Journal of
Applied Research in Water and Wastewater
Journal homepage: www.arww.razi.ac.ir

Original paper

The effect of *Typha Latifolia L.* on heavy metals phytoremediation at the urban and industrial wastewater entrance to the Meighan wetland, Iran

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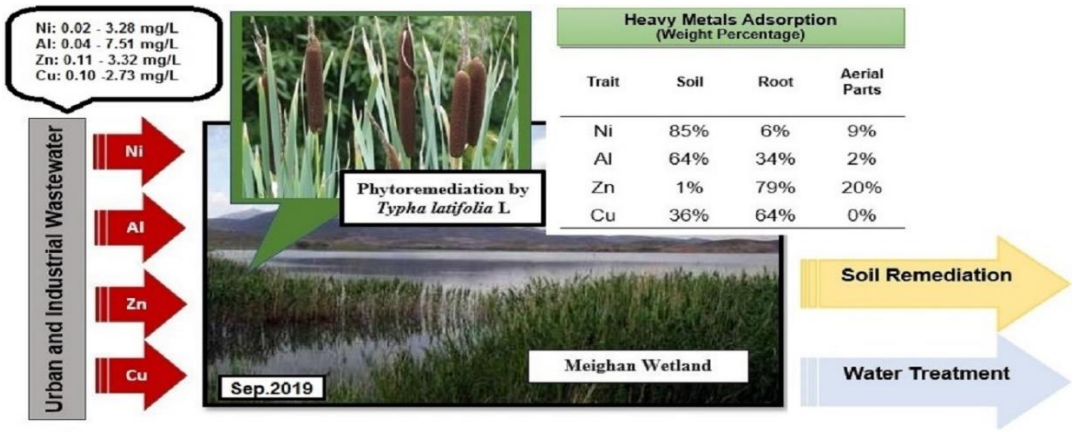
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 September 2020
Reviewed 10 November 2020
Received in revised form 13 December 2020
Accepted 14 December 2020

Keywords:
Phytoremediation
Heavy removal
Typha Latifolia L.
Meighan wetland
Water treatment

ABSTRACT

A mixture of urban and industrial wastewater is discharged into the Meighan wetland, Arak, Iran. The heavy metals containing wastewater leads to environmental hazards whereby phytoremediation can be used for removing pollutants from contaminated water. An essential native plant that is abundant in the Meighan wetland is *Typha Latifolia L.* The effect of *T. Latifolia L.* was investigated on the removal of heavy metals (aluminum, zinc, copper, and nickel) at the inlet of Arak wastewater treatment plant into Meighan wetland. The soil (0-30 cm in depth) and plants (aerial and root biomass) were sampled in September 2019. In the laboratory, heavy metal accumulation in samples was measured via atomic absorption method. The results of analysis of variance (ANOVA) revealed that the concentrations of all four metals were significantly different across the soil, root, and aerial biomass. The results of mean comparison by Duncan's test indicated that the highest concentration of aluminum was in the soil, while the lowest in aerial parts. Copper was maximum in the root, and the minimum was found in the aerial parts. Also, the concentration of zinc was the highest in the root, and the lowest in the soil. And, nickel was maximum in the soil while being minimum in the root. Data showed that the concentrations of aluminum in the soil, root, and aerial biomass of *T. Latifolia L.* were multiple times higher than permissible limit. The concentration of nickel in the soil was slightly higher than the acceptable limit, but the concentrations of other heavy metals in the soil, in aerial biomass, and in the root of *T. Latifolia L.* were within the acceptable range. The results indicated *T. Latifolia L.* is a suitable plant for the phytoremediation and water treatment in the Meighan wetland.

1. Introduction

Wetlands and rivers are used as the main site for discharging wastewater, and there is always possibility of contamination beyond the self-clearance capacity of the receiving waters. In aquatic ecosystems, aquatic plants are suitable biological indices for monitoring heavy metals, as they live in water and can determine the level of contamination (Firouzshahian et al. 2019). Among different

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environmental contaminants, heavy metals are really important in the ecology of various systems which eventually enter the human food ctypehain. The reasons of this importance are toxicity, high persistence, non-biodegradability, and potential for bioaccumulation (Ghasemi 2013). Indeed, the most important contaminating minerals are heavy metals including lead, mercury, zinc, cadmium, chromium, copper, nickel, aluminum, etc. The contamination of wetland ecosystems with heavy metals is major challenge that has recently attracted much attention. These contaminants enter the ecosystem through human activities, occurring via direct or indirect discharge of different contaminating sources (Norouzi Nodeh. 2009).

Phytoremediation technology is a sustainable, inexpensive, and environmentally friendly process, allowing for concurrent use of plants and microorganisms for cleaning contaminated bed. From among various phytoremediation methods, three methods including phytostabilization, phytoextraction, and phytovolatilization are used for the clearance of soils contaminated with heavy metals. High biomass production, well-developed root system, and a powerful defense system are the most important general criteria for plants in order to succeed in the phytoremediation process of soils contaminated with heavy metals (Salehi, 2019). Vahid Dastjerdi et al. (2010) investigated and compared to activation of heavy metals in water, soil, and plants of Gavkhuni international wetland. They found that the mean concentration of Cd, Cr, Cu, Mn, Ni, Pb, and Zn was higher than the standard set for drinking water as well as well as suitable water for agriculture at the points determined from Varzaneh bridge until Black Mountain of the wetland. Also, the concentrations obtained for the mentioned metals were higher than the standard limit in the soil and plant. The values related to electric conductivity and water chloride of the wetland also reflected heavy water contamination. If accumulation of contaminants persists, the entity of the wetland will be affected and destructed. Norouzi Nodeh (2009) examined the potential of Aegilops columnaris and Alyssum longistylum species in clearing Arak urban wastewater. They reported that irrigation with wastewater resulted in increased biomass and soil fertility because of the large mineral content. However, the water content percentage of the aerial and root parts of the plants diminished with the wastewater treatment in comparison to the control group. Elevation of sodium, potassium, chloride, and calcium in the aerial and root parts of these species in the wastewater treatment compared to the control suggests their absorption off the environment. Ghasemi (2013) researched accumulation of heavy metals including lead, cadmium, and zinc in the sediments as well as in *Typha Latifolia L.* in Anzali international wetland. They observed that the maximum extent of contamination occurred in the eastern part of the wetland. The largest bioconcentration factor (BCF) was found for zinc, while the maximum transmission factor (TF) was observed for cadmium. The results showed that *Typha Latifolia L.* is a suitable biological indicator for investigating the contamination with the mentioned metals across different parts of the wetland. Considering the wide distribution of this plant in the wetland and high rate of accumulation of heavy metals in Typha, this plant plays an important role in modifying and reducing contamination in the wetland, and is considered as a phyto-remediating species in Anzali wetland.

The results of different studies have indicated that among different plants, tree species especially fast-growing trees belonging to the Salicaceae family (Salix and cottonwood) are suitable options to be used in phytoremediation. These three species produce large amounts of biomass, are not food sources for quadrupeds compared to farming and rangeland species, have a developed root system to absorb heavy metals from the soil, and show great evaporation and perspiration rate, causing increased water flow in the tree and transfer of metals into the aerial parts (Salehi, 2019). Ghanadpour (2010) examined accumulation of heavy metals including lead, zinc, nickel, and cadmium in *Typha Latifolia L.* plant as well as the sediments of Arvand and Bahmanshir rivers. They observed that the mean concentration of metals in the sediments of region was lower than the Canadian environmental standards as well as the American sediment quality standards. Further, from among the mentioned metals, the mean concentration of zinc and cadmium was higher in the rhizome compared to the leaf and sediments; thus, rhizome of *Typha Latifolia L.* can be used as an index of contamination with cadmium and zinc in the soil and sediment of the region. In this research, TF index was used to determine the bioaccumulation rate of metals in Typha, where the following results were obtained: TF-Ni>Cd>Zn>Pb. Ebadat. (2005) investigated phytoremediation of heavy metals including lead, zinc, copper, and cadmium by Phragmites australis, Typha angustifolia, and Potamogeton crispus in Miankaleh international wetland. The results showed that the maximum accumulation of these metals was observed in the root of Phragmites australis and Typha angustifolia, while accumulation of metals did not differ significantly between the aerial parts and root for Potamogeton crispus. Firouzshahian et al. (2019) explored the level of heavy metals including cadmium, nickel, and vanadium in Phragmites australis and *Typha Latifolia L.* aquatic plants in Hourolazim wetland. They indicated that the maximum cadmium level was obtained in the root of Phragmites australis (1.486), while the minimum Cd level was found in the stalk of Typha Latifolia L. (0.036). The largest and lowest Ni contents were observed in the root of *Typha Latifolia L.* (4.77) and stalk of Phragmites australis (0.843 mg/kg) respectively. The concentration of Cd, Ni, and Vd was higher in the root of these two plants, as compared to the stalk and leaves. Cd was higher in the root, stalk, and leaves of Phragmites australis compared to *Typha Latifolia L.*, but Ni and Vd levels were higher in the root, stalk, and leaves of *Typha Latifolia L.* compared to Phragmites australis. Sasmmas et al. (2008) investigated the role of Typha Latifolia L. plant in accumulation of heavy metals in Kehli River. They observed that the heavy metal levels were higher in the root than in the leaves. Further, Cd level was higher in the root than in the sediments, while it was lower in the leaf compared to the sediments. Thus, they concluded that Typha root can be used as an index for Cd contamination in soil. Juan et al. (2008) examined the role of *Typha Latifolia L.* plant in reducing hexavalent chromium from the wetland sediments. They indicated that the longitudinal microorganisms released from the organic part of the plant root resulted in elevated sulfide concentration in the sediments of clear water, thus reducing the hexavalent chromium. Wrigh and Otte (1999) investigated the effect of wetland plants on the bio-geo-chemical status of heavy metals around the root (rhizosphere). They showed that Typha causes reduced pH and elevated zinc of the solution close to the roots. Liu et al. (2007) tested the extent of absorption of Cd, Pb, and Zn by 19 wetland plant species irrigated with synthetic wastewater in a small-scale experimental design. They observed that the removal efficiency of the mentioned metals was around 90%. On average, all plants absorbed around 19.58 %, 22.5 %, and 23.75 % of Cd, Pb, and Zn, respectively. Khan et al. (2009) used a surface floating wetland system along with 11 plant species to remove Cd, Fe, Ni, Cr, Cu, and Pb from the wastewater of industries in Savabi, Pakistan. Eventually, the mean removal efficiency for Cu, Cr, Ni, Fe, Cd, and Pb was obtained as 48.3, 89, 40.9, 74.1, 91.9, and 50 %, respectively. Hejna et al. (2020) conducted a research entitled bioaccumulation of heavy metals from wastewater through a phytoremediation system consisting of *Typha Latifolia L.* and Thelypteris palustris performed as experimental plots in a botanical garden. They found that *T. Latifolia L.* and T. palustris plants could displace accumulation of Zn and Cu from the contaminated wastewater to the plant tissues; the extent of absorption of these metals in different parts of plants had a direct association with the time of exposure to the wastewater. They stated that usage of this phytoremediation system can be recommended as an environmental approach to neutralize the negative effects of heavy metals in wastewaters.

In Meighan desert wetland, natural plant coverage is covered with hydrophytic species including *Typha Latifolia L.* around the input wastewater channel entering the wetland. The aim of this study is to investigate the extent of accumulation of Al, Zn, Cu, and Ni in superficial soil (0-30 cm) of the habitat as well as in the aerial parts and root of *Typha Latifolia L.*

2. Materials and methods

This research was performed in the inlet of Arak wastewater treatment plant into Meighan wetland. The studied region is located in the national lands of the south of Meighan desert wetland, 8 km away from Northeast of Arak city (the northern beltway), 1.75 km away from the Northeast of Mobarakabad village and 1.5 km northeast of Arak wastewater treatment plant. The coordinates of the studied region include 49° 48' 55" eastern longitude and 34° 08' 30" northern latitude, 1655 m above sea level (Figs. 1 and 2). Climatically, the rainfall in the studied region is around 300 mm, and the mean annual temperature is about 14.7 °C. According to Ambreje method, this region has a semi-dry cold climate (the meteorology administration of Markazi province, 2017).

The species studied in this research is a Monocotyledon plant called *Typha Latifolia L.* belonging to the Typhacea family, naturally growing on the pathway of inlet wastewater transfer channel of the Arak wastewater treatment plant into Meyyghan desert wetland. This plant

Please cite this article as: M. Heidarzadeh, N. Abdi, J. Varvani Farahani, A. Ahmadi, H. Toranjzar, The effect of *Typha Latifolia L.* on heavy metals phytoremediation at the urban wastewater entrance to the Meighan wetland, Journal of Applied Research in Water and Wastewater, 7 (2), 2020, 167-171.

enjoys high frequency and coverage especially in the estuary of the wetland inlet channel (sampling site). *Typha Latifolia L.* is a perennial plant, which is aquatic or semi-aquatic with straight and reed-like stalks, 2 m high, covering a wide range due to reproduction through rhizome. This plant is native to temperate regional marshes, and grows in wide areas of the country including Hirkani, Iran, and Tourani regions (Karimi. 2009).

Fig. 1. The map of location of Meighan desert wetland in Markazi province and the site of conducting the research.

Number	1	2	3	4	5
Location	Sampling location	Mobarakabad village	Effluent channel to the wetland	Arak Wastewater Treatment Plant	Airport runway

Fig. 2. The map of location of sampling site in relation to the Arak wastewater treatment plant and Mobarakabad village

In September 2019, after visiting the Typha habitat (Fig. 3), three points were sampled randomly, whereby one soil sample was taken from each point from 0-30 cm depths with several types of the plant. Attempts were made to take the entire aerial part and root of the plant as much as possible using a spade. The samples were placed in plastic bags and transferred to laboratory.

Fig. 3. A view of *Typha Latifolia L.* in the studied region.

In the laboratory, after separating the aerial parts and root, cleansing the samples with distilled water, as well as drying the plant and soil samples in an oven at 75 °C, the samples of each replication were mixed together and then milled. Next, 1 g of each replicate was poured into crucibles inside an electric furnace, and exposed to 550 °C for 480 min. Once the samples were cooled, they were poured into flasks, to which some distilled water and 5 ml HCl 2 M were added. Next, the samples were placed on a heater at 70 °C for 1 h, whereby the volume of the solution in the flask was diluted to 50 ml. The levels of Al, Cu, Zn, and Ni were read using flame atomic absorption spectrometer (GBC 932). Also, the soil parameters including acidity (pH), electric conductivity (EC), total suspended solids (TDS), nitrogen (N), organic carbon (OC), organic matter (OM), clay, silt, and sand were determined according to the pedology laboratory instructions (Jafari Haghighi, 2003). Concerning the objective of the research, using analysis of variance (ANOVA) and mean comparison (via Duncan method), the concentration of the four metals of interest were examined and compared in the soil of habitat as well as in the aerial and root parts of *Typha Latifolia L.* Data preparation as well as graph plotting were done using Excel software, while the data were analyzed by SPSS.

3. Results and discussion

The mean levels of the measured soil attributes in the studied region are reported in Table 1.

Table 1. The mean values of the attributes measured in the soil of the studied region.

Attributes	Description of attributes	Mean
pH	Acidity	7.7
EC	Electric conductivity, ds/m	2.29
TDS	Total suspended solids, mg/kg	1190
SAR	Exchangeable sodium, %	8.6
N	Nitrogen, %	0.15
OC	Soil organic carbon, %	1.5
OM	Soil organic matter, %	2.59
Clay	Clay, %	35.6
Silt	Silt, %	37.6
Sand	Sand, %	26.8
Texture	Soil texture	Clay-loamy
Al	Aluminum, ppm	10890
Cu	Copper, ppm	2.9
Zn	Zinc, ppm	2.2
Ni	Nickel, ppm	57.2

The results of analysis of variance (Table 2) showed that the studied treatments had a significant difference with each other in terms of heavy metal content.

Table 2. The ANOVA of the heavy metal data in the habitat soil, as well as the root and aerial parts of *Typha Latifolia L.*

Source of changes	Degree of freedom	Sum of squared	F value
Al	2	82224060	175594**
Cu	2	20	2271**
Zn	2	15040	42820**
Ni	2	2739	13913**

** Means remarkable

Based on Duncan multiple range test, the mean concentration of heavy metals was compared across the treatments, with the results presented in Tables 3 and 4.

Table 3. Mean comparison of the various traits measured using Duncan test.

Traits	Soil		Root Typha		Aerial parts Typha	
Al, ppm	10890.00	a	5737.50	b	419.90	c
Cu, ppm	2.90	b	5.20	a	00	c
Zn, ppm	2.20	c	137.50	a	33.65	b
Ni, ppm	57.20	a	3.65	c	6.17	b

The mean of traits with the same letters in each row indicate no significant difference at 5 % level.

Based on comparison between the heavy metal levels examined and their maximum allowable limit in soil and plant (Davodpour. 2018) in Table 4, it was found that:

- The level of aluminum is beyond the standard limit in the soil of the habitat and in the body of Typha plant.
- Cu concentration is less than the maximum allowable limit, and has been trivial in the soil and plant samples. In other words, the effluent of Arak wastewater treatment plant is not contaminated with copper.
- Ni concentration is slightly beyond the standard limit in the soil of the habitat, but Ni accumulation in the body of the plant is less than the allowable limit. Nevertheless, over time Ni accumulation will increase in this habitat.
- Zn concentration is below the maximum allowable limit in the soil of habitat, but is beyond that limit in the root of Typha, while it has lied within the allowable range in the aerial parts.

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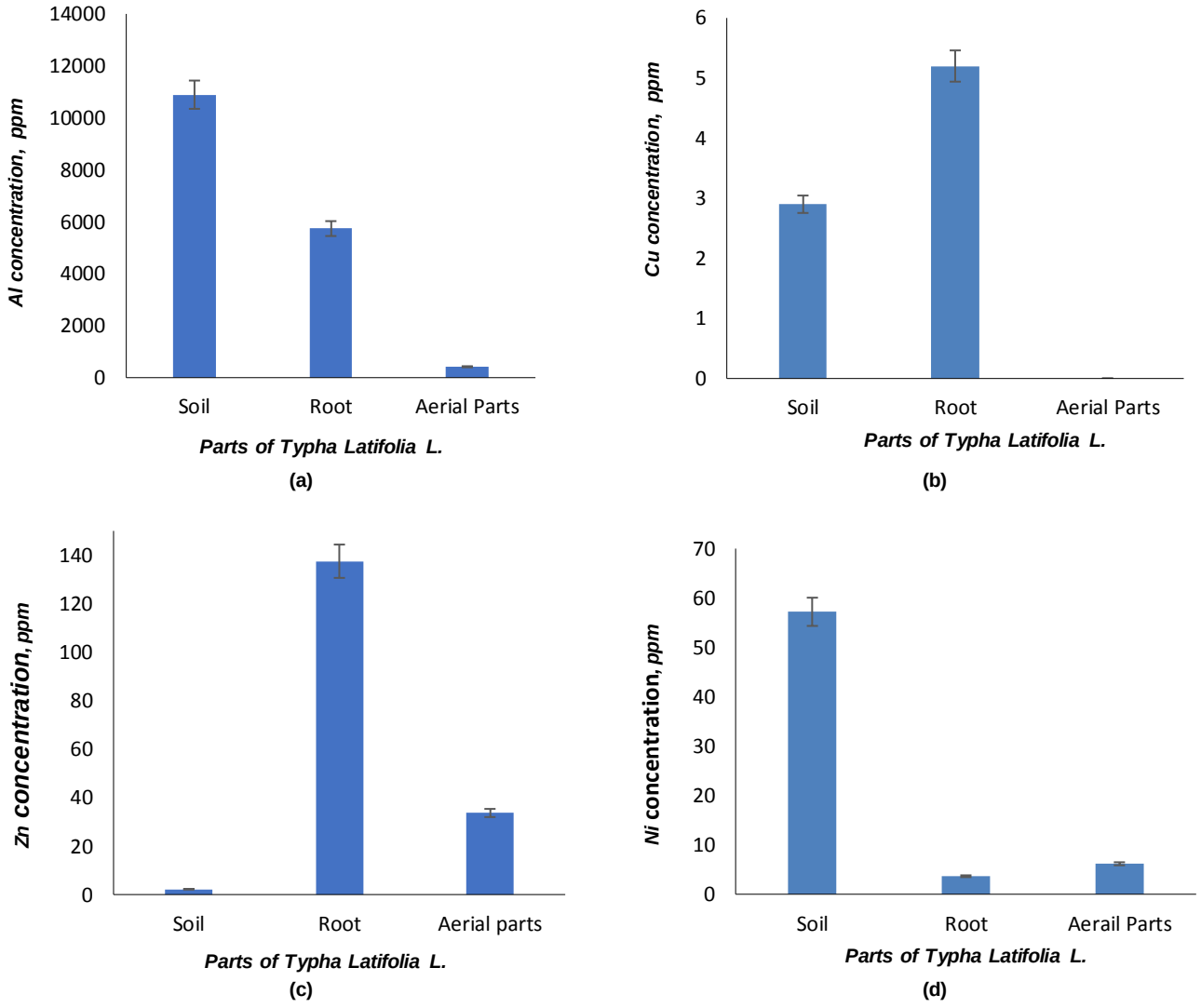

Fig. 4. Comparison of the mean heavy metal levels in the soil samples of the habitat as well as the root and aerial parts of *Typha Latifolia L.*, (a) Al; (b) Cu; (c) Zn; (d) Ni.

The results of Table 3 and diagram of Fig. 4 indicated that the maximum Al was found in soil (10890 ppm). Also, the root of this plant had a greater ability in Al accumulation compared to the aerial part, while the aerial parts with the numerical value of 419.9 ppm had the minimum Al accumulation, and ranked the last in Duncan grouping. The concentration of Aluminum toxic metal was beyond the allowable limit in the wetland habitat soil. This is due to the entrance of Arak wastewater treatment plant effluents containing urban wastewater as well as the wastewater produced by major industries (Arak aluminum production Company, machining, AzarAb Co., etc.) into the wetland, which is one of the major threats for the wetland (The administration for environmental protection of Markazi province, 2016; Hajihosseini et al. 2013). Regarding Cu, the plant root claimed the most considerable level, followed by the habitat's soil. The level of this metal was zero in the aerial parts. Considering the extent of Cu accumulation in the root (with the numerical value of 5.2 ppm) in comparison with its level in soil (2.9 ppm), it can be stated that *Typha Latifolia L.* enjoys a great potential in Cu phytoremediation. The success of this plant in Cu phytoremediation has also been confirmed by Khan et al. (2009). The results of comparing the mean Zn level in the plant and soil samples showed that the root containing 137.5 ppm was the best Zn accumulator, followed by aerial parts and soil. The Zn level in the root was 62.5 times as large in the soil (2.2 ppm), which concurred with the results of Ghasemi (2013). The finding in Anzali wetland indicated that the trend of Zn changes in the aerial parts of *Typha Latifolia L.* was as follows: root > stalk > leaf, where the maximum bio-concentration factor (BCF) in this plant belonged to Zn. Concerning accumulation of nickel, the results showed that the soil samples had the maximum levels, followed by aerial parts and root of the plant. Ni concentration was higher in the soil sample compared to the plant sample. Comparison of the Ni content in the soil (57.2 ppm) with the maximum allowable limit of Ni in soil (50 ppm) (Table 4) indicated that the level of this element is slightly above the standard limit. The high level of Ni in soil and its low level in the plant parts may be due to the fact that *Typha Latifolia L.* does not enjoy a high potential for absorbing this heavy metal in the Meighan wetland habitat. This is because the concentration of this metal was not low in the soil, and even slightly above the standard limit. Note that the success of *Typha Latifolia L.* in phytoremediation of Ni was lower than in the research by Khan et al. (2009).

Table 4. Results of comparison of mean heavy metal levels in the plant and soil samples of Meighan wetland with the maximum permissible limit (Adapted from Davodpour. 2018).

Trait	Soil standard	Plant standard	Habitat soil	Typha root	Typha aerial parts
Al, ppm	700	300	10890.00	5737.50	419.90
Cu, ppm	63	20	2.90	5.20	00
Zn, ppm	300	100	2.20	137.50	33.65
Ni, ppm	50	20	57.20	3.65	6.17

Based on the comparison of heavy metal levels examined with their maximum allowable limit in the soil and plant, it was found that the level of Al was beyond the allowable limit in the soil of habitat and in the body of the plant; Cu concentration was trivial in the soil and plant sample and below the allowable level; Ni concentration was slightly above the standard limit in the soil of habitat, but it accumulation was below the standard limit in the plant's body. The concentration of zinc was beyond the allowable limit only in the root of *Typha Latifolia L.*

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The results showed that there was partial absorption for all metals in the aerial part and root samples of the *Typha Latifolia L.* (except for copper, whose content in the root sample of Typha was zero). The accumulation of Al, Cu, and Zn was greater in its roots than in aerial parts, which concurs with the findings of Ebadati et al. (2005). *Typha Latifolia L.* is a wetland and aquaculture macrophyte with a high biomass. It is more adaptable to freshwater wetland conditions, and has developed naturally in the region. Alongside Phragmites australis plant, it is one of the major elements of the canebrake created at the inlet of Arak wastewater treatment plant entering the Meighan wetland. Also, due to its good phytoremediation ability, it can be protected and developed in Meighan wetland management plans regarding phytoremediation. This result is in line with the findings of Ghasemi (2013).

4. Conclusions

The results obtained from this research suggest that the soil of the studied habitat and the biomass of *Typha Latifolia L.* in this habitat play a significant role in the phytoremediation of aluminum, and can suitably control the entrance of this toxic metal into the Meighan desert lake. Also, the root of *Typha Latifolia L.* enjoys high accumulation potential for aluminum. Despite the toxicity caused by high levels of aluminum for plants, these plant species enjoy good phytoremediation potentials due to their high succulence.

Acknowledgments

The authors highly appreciate Department of Environment-Markazi province for cooperation and support to conduct this study.

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