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Role of *Phragmites Australis* for Biomonitoring and Phytoremediation of Heavy Metals Pollution in Badavar River, Lorestan Province (Iran)

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A-B-S-T-R-A-C-T

Background & Aims of the Study: Aquatic macrophytes, given their rapid growth and high bio mass production, great potential to accumulate heavy metals in their organs. *Phragmites australis* as a hyper-cumulative plant plays an important role in the bioremediation. The Badavar River of Noorabad, Lorestan province, is exposed to urban wastewater and agricultural runoffs. In order to heavy metals (Pb, Zn and Cu) monitoring by aquatic macrophyte *Phragmites australis*, the concentration of these metals were investigated in this plant and river sediments in 2016.

Materials & Methods: The concentration of the metals after acid digestion was measured by Atomic Absorption Spectrometer. The contamination factor was used to study the sediments pollution.

Results: Comparison of metal concentrations with American and Canadian standards does not indicate the critical status of sediment contamination. The accumulation of metals in *Phragmites australis* plants showed a decreasing trend in root, leaf and stem respectively. On the other hand, the higher the transfer factor from the numerical value of one for zinc and copper metals indicates the high ability of these metals to be transferred to the plant.

Conclusions: Finally, it can be noted that the shoots and roots of the plant in relation to the metals studied as an hyper accumulative organs and since there is a positive and significant relationship between the concentration of copper in sediment and root, it is likely that the roots organ of the plant is an appropriate bio indicator for its contamination in the sediments of the area.

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Background

High amounts of heavy metals are introduced into aquatic ecosystem under anthropogenic and natural processes (1). Human activities, such as urbanization, industrialization, and land use change, increase dangerously pollutants such as heavy metals in the environment (2). Mineral contaminants such as heavy metals are biodegradable and have prolonged persistence. Some of these metals have vital functions in the body, but higher levels of each of these metals cause serious damage to the health of humans and other living organisms (3). In this regard, the phytoremediation is considered as a low-cost alternative to physicochemical methods and less human resource requirements (4). This approach has begun since the 1990s and is considered as an appropriate solution to reduce the pollution from heavy metals in the environment (5). *Phragmites australis* is a wide-spread, ubiquitous macro-macrophyte species that grows from the best plant species to reduce the accumulation of heavy metals and their effects on water, soil and, consequently on food chain. (6). Therefore, many studies have been carried out to determine the concentration of heavy metals in the aquatic ecosystems and their plants. Mortazavi et al. (2016) studied the role of *Phragmites australis* contamination of heavy metal pollution in the Dez River. The results showed that the highest and lowest concentration of metals in the sediment was related to zinc and cadmium, and also the highest aggregate of metals in the roots organs of the plant was obtained (7). Ebrahimi et al. (2012) used the *Phragmites australis* in the Lia-Qazvin industrial zone for the treatment of heavy metals contaminated with Zn, Copper and Chromium. The results showed that the lowest aggregate in the plant organs, soil and water was related to the copper element and the highest aggregate of the three studied metals was observed in the root of the plant. Finally P. australis species was introduced as a biological indicator for the treatment of heavy metals contaminated with soil (6). Liu et al. (2016) studied the contamination and distribution of heavy metals in the Luanhe River estuary in the northwest of the Bohai Sea in China. Bioconcentration factor (BCF) showed that there was a low level of contamination of lead, mercury, arsenic and cadmium in the studied areas, while mercury showed a moderate to high level of pollution in the mouth and southern regions of the river (8). Mazal (2015) investigated the ability of Neumann's plant to absorb phosphorous and nitrogen nutrients, as well as the ability to remove heavy metals from cadmium, mercury and chromium from contaminated water in an artificial wetland. The results showed that the highest accumulation of heavy metal cadmium and mercury in the stem and the highest chromium concentration in the root (9). Phillips et al. (2015) tested the concentration of heavy metals in the tissues of three aquatic plants (*Phragmites australis*, Typha capensis, Spartina maritime) and based on the results, these plants were introduced as an indicator of the presence and level of contamination in the aquatic ecosystems (10).

Aims of the study:

Considering the importance of the Badavar River in supplying the water used by the agricultural sector and catching fish by the natives of the region and against the entry of urban wastewater and heavy runoff agricultural runoffs, the present study aims to measure the concentration of heavy metals in sediments, roots, stems of *Phragmites australis*. Because aquatic plants are suitable biological indicators for identifying the status of contamination of metals in the aquatic ecosystems, the role of bioindicator and the ability to refine *Phragmites australis* as the dominant species of the river with the help of transfer indices and biomass factor it placed.

Materials & Methods

Study area

The Badavar basin is one of the sub-basins of Semareh, with geographical coordinates '30˚47 'to 15˚48' E and 34˚ to 15˚45 N located in the northwest of Lorestan province. The river flow from Niaz and Galam Bahri, located on the mountain ridge, and finally passes through the city of Nurabad to the Seymareh River (Figure 1).

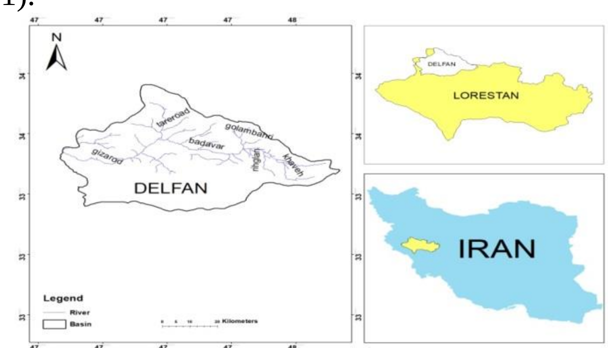

Figure 1) Map of study area

Sample preparation and analysis

After field studies, to determine the status of the Badavar in the area, the river entrance and exit to the city of Noorabad were identified and 6 sampling stations from the surface sediments of the river and strain species in the pre-entering habitats, the length of the city and the outlet range of the city were selected. It should be noted that sampling of sediment and herbaceous material was carried out in spring. In order to collect sediment samples at each 1 × 1 meter plotted station, three sediment samples were collected from each plot (0-20 cm) from each plot, and at each station, according to the sites of *Phragmites australis* 6 seedlings were also harvested. The geographic coordinates of the stations studied are given (Table 1).

Table 1) The coordinates of the sampling points

Station number	Altitude (m)	Latitudel (N)	Longitude (E)	Site description
1	1787	34° 05′ 03.9"	47° 59′ 23.4"	Azizabad village
2	1787	34° 04′ 45.3"	47° 58′ 34.2"	Municipality
3	1786	34° 04′ 46.4"	47° 58′ 12.3"	Laleh park
4	1781	34° 04′ 48.0"	47° 57′ 42.3"	Slaughterhouse
5	1784	34° 05′ 10.3"	47° 57′ 27.2"	Kamal-Jamal tomb
6	1783	34° 05′ 14.3"	47° 57′ 04.4"	Agricultural land

The sediment and plant samples were dried in an oven at 105 ° C for 72 hours and poured into powder in mortar. At this stage about one gram of dried specimen (sediment and plant) was prepared by combining nitric acid, perchloric acid and chloride acid in a ratio of 6: 2: 2 in a digestive tract initially at a low temperature (40 °) for one hour, and It was then digested at 140 ° C for three hours and filtered with Wattem No. 1 filter paper and distilled twice with distilled water (DDW) in a volume of 25 ml. Finally, the concentration of metals in the samples was measured by atomic absorption (11).

Data analysis

Statistical analysis was performed using SPSS 21 software. Excel charts were drawn using Excel software. At first, the normality of the concentration of metals in sediments and strain species was investigated and one-way ANOVA test was used to compare the normal data and the Kruskal-Wallis non-parametric test to compare the abnormal results at stations Different were used. The correlation between the concentration of metals in the sediments and the amount of elements measured in the species were determined using the Pearson correlation test for normal data and Spearman correlation test for abnormal results. Also, one sample T-test was used to compare the concentration of metals with the universal standards. Finally, the average concentration of metals in the sediments of the region was compared with the Interim sediment quality guidelines and National Oceanic and Atmospheric Administration sediment quality guidelines.

Calculation of Bioconcentration factor (BCF)

The Bioconcentration factor coefficient indicates the ability of plants to tolerate and accumulate heavy metals in their organs, which are calculated according to the following relationships (12).

Roots Bioconcentration factor = Concentration of metal in the root/ Concentration of metal in soil

Shoots Bioconcentration factor = Concentration concentration in shoots / metal concentration in soil

Translocation Factor (TF)

Translocation Factor coefficient indicates the ability of plants to absorb and transfer metals from sediments and then store them in upper parts of the earth (12, 13).

Transfer factor from roots to shoots= Concentration of metals in shoots/ Concentration of metal in root

Contamination Factor (CF)

The Contamination Factor coefficient describes the contamination of the metal in question. Hackson's contamination coefficient (14) is obtained from the following equation:

$$Cf = \frac{Mx}{Mb}$$

Where, Mx is the concentration of the metals in the sample and Mb is the concentration of the same metal in the reference material.

Results

According to (Figure 2), the mean concentration of lead metal in the studied stations is presented in the plant organs and sediments:

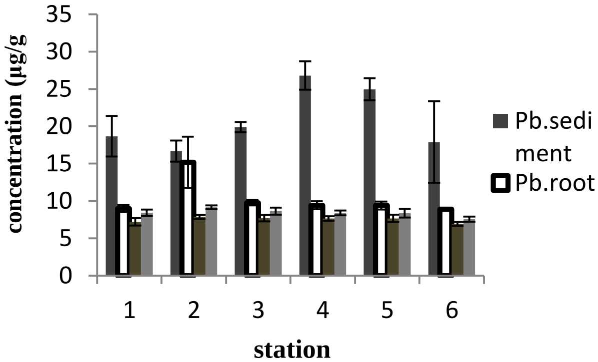

Figure 2) Comparison of concentration of lead in sediment, roots, stem and leaf of plants

Regarding normal and homogeneity of results (P <0.05) in sediment, one-way analysis of variance showed no significant difference (P> 0.05) between the concentration of this metal in sediments of different stations. Also, due to the normalization and homogeneity of the results (P <0.05) in leaf and stem *Phragmites australis* according to ANOVA, there was no significant difference between the lead concentrations in leaf and stem of the plants in the studied stations. There was no significant difference between the concentrations of this metal in the roots organs due to the lack of normal results according to Kruskal-Wallis test (P <0.05).

According to (Figure 3), the mean concentration of zinc metal in the studied stations is given in plant organs and sediments.

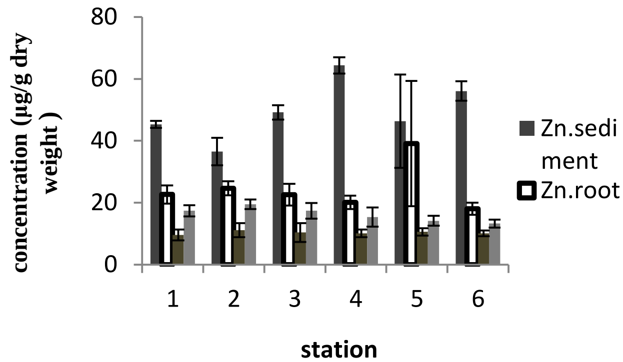

Figure 3) Comparison of zinc concentration in sediments, roots organs, stems and leaves of plants

Regarding to normal and homogeneity (P <0.05) zinc results in sediment, the results of one-way analysis of variance analysis showed no significant difference between the concentration of this metal in sediments of different stations (P<0.05)) Also, due to the normal and non-homogeneity of the data (P <0.05), there was no significant difference between Zn concentration in plant stems in different stations according to Dunnetts T3 (P <0.05). There was no significant difference in the leaves of the plants at the stations due to the homogeneity of the data (P <0.05) according to the ANOVA test (P> 0.05). There was no significant difference in the root of the *Phragmites australis* as a result of abnormal results at different stations according to Kruskal-Wallis test (P <0.05).

According to (Figure 4), the average concentration of copper in the studied stations is shown in the plant organs and sediments.

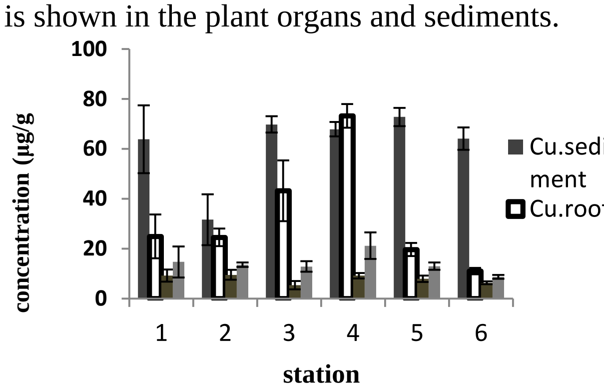

Figure 4) Comparison of the concentration of copper in sediments, roots organs, stems and leaves of plants

According to the normal and homogeneous results of copper in sediments (P <0.05), one-way analysis of variance showed that there was a significant difference between the concentration of copper in the sedimentation of different stations at 95% (P <0.05) there is. According to Duncan test, at 95% level (P <0.05), there was a significant difference between the concentration of copper in station two with other stations studied. Regarding the non-normal results of copper in roots and leaves of *Phragmites australis* (P<0.05), Kruskal-Wallis test showed a significant difference (p<0.05) between the concentration of copper in the root of the plant) There is. Also, there was no significant difference between the concentrations of this metal in the leaf of the *Phragmites australis*. On the other hand, due to the normal and homogeneous results of copper in the stem of *Phragmites australis* (P<0.05), according to ANOVA, there is not a significant difference between the concentration of copper in the studied stations in the stem of the *Phragmites australis*.

The values obtained from the bioconcentration factor and the transfer factor is shown (Table 2).

Table 2) Calculated values of bioengineering and transfer factor

Index	Cu	Zn	Pb
BCF(Aerial organs)	0.433	0.494	0.493
BCF(Underground organs)	0.355	0.527	0.764
TF of underground organ to aerial organ	1.550	1.067	0.819

The calculated values of the coefficient of pollution are presented (Table 3).

Table 3) Estimated values of contamination factor in sediment of studied stations

Station	Cu	Zn	Pb
1	1.418	0.477	0.933
2	0.702	0.384	0.833
3	1.549	0.517	0.995
4	1.508	0.677	1.339
5	1.617	0.487	1.247
6	1.424	0.590	0.894
Mean	1.370	0.522	1.040

Discussion

The results showed that the concentration of metals in the Badavar sediment has a decreasing trend of Cu, Zn and Pb, respectively which is consistent with studies by Gurumoorthi and Venkatachalapathy (2016) on the deposits of the Kaniakumari coast in India (15) as well as Chen et al. (2016) in the deposits of the Lean River in China (16).

In order to investigate the sediment pollution of the Badavar River from heavy metals of lead, zinc and copper, the average concentration of metals in the sediments of the region was compared with the ISQGs and NOAA sediment quality guides in (Table 4).

Table 4) Mean comparison of concentration of heavy metals in terms of (μg/ g dry weight) in sediment with some world standards

Case study	NOAA (17)		ISQGs (18)		element
	ERM[4]	ERL[3]	TEC[2]	PEC[1]	
18.662	218	46.7	35.8	128	Pb
49.638	410	150	121	459	Zn

[1]-Probable Effect Concentration
[2]-Threshold Effect Concentration
[3]-Effect Rang Love
[4]-Effect Rang Medium

61.641	270	34	31.6	149	Cu

The results presented in Table 4 showed that concentrations of Zn and Pb in river sediments were lower than those of the Canadian and

Contamination	CF value
Low	CF<1
Moderate	1≤CF≤3
High	3≤CF≤6
Very High	CF≥6

American environmental standards, while the standard tensile metal (TEC) ISQGs and the standard threshold (ERL) NOAA is more. This indicates a critical situation of heavy metals in the sediments of the region.

To estimate the contamination rate of heavy metals, contamination factor (CF) was used (Table 3). The results indicated that the highest contamination was for copper and the least for zinc. According to (table 5), the contamination factor for zinc metal in all studied stations is less than one, which indicates the low pollution of zinc metal in river sediments. In relation to lead metal, at stations 1, 2, 3 and 6, the contamination rate was less than one, indicating low pollution of these metals at these stations, while at stations four and five contamination factors between one and three, which indicates the average contamination of lead in these stations. Regarding the role of fossil fuels in increasing the concentration of lead in the environment and the proximity of the four and five stations with sub roads, as well as the entry of urban wastewater and agriculture, which are the main causes of high lead concentrations (19). The higher the lead metal content in these two stations than the other stations can be justified. Pollution factor for copper in all stations is in the middle class, except at station 2, which has a contamination level of less than one and indicates low contamination. The lower amount of copper in station 2 compared to other stations due to high plant density in this station and the effective role of this plant in absorbing copper from sediments.

Table 5) Pollution factor classification (14)

The results of the transfer coefficient presented in (Table 2) show that the highest value of this factor is related to copper metal and the least is related to lead metal. The TF (Transfer Factor) or transfer coefficient, known as the rate of biological accumulation, meets the plant's ability to meet the planting goals (13, 20). According to kabata-Pendias and Pendias in 2000, if the transfer factor ranges 0.01-1, it means that the accumulation and access in the plant is moderate (21). In the present study, the transfer factor in relation to lead is less than one, indicating the accumulation and access of this metal by the plant is moderate. But in relation to copper and zinc, the values of this factor are greater than one, which indicates the accumulation and high availability of copper and zinc in the plant.

Based on the results of calculating the bio-concentration factor, according to (Table 2) and comparing it with the proposed classification of Ma et al. (2001) for plants with BCF equivalent to Excluder, plants with BCF less than one (Accumulator) and for species with BCF, it considers more than one (Hyper accumulator) (22), a plant that has been used as an absorbent plant for lead, zinc and copper in the air and roots organs.

The values for correlation coefficient between sediments and plant organs are presented (Table 6).

Table 6) Correlation values of metals between sediments and plant organs

Plants organs	Metal	root	stem	leaf
stem	Pb	0.186		
	Zn	0.075		
	Cu	0.322		
leaf	Pb	0.331*	0.718**	
	Zn	0.024	0.472**	
	Cu	0.524**	0.549**	
sediments	Pb	-0.029	0.146	0.392
	Zn	0.085	0.438	0.364
	Cu	0.476*	0.219	0.207

Given results obtained for the transfer factor it is worthy to note that this plant, in particular with regard to the metals studied, has a high ability to transfer Zn and Cu from the roots to the shoots. In this way, there is a positive and significant correlation between leave and stem. There is also a positive and significant relationship between leaf and root in relation to lead and copper metals. When the metal passes through the roots body to the plant when it is transported to the airspace, it mainly accumulates in the body due to the presence of vacuoles in the leaves. Therefore, it is logical to have a positive and significant relationship between the leaf and the roots for the reasons mentioned. Between plant organs and sediments, only in relation to copper element, there is a positive and significant relationship between sediment and root. Therefore, it can be suggested that the organ plant in the study area as an indicator.

The average concentration of zinc metal in the roots, stems and leaves of Common reed plant is less than the zinc metal (500-2000 μg / g) (23). Pb is a trace element that has toxic effects on plants; however, lead toxicity is less for plant growth than zinc toxicity in plants. The average concentration of lead metal in the roots, stems and leaves of the *Phragmites australis* was lower than the lead toxicity (300-30 μg / g) based on Figure 1 (24). The average concentration of copper in the roots, stems and leaves of the plant according to (Figure 3) is lower than the plant toxicity level by copper (25-40 μg / g) (23). In the present study, the descending roots, leaves and stems were shown for all three studied metals. The concentration of metals in the stems and leaves showed a downward trend of Zn, Cu, Pb and in the root of the decreasing trend of Cu, Zn and Pb, respectively, in relation to zinc and copper metals in the roots, stems and leaves of the study. By Ohimian et al. (2009), is consistent with the mangrove plants in the gutta bay (25). The results of this study showed that the concentration of heavy metals in P. australis species is highly dependent on plant organs and anthropogenic sources (26). The roots of the plant had the highest aggregate of the studied metals compared to the shoots parts, which probably related to the availability of these elements in sediments. P. australis root can accumulate large quantities of metals due to parenchymal tissue with high intercellular spaces filled with air (24).

Conclusion

Finally, it can be noted that water and soil pollution with heavy metals is one of the environmental issues. Heavy metals and other inorganic pollutants are the most common pollutants in sewage, and their removal from water and contaminated soils requires very complex and difficult technologies. Research has shown that the plant is a highly environmentally friendly and low technology alternative to existing methods, hence one of the most suitable methods to deal with the accumulation of heavy metals in the environment.

Considering the continued discharges of poisonous pollutants into the Badavar River, there is a high potential for rapid and cumulative increase in the concentration of toxic heavy metals and their concentration reaching a critical level. Therefore, in order to prevent further contamination from heavy and toxic metals in the river, it is suggested to control fertilizer and pesticide application by farmers, to use biological pest control methods, establishing a suitable collecting system for rural-urban wastewater and preventing their entering into the Badavar River.

Footnotes

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