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Accumulation of Mercury and Arsenic in Three Species of Aquatic Plants in Dezful, Iran

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Abstract: Aquatic plants were used for the removal of 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 treated spices consist of 50, 100 and 200 mg.kg^{-1} As and Hg in soil under the semi-arid conditions of Dezful, Southwest of Iran were investigated. Data obtained from the 60 days treated, the growth indicated that each of 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 (measured 119.55 mg kg^{-1}) was observed for *Phragmites australis* in the treatment of 200 mg. kg^{-1} As in the soil, followed by 65.25 and 47.86 mg kg^{-1} for *Bulrush (Scirpus)* and *Typha latifolia,* respectively. Maximum accumulation for Hg in below-ground tissues was observed in *Phragmites australis* determined 6.23 mg kg^{-1} in 200 mg kg^{-1} Hg in the treated soil, followed by *Typha latifolia* and *Bulrush (Scirpus)* measured 2.23 and 1.45 mg kg^{-1}, respectively. Results indicated that As and Hg accumulations in below-ground tissues were higher than those for the above-ground tissues for all plants. Results also indicated highest below-ground to above-ground tissues rations (BG/AG) for As and Hg in *Phragmites australis* and *Bulrush (Scirpus)* in the range of 85.3-108.8 and 19.7-39 mg. kg^{-1}, respectively. Data obtained from this research confirmed with the Exponential Association Model. The overall conclusion being that the three aquatic plants selected in this study were used as effective catalysts for the removal of heavy metals from the industrial wastewater under arid and semi-arid conditions.

Key words: Arsenic · Mercury · *Phragmites australis* · *Typha latifolia* and *Bulrush (Scirpus)*

INTRODUCTION

Contamination of water and wastewater with heavy metals is emerging as a global environmental challenge that has attracted attention of many researchers and decision-makers on methods to overcome the problem and eliminate the causes of creation of such phenomenon. One a pproach is to treat the contaminated wastewater in order to remove the heavy metals contents and reuse it for agricultural irrigation. The aquatic plants can reportedly be used as natural catalysts to absorb and accumulate heavy metals in plants tissues [1]. Several researches have been conducted on harmful effects of the heavy metals in 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 wastewater; therefore to mitigate their harmful consequences [2-6].

Generally, the metals are absorbed by the plant root and shoot systems. However, in many cased reported that absorption of heavy metals and accumulation of toxic compounds were detected in the aquatic plants' tissues [7, 8]. The highest concentrations of heavy metals were reported in the root systems followed by those in the

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stems and leaves. Some heavy metals such as arsenic and mercury however, may be discharged through the wastewater bodies into environment even in 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 [9] who also found the potential of these plants in biologically converting the absorbed metal into gaseous form that may be then easily released in to environment. Accumulation of metals in plants depends on various factors such as concentration of metals in the soil, the nature of plant species and special chemical structure of metals in soil solutions [10]. The accumulation rate of arsenic and mercury has been evaluated on the above and below the ground tissues of many aquatic plants species [11-15].

Results of field studies on plants such as *Phragmites australis* showed that the highest metal concentration was in below the ground tissues with smaller translocation on above the ground [16]. The bioremoval process using aquatic plants consists of two uptake processes; such as biosorption and bioaccumulation. Hybrid generation in conjunction with atomic fluorescence spectrometry (HG–AFS) was used to determine the total arsenic concentrations of the *bamboo* shoots [17]. It was found that, *Bamboo* (*Phyllostachys pubescens* Mazel) is one of the aquatic plants have the ability to absorb arsenic elements from industrial wastewater. The ability of Typha *angustifolia* plant was investigated in removal of the heavy metals such as chromium, copper and zinc from tannery sludge [18]. Observations showed that significant absorptions of these chemical elements by the aquatic plant have been investigated [18]. *Phragmites austrulis* and *Typha spp.* are among the most common aquatic plants used for wastewater treatment in constructed wetlands [1].

Several aquatic plant species thrive in arid and semi-arid regions of Khouzestan in Iran, were studied for their ability to uptake heavy metals from industrial wastewater. Once heavy metals are removed, the treated wastewater can be used for agricultural purposes. Literature review on phytoremediation showed limited knowledge on 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 by three aquatic plant species known as *Phragmites australis*, *Typha latifolia* and *Scirpus (Bulrush)* under simulated arid and semi-arid conditions of Dezful region in southwest of Iran.

MATERIALS AND METHODS

Study Location and Plant Selection: Three aquatic plants of *Phragmites australis*, *Typha latifolia* and *Scirpus* (*Bulrush)* were selected because of their availability and accessibility in the selected region. During spring of 2008, samples were collected from Dez River, Safiabad and Senjar main drains and other stream margins in Dezful, Iran. These samples were replanted in nurtured environment of the plastic pot bed with 100 cm diameter and 60 cm height. Approximately 200 kg of river sediment materials (sands) 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. For 10 days after experimental re-plantation, one herb from each pot was disposed of every ten days. All experiments were conducted at 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 were in the range of 24 to 50 degrees Celsius.

Simulated Wastewater: After filling the pots with the specified amount of sands, chemical fertilizers (N, P and K) and water for irrigation were then applied prior to replanting the samples. Three level of As and Hg consist of 50, 100 and 200 mg. kg^{-1} in soils were added at three replications for each plant in ten days after re-plantation. In order to conduct this research; two chemical substances of $As(NO_3)_3$ and $Hg(NO_3)_2$ represented as two main sources of pollutions in the wastewater were used in the actual experiments as the necessary contaminants for AS and Hg.

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 data analysis, 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 in a ball mill. The APHA [19] 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. The APHA [19] procedures were used as an analytical framework for the analysis of plant chemical accumulation.

Statistical Analyses and Uptake Curves: The statistical analyses were based on a totally random design using the SPSS (Version 13).

Curveexpet software (version 1.3) was used in this study to draw the accumulation of Hg and As in plant tissues with respect to time.

RESULTS AND DISCUSSION

Plants Uptake: The metal concentrations in the above and below the ground tissues of all plant samples showed increasing trend with respect to time. Analysis of As and Hg accumulations in the aquatic macrophytes in three treatments (50, 100 and 200 mg. kg^{-1} of As and Hg in soil) revealed that accumulation rate in the below-ground (BG) samples were higher 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^{-1} in soil; amount of 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^{-1} in the dry matter of below-ground tissues. Afterwards *Typha latifolia* and *Scirpus* (*Bulrush)* were 1.26 and 0.62 mg. kg^{-1} in the dry matter of below-ground tissues at sixth decade, respectively. Hg accumulation in all plants above-ground tissues was lower than below-ground tissues; which Hg accumulation in *Phragmites australis*, *Typha latifolia* and *Scirpus* (*Bulrush)* determnined 0.033, 0.075 and 0.022 mg. kg^{-1} in the dry matter of above-ground tissues, respectively. The accumulation of HG in *Typha latifolia* was highest at end of experiment (Table 1). When plants accumulate metals, the roots and rhizomes generally show higher concentrations than the shoots [20]. In treatment of 100 and 200 mg. kg^{-1} Hg in soil, results indicated that accumulation of Hg in samples with medium and high treatment were higher than in low traetment (Table 2 and 3). In medium treatment (100 mg. kg^{-1} Hg in soil), the highest accumulation of Hg was recorded in *Phragmites australis* with 4.76 mg kg^{-1} in the dry matter in below-ground tissues, afterwards *Typha latifolia* and *Scirpus* (*Bulrush)* were 1.43 and 1.12 mg. kg^{-1} in the dry matter, respectively. For 200 mg. kg^{-1} Hg in soil treatment, these amounts were 6.23, 2.23 and 1.45 mg. kg^{-1} in the dry matter of below-ground tissues with *Phragmites australis*, *Typha latifolia* and *Scirpus* (*Bulrush)*, respectively.

Arsenic: In treatment of 50 mg. kg^{-1} As in soil, all plants absorption increased with respect to time duration of experiment. In this treatment, maximum absorption was devoted to *Phragmites australis* with 30.18 mg. kg^{-1} in the dry matter in its below-ground tissues. Afterwards, *Bulrush (Scirpus)* and *Phragmites australis* were 17.56 and 14.26 mg. kg^{-1} in the dry matter of below-ground tissues, respectively (Table 1). In other treatments (100 and 200 mg. kg^{-1} As in soil), the accumulation of As in all plants increased with respect to time and also concentration in soil (Table 2 and 3). As accumulation in all plants above-ground tissues increased with respect to time, but were lower in these tissues of plants rather below-ground tissues. In above-ground tissues, the highest As accumulation was 3.26 mg. kg^{-1} in dry matter for *Typha latifolia* at 50 mg. kg^{-1} As in soil, afterwards *Phragmites australis* and *Bulrush (Scirpus)* were 1.78 and 0.89 mg. kg^{-1}, respectively. While increasing in As concentration in soil, also its accumulation increased in plants. In treatment of 100 and 200 mg. kg^{-1} arsenic in soil, As accumulation in below-ground tissues of *Phragmites australis*, *Bulrush (Scirpus)* and *Typha 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 the As accumulation in below-ground tissues.

Ratios: The ratios for arsenic concentration in below-ground was higher than the above-ground for *Phragmites australis* which was in the range of 85.3-108.8, followed by *Bulrush (Scirpus)* and *Typha latifolia* were 28.2-31.5 and 16.8-22.3, respectively (Table 4). This ratio for mercury was higher for *Bulrush (Scirpus)* in the range of 19.7-39; afterwards *Phragmites australis* and *Typha latifolia* were 16.9-25 and 4.4-6.6, respectively (Table 4).

Results showed significant statistical differences between the arsenic accumulation ratios on the below-ground to above-ground tissues of *Phragmites australis* and two other varieties. While this accumulation ratio for Hg in *Typha latifolia* is lower than the *Phragmites australis* and *Bulrush (Scirpus*) varieties, which had no significant difference in their accumulation ratios (Table 4).

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

Element macrophytes	Arsenic AG	Arsenic BG	Mercury AG	Mercury 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^{-1} dry weight ±S.D.) of heavy metals in above-ground (AG) and below-ground (BG) tissue of plants sampled in medium treatment

Element macrophytes	Arsenic AG	Arsenic BG	Mercury AG	Mercury 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^{-1} dry weight ±S.D.) of heavy metals in above-ground (AG) and below-ground (BG) tissue of plants sampled in high treatment

Element macrophytes	Arsenic AG	Arsenic BG	Mercury AG	Mercury 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

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

Element macrophytes	As BG/AG	Hg 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: Comparison of the Arsenic uptake constants of various macrophytes

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

Table 6: Comparison of the Mercury uptake constants of various macrophytes

Macrophytes	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

Plants Uptake Curves: Due to higher absorption of 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.

To draw 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 (Table 5 and 6). General form of the equation can be expressed as follows:

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

Where y is As or Hg accumulation with below-ground tissues of each aquatic plants (mg. kg^{-1}), t is 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 *Phragmites australis*, *Bulrush (Scirpus)* and *Typha latifolia* are shown in Table 5 and 6. The curves were drawn in order to work out on accumulation of As and Hg (mg. kg^{-1}) in plants tissues below-ground respect to time (0, 10, 20, 30, 40, 50 and 60 days). Where; r and s are regression coefficient and standard errors, respectively (Table 5 and 6).

Results showed the highest a and b coefficients for As in *Phragmites australis* ranging between 38.24-133.91, followed by *Bulrush (Scirpus)* and *Typha latifolia* ranging between 26.56-67.51 and 18.74-52.43, respectively. The regression of these curves showed high accuracy for this study. For Hg, the coefficients a and b were also highest values in *Phragmites australis*, ranging between 3.85-6.86 and 0.038-.043, followed by *Typha latifolia* and *Bulrush (Scirpus)* ranging between 1.67-3.14 and 0.64-1.91, respectively.

DISCUSSION

Phragmites australis, *Typha 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 the present study was increased with initial concentration and duration time of experiment. All species of plants were experimented, despite differences in physiology, removed much of the effective concentration of arsenic and mercury in the simulated wastewater for the duration of 60 days. Most of heavy metal accumulation occurred at first 10 days in this research, as the trend rate of plant absorption was slow down in the later stage. 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 was due to high mobility of As rather than Hg. The absorption of organic and inorganic mercury than some heavy metals from soil by plants is low [21] 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 soluble 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^{-1} in the dry matter, respectively. The results showed significant difference in Hg accumulation in *Phragmites australis* below-ground tissues than *Typha latifolia* and *Bulrush (Scirpus)* in three treatments. 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 [22]. Unfortunately, regarding plants tested in our study literature data on Hg accumulation are poor. In some aquatic plants, Hg is removed at a higher efficiency than other heavy metal contaminants, such as iron, zinc and copper [23]. It was also founded by Kamal and his coworkers [23] that the removal rate of mercury was dependent on contamination. 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 [24]. Hg accumulation in above-ground tissues and below-ground tissues of four constructed wetlands in tCzech Republic with *Phragmites australis* were 0.016 and 0.025 mg. kg^{-1} , for As these amounts were 0.17 and 1.95 mg. kg^{-1} [25]. In present study As accumulation in all plants increased, however *Phragmites australis* was highest concentrations in below-ground tissues with value of 119.55 mg. kg^{-1} in 200 mg. kg^{-1} As in soil. There were significant difference than *Typha latifolia* and *Bulrush (Scirpus)*. As accumulation by *Phragmites australis* in contaminated areas with 1225.6 mg. kg^{-1}

As in sediments was 688 mg. kg^{-1} [26]. Bonanno and his research team [11] have 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 [11, 27, 28]. In this study indicated to confirm this result for *Phragmites australis*, *Typha latifolia* and *Bulrush (Scirpus)*, as ratio below-ground to above-ground for Hg and As accumulation were high particularly about *Bulrush (Scirpus)* and *Phragmites australis*, respectively.

CONCLUSION

There are a great numbers of small industrial plants and other sources of heavy-metals emitting source in Khouzestan province of Iran, which is home of various aquatic plants species such as *Phragmites australis*, *Typha latifolia* and *Bulrush (Scirpus)* that grow in the river banks margins of streams and drains. The arsenic and mercury accumulation by these plants were investigated in a field during a period expanded 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. Results indicated the appropriateness of below-ground tissues for metals accumulation. *Phragmites 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 *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 also facilitate the reuse of resources, otherwise contaminated industrial wastewaters on the other.

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