of the Arsenic uptake constants of various macrophytes.

Macrophyte	Treatment	a	b	r	s
	50	38.24	0.027	0.999	0.404
Phragmites australis	100	90.64	0.024	0.999	0.699
	200	133.91	0.038	0.999	0.898
	50	18.74	0.026	0.997	0.431
Typha latifolia	100	31.63	0.029	0.998	0.55
	200	52.43	0.040	0.999	0.637
	50	26.56	0.018	0.994	0.789
Bulrush (Scirpus)	100	52.14	0.027	0.997	1.16
	200	67.51	0.056	0.998	1.33

r and s are regression coefficient and standard error; a and b are coefficients.

Bulrush (Scirpus) and *P. australis* were 17.56 and 14.26 mg/kg in dry matter of below-ground tissues, respectively (Table 1). 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 (Tables 2 and 3). As accumulation in all plant above-ground tissues increased with time, but were lower in the tissues of plants rather than below-ground tissues. In the above-ground tissues, the highest As accumulation was 3.26 mg/kg in dry matter for *T. latifolia* at 50 mg/kg As in soil; afterwards *P. australis* and *Bulrush (Scirpus)* were with 1.78 and 0.89 mg/kg, respectively. With increase in As concentration in soil, its accumulation was enhanced by plants. In treatment of 100 and 200 mg/kg arsenic in soil, As accumulation in below-ground tissues of *P. australis*, *Bulrush (Scirpus)* and *T. latifolia* were 69.66, 41.23, 26.15 and 119.55, 65.25, 47.86, respectively. In these treatments, As accumulation in above-ground tissues was lower than below-ground tissues too.

Ratios

Below-ground to above-ground tissues ratios for arsenic was higher for *P. australis* with range of 85.3 to 108.8, followed by *Bulrush (Scirpus)* and *T. latifolia* with ranges of 28.2 to 31.5 and 16.8 to 22.3, respectively (Table 4). This ratio for mercury was higher for *Bulrush (Scirpus)* with ranged 19.7 to 39; afterwards *P. australis* and *T. latifolia* were with ranges 16.9 to 25 and 4.4 to 6.6, respectively (Table 4). The results showed significant statistical differences between the arsenic accumulation ratios on the below-ground to above-ground tissues of *P. australis* and two other varieties. While this accumulation ratio for the Hg in *T. latifolia* is lower than the *P. australis* and *Bulrush (Scirpus)* varieties, which had no significant difference in their accumulation ratios (Table 4).

Plants uptake curves

Due to the higher absorption of the two metals by the below-ground plant tissues compared to those in the above-ground ones, the methodology involved drawing a curve for the As and Hg accumulation for below-ground plant tissues. Drawing the Hg and As accumulation in below-ground tissues of plants based on time, the observation data were plotted against time and the relationship was fitted with a exponential association equation (Tables 5 and 6). General form of the equation can be expressed as follows:

$$y = a(1 - e^{-bx})$$

Where, y is the As or Hg accumulation with below-ground tissues of each aquatic plants (mg/kg), x is the time from the experimental set up (days), a and b are constants.

The results obtained for As and Hg accumulation in below-ground tissues of *P. australis*, *Bulrush (Scirpus)* and *T. latifolia* are shown in Tables 5 and 6. The curves

Table 6. A comparison of the Mercury uptake constants of various macrophytes.

Macrophyte	Treatment	a	b	r	s
Phragmites australis	50	3.85	0.043	0.999	0.045
	100	5.46	0.031	0.997	0.118
	200	6.86	0.038	0.999	0.077
Typha latifolia	50	1.67	0.024	0.998	0.027
	100	1.83	0.026	0.999	0.011
	200	3.14	0.020	0.999	0.006
Bulrush (Scirpus)	50	0.64	0.058	0.996	0.021
	100	1.18	0.045	0.998	0.022
	200	1.91	0.025	0.998	0.032

r and s are regression coefficient and standard error; a and b are coefficients.

were drawn in order to work out the accumulation of As and Hg (mg/kg) in plants tissues below-ground relative to time (0, 10, 20, 30, 40, 50, and 60 days). Where; r and s are regression coefficient and standard error, respectively (Tables 5 and 6). The results showed highest a and b coefficients for As in *P. australis* ranging between 38.24 to 133.91, followed by *Bulrush (Scirpus)* and *T. latifolia* ranging between 26.56 to 67.51 and 18.74 to 52.43, respectively. The regression of these curves showed high accuracy in this study. For Hg, a and b coefficients was highest in *P. australis* too, ranging between 3.85 to 6.86 and 0.038 to 0.043, followed by *T. latifolia* and *Bulrush (Scirpus)* ranging between 1.67 to 3.14 and 0.64 to 1.91, respectively.

DISCUSSION

P. australis, *T. latifolia* and *Bulrush (Scirpus)* 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. 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 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 is, showing that 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 (Lodenius, 1990) 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. *T. latifolia* showed that it could be capable of 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 *P. australis* below-ground tissues than *T. latifolia* and *Bulrush (Scirpus)* in three treatments. The reason for higher heavy-metals accumulation in the tissues of *P. 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 (Bargagli, 1998). Unfortunately, regarding plants tested in this study, literature data on Hg accumulation are poor. Kamal et al. (2004) 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 found by Kamal et al. (2004) that the removal rate of mercury was dependent on the contamination. Skinner (2007) 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. Vymazal (2009) reports that the Hg accumulation in above-ground tissues and below-ground tissues of four constructed wetlands in the Czech Republic with *P. australis* were 0.016 and 0.025 mg/kg, for As these amounts were 0.17 and 1.95 mg/kg. In this study, the As accumulation in all plants increased, however, *P. 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 *T. latifolia* and *Bulrush (Scirpus)*. Baroni et al. (2004) reported that As accumulation by *P. australis*in contaminated areas with 1225.6 mg/kg As in sediments was 688 mg/kg. Research by Bonanno and Giudice

(2010) has shown that below ground 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 through their leaves and stems because they latter provide an expanded area to trap particular matter, sorb metal ions, and accumulate and sequester pollutants (ward, 1987; Levine et al., 1990; Bonanno and Giudice, 2010). Our study confirmed this result for *P. australis*, *T. latifolia* and *Bulrush (Scirpus)*, as ratio below-ground to above-ground for Hg and As accumulation were high particularly about *Bulrush (Scirpus)* and *P. australis,* respectively.

Conclusion

There are great numbers of small industrial plants and other sources of heavy-metals emitting source in Khouzestan province of Iran, which is the home to various aquatic plants species such as *P. australis*, *T. latifolia* and *Bulrush (Scirpus)* that grow in the river margins and drains. The arsenic and mercury accumulation by these plants were investigated in a field during a period expanding over 60 days. 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. The results indicated the appropriateness of below-ground tissues for metals accumulation. *P. australis* showed highest accumulation of As in the above-ground and below-ground tissues than other plant varieties. Highest Hg accumulation was found in below-ground tissues of *P. australis*, whereas the highest Hg accumulation was found in above-ground tissues of the *T. 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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