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Full Length Article:

Investigation of Heavy Metals Accumulation in Plants Growing in Contaminated Soils (Case Study: Qazvin Province, Iran)

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Abstract. Environmental pollution with heavy metals is a global disaster that is related to human activities. This study was conducted to determine the extent of heavy metals accumulation by plant species in Lia industrial city (Qazvin, Iran) and to investigate the remediative capacity of native plant species grown in the contaminated soils. Soil and industrial wastewater sampling was done radially along transects with 300 m intervals from exit point of wastewater at three sites. In each sampling point, along 100 m transects within 5×2 m plots, the plant samples and soil samples were collected in depth of 0-20 cm and 20-40 cm from rhizosphere zone. Concentration of copper, zinc and chromium in root and shoot of 11 plant species, soil and wastewater were analyzed in three sits for mentioned metals. Bio Concentration factors and translocation factor were determined to ensure phytoremediation availability. Results showed that the concentrations of metals in the soil and wastewater greatly exceeded the threshold limit values. The contents of metals in soils ranged in the order of Cr>Zn>Cu and in wastewater were in the order of Zn>Cr>Cu, respectively. Results showed that Scirpus maritimus L. and Phragmites australis (Cav.) Trin. ex Steudel presented the highest accumulation of Zn, Cu and Cr in their root tissues which were suitable for phytostabilization (with a high BCF couple with low TF). The lowest extractable Zn (7.24 and 3.29 mgkg⁻¹ for shoot and root respectively, BCF=0.07) and extractable Cu (2.56 and 2.80 mgkg⁻¹ for shoot and root respectively, BCF=0.14) were related to *Hordeum glaucum* L. Moreover, the relatively lowest values of Cr were measured for *Taraxacum officinale* L. Results indicated that the species, which had low metal bioaccumulation in their roots and high TF, could play important roles for removal of heavy metals through phytoextraction.

Key Words: Phytoremediation, Heavy metals, BCF, TF, Lia industrial city

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Introduction

Contamination of soils with toxic heavy metals is a major environmental problem that can affect both plant productivity and safety as food and feed crops (Alloway, 1990; Kabata-Pendias, 2001). In fact, heavy metals have a significant toxicity for humans, animals, microorganisms and plants (Hernandez-Ochoa et al., 2005; Bodar et al., 2006; Fotakis and Timbrell, 2006; Ok et al., 2011). Many methods, including removal, incineration removal followed by thermal desorption, have been used for the cleanup of contaminated soils (Joner and Leyval, 2001). In this way, phytoremediation, using plant species to restore the deteriorated soils, is a promising technology in cleanup of polluted sites due to less destructive, low cost and environmentally friendly nature (Wang et al., 2012) and it can be categorized into two different approaches: phytoextraction, metal accumulating species are planted on a contaminated soil and later harvested in order to remove metals from the soil (Salt et al., 1995) ;Yoon et al. 2006; Usman and Mohamed, 2009), and ii) phytostabilization, metaltolerant species are used to reduce the mobility of metals, thus, the metals are stabilized in the substrate (Salt et al., Abdel-Ghani et al., 1995: 2007; Antosiewicz et al., 2008).

Some metallophytes called are hyperaccumulators, as these plant species accumulate very high concentrations in their aerial tissues, besides normal levels found in most species (Baker and Brooks, 1989). There are also some plant species called excluders that can restrict the uptake and transport of elements between roots and shoots, maintaining low metal levels inside plant body over a wide range of external concentrations (Baker, 1981). Depending on the ability of plant species used as a phytoremediation to accumulate and tolerate heavy metals, plants are classified into three categories

hyperaccumulators, indicators and excluders (Ghosh and Singh, 2005).

Hyperaccumulators can accumulate very high metal concentrations in their aerial tissues, besides normal levels found in most species (Baker and Brooks, can 1989). Indicators uptake transport heavy metals to aerial tissues regularly, so that tissue concentrations are proportional to environmental concentrations (Greger, 1999; Ghosh and Singh, 2005). Excluders can restrict uptake and transport of elements between roots and shoots, maintaining low metal levels inside plant body over a wide range of external concentrations (Baker, 1981). The ability of selecting species of plants, which are either resistant to heavy metals, or can accumulate great amounts would certainly facilitate of them. reclamation of contaminated (Chehregani et al., 2009; Lasat, 2002). Since native plant species can survive better under toxic metal stress compared to invasive plant species, it is possible to identify hyperaccumulators among native species in contaminated areas (Yoon et al., 2006; Nouri et al., 2011). This study conducted to determine concentrations of Zn, Cu and Cr in species growing in contaminated soils of Lia industrial city and to identify the remediation ability of native plant species for phytoremediation purpose.

Materials and Methods Study area

The study area was located at Lia industrial city, Qazvin Province, Iran. This area was designed for agriculture and industrial purposes. The coldest and hottest months are February and July whose mean temperatures are, 7.2°C and 21.7°C, respectively, with an annual mean temperature of 13.9°C. The annual mean rainfall is 321.5 mm and the soil is classified as aridisols. Industrialization in this area has exposed the soil to various effluent inputs including heavy metals. This has resulted in contamination of the soils which need to be improved.

Sampling method

Sampling was carried out from Mav (2009) to February (2010). Sampling was done along transects with 300 m intervals in three locations due to the widespread of wastewater in the area. Samples were taken from plant, industrial wastewater and sediment. In each sampling point, based on the vegetative state and cover area, a total of 11 plant species were collected from three locations (along 100 m transects within 5×2m plots (according to dominant plant species, Bonanno et al., 2010) (Table 1) with their corresponding soils. The collected plant samples were washed with tap water to remove sediments and quickly transported in plastic bags to the laboratory for analysis.

Industrial wastewater and sediment samples were collected at each sampling point. Industrial wastewater samples were collected in 0.5L clean polyethylene bottles and kept at 3°C until analysis (Bonanno *et al.*, 2010). Sediment samples were collected using a stainless steel collector at about 0–20cm and 20–40cm depths of soils from each sampling point.

Metal content analysis

The washed plant samples were divided into roots and shoots to recognize the different bioaccumulation capability. As the second step, samples were oven dried at 70 °C to a constant weight for approximately 48h and ground into fine powder (Yang et al., 2009). Copper (Cu), Zinc (Zn) and Chromium (Cr) were analyzed after mineralization of 400 mg dry shoot material in a microwave oven with 5 ml of nitric acid (69% v/v), 5 ml deionized water and 2 ml H₂O₂ (30% v/v). The digest was made to 25ml final volume with deionized water, filtered (0.45 mm, Millipore) and then analyzed for Zn, Cu and Cr using ICP/OES (Du Laing et al., 2003). Prior to analysis, industrial wastewater samples were passed through Whatman filters. Dried sediment samples were passed through a 2mm diameter sieve. About 100 mg dry sediment was digested with HNO3 and HCl (3:1) in a microwave oven. (Du Laing *et al.*, 2003). After mineralization, the samples were diluted, filtered and analyzed. Metal concentrations (Zn, Cu, Cr) of sediment samples (Table 2) and wastewater (Table 3) were measured as described for the plant samples. All the analyses were performed in five replicates.

Phytoextraction efficiency

The Bio Concentration Factor (BCF) and Translocation Factor (TF) calculated to determine the heavy metals efficiency phytoextraction (Wilkins, 1978; Zayed et al., 1998; Mattina et al., 2003; Yoon et al., 2006). The BCF expresses the ability of a plant to accumulate metal from soils (Metal in root DW (Dry Weight)/Metal in soil DW (Dry Weight)) (Yoon et al., 2006) and TF is the capacity of a plant to transfer metal from its roots to shoots (Metal in shoot DW/Metal in root DW) (Santillan et al., 2010).

Statistical analysis

All data were checked for their normality and homogeneity of variance, and where necessary, data were log-transformed before statistical analysis. Statistical significance was computed by analysis of variance (ANOVA) at p<0.05. Regarding plants data, the statistical model was based on two groups (root and shoot), and aimed to show whether plant organs triggered a different accumulation pattern of a given trace element. Duncan test post hoc analysis was performed to define specific which mean pairs significantly different. The ANOVA for industrial wastewater and sediment considered three groups (Zn, Cu, Cr) in order to detect significant different levels of concentration within the same kind of difference of metals The concentration between soil sampling depths was analyzed by Student's t-test. statistical calculations performed using SPSS 16.0 software.

Table 1. Native plant species in surrounding area of Lia region

Family	Plant Species
Cyperaceae	Scirpus maritimus
Poaceae	Phragmites australis, Hordeum glaucum, Bromus tectorum, Poa bulbosa
Polygonaceae	Polygonum lapathifolium
Alismataceae	Alisma plantago-aquatica
Brassicaceae	Cardaria draba
Asteraceae	Lactuca orientalis, Taraxacum officinale
Euphorbiaceae	Euphorbia helioscopia

Results

Soil and wastewater Characteristic

Chemical analysis of soil including cation exchange capacity (Bower and Hatcher method; Bower and Hatcher, 1966), total N (Kjeldahl method; Black, 1965), organic carbon content (Walkley-Black method; Walkley and Black,1934), EC (solid: deionized water = 1:2 w/v; Rowell, 1993), pH (1:1 soil/ water ratio; Thomas, 1996) and CaCO₃ equivalent (Drouineau and Galet method; Loeppert and Suarez, 1996) are listed in Table 2.

Acidity (pH) and Electrical Conductivity (EC) showed that the soils are categorized in salin sodic soils and are poor in terms of organic matter (less than one percent). The texture of the soils was clay to clay loam and due to low organic materials, clay particles had a

special role in the soils. The metals content in soil of the studied area greatly exceeded the threshold limit value (2758.50-74.36 mgkg⁻¹ Zn, 436.76-85.38 mgkg⁻¹ Cu and 1549.6-215 mgkg⁻¹ Cr (Table 3). The concentration of metals decreased in industrial wastewater and was in order of Zn > Cu > Cr (Table 4) the level of Zn was higher than the levels of Cu and followed by Cr. Although analyzed metal contents in soils and wastewater were highly variable, no significant variation concentration levels were observed for the metals during the experimental period among locations (sites 1, 2 and 3). Metal concentrations in the 0-20 cm depth showed higher concentrations of metals than that for the top soil (Table 3).

Table 2. Chemical and physical characteristics of soil

Site	Texture	Depth	CEC	N	OC	EC	рН	CaCO ₃
		(cm)	(meq)	(%)	(%)	(dSm^{-1})		(%)
1	Clay loam	0-20	36.00	0.10	0.12	2.24	8.41	12.28
	Clay	20-40	45.04	0.06	0.15	3.46	8.30	14.04
2	Clay loam	0-20	26.08	0.15	0.13	2.37	8.37	13.95
	Clay	20-40	47.39	0.13	0.15	3.33	8.85	16.46
3	Clay	0-20	48.69	0.08	0.13	2.78	8.42	13.27
	Clay loam	20-40	29.34	0.06	0.16	3.05	8.89	15.33

Table 3. Mean concentrations of Zn, Cu and Cr (mg kg⁻¹) in soil sediments

Site	Depth (cm)	Cr (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)
1	0-20	$1549.60 \pm 9.50 $ a (a)	$436.76 \pm 4.50 \text{ a (c)}$	725.04 ±7.50 a (b)
	20-40	$369.36 \pm 7.50 \text{ b (a)}$	$209.16 \pm 6.20 \text{ b (b)}$	$375.70 \pm 6.00 \text{ b (a)}$
2	0-20	$674.44 \pm 7.50 \text{ a (a)}$	$269.68 \pm 60.0 \text{ a (b)}$	$265.20 \pm 6.00 \text{ a (b)}$
	20-40	$215.00 \pm 6.20 \text{ b (a)}$	$85.38 \pm 3.20 \text{ b (b)}$	$74.36 \pm 0.10 \text{ b (b)}$
3	0-20	$725.08 \pm 7.50 \text{ a (b)}$	$280.16 \pm 6.00 \text{ a (c)}$	2758.50 ± 9.20 a (a)
	20-40	$229.49 \pm 6.00 \text{ b (a)}$	$106.04 \pm 3.20 \text{ b (b)}$	$181.94 \pm 6.00 b (a)$

Note: Mean values are reported with SD (Standard Deviation)

Means with the same letter for soil sampling depth (column) are not significantly different (p<0.05)

Means in parenthesis with the same letter for element concentration (row) are not significantly different (p< 0.05)

Table 4. Mean concentrations of metals (mg L⁻¹) in industrial wastewater

Site	Cr (mg L ⁻¹)	Cu (mg L ⁻¹)	Zn (mg L ⁻¹)
1	$20.07 \pm 0.05 \text{ a (b)}$	$11.77 \pm 0.02 a (c)$	$31.61 \pm 0.02 \text{ a (a)}$
2	24.35 ± 0.05 a (b)	$14.99 \pm 0.01 a (c)$	34.35 ± 0.01 a (a)
3	21.83 ± 0.01 a (b)	13.53 ± 0.06 a (c)	40.94 ± 0.40 a (a)

Note: Mean values are reported with SD (Standard Deviation)

Means with the same letter for soil sampling site (column) are not significantly different (p< 0.05)

Means in parenthesis with the same letter for element concentration (row) are not significantly different (p< 0.05)

Metal concentration in plant tissues and BCF and TF coefficients

Eleven plant species were identified in the study area (Table 1) that belong to different plant families. The data showed that the metal contents in the plant tissues differed among species at the polluted sites indicating their different capacities for metal uptake (Tables 5, 6, 7). The species with the highest Zn, Cu and Cr concentration for shoots and roots were *S. maritimus* and *P. australis*, respectively and metal concentrations were above the phytotoxic range in both species

Zinc concentration in roots of the plants varied from 3.29–238.50 mg kg⁻¹ and the level of Zn in shoots was from 7.24–93.16 mg kg⁻¹ that the highest BCF of Zn was obtained for *S. maritimus* and *P. australis* with average values of 5.4 and 4.7 and the lowest values of TF were 0.41 and 0.39 respectively. The lowest extractable Zinc with average values of 7.24 mgkg⁻¹ and 3.29 mgkg⁻¹ was

obtained for shoot and root in *H. glaucum*, respectively.

With regard to copper concentrations, in shoots of the plants, Cu varied from 2.56-43.40 mg kg⁻¹ and in root tissues, whereas the level of Cu ranged from 2.80-73.06 mg kg⁻¹ in the root tissues. The highest BCF values of Cu with average values of 3.85 and 3.05 were obtained for S. maritimus and P. australis, respectively. P. australis with average values of 0.74 had the lowest TF coefficient. The lowest extractable Cu with average values of 2.56 and 2.80 mgkg⁻¹ were obtained for shoot and root in H. glaucum, respectively (BCF=0.14). The Chromium concentrations in shoot and root ranged from 2.90–8.50 mgkg⁻¹ and 3.44 to 296 mgkg⁻¹, respectively coupled with the highest BCF values of 19.70 and 4.26 for S. maritimus and P. australis, respectively. The lowest Bio Concentration Factor (BCF) of chromium with average values of 0.22 was obtained in T. officinale.

Table 5. Zinc concentration (mgkg⁻¹) and coefficient in plant samples

Species	Shoot	Root	TF	BCF
Phragmites australis	86.59	210.35	0.41 c	4.70 a
Scirpus maritimus	93.16	238.50	0.39 c	5.40 a
Polygonum lapathifoli um	51.80	31.21	1.66 b	0.70 b
Cardaria draba	14.45	41.16	2.80 b	0.93 b
Alisma plantago-aquatica	66.40	13.64	4.80a	0.31c
Hordeum glaucum	7.24	3.29	2.20 b	0.07e
Bromus tectorum	52.60	42.07	1.24 b	0.95 b
Lactuca orientalis	51.20	41.09	1.24 b	0.73 b
Poa bulbosa	34.60	19.86	1.74 b	0.45 bc
Taraxacum officinale	59.30	41.70	1.42 b	0.82 b
Euphorbia spp	66.24	45.78	1.44 b	0.63 b
		22 (2.5)		

Means with the same letter in each column are not significantly different (p< 0.05)

Table 6. Copper concentrations (mgkg⁻¹) and coefficient in plant samples

Species	Shoot	Root	TF	BCF
Phragmites australis	43.40	58.00	0.74 c	3.05 a
Scirpus maritimus	32.55	73.06	0.44 c	3.84 a
Polygonum lapathifolium	6.40	4.47	1.43 b	0.22 c
Cardaria draba	26.40	9.30	2.83 b	0.48 c
Alisma plantago-aquatica	16.71	3.40	4.90 a	0.20 c
Hordeum glaucum	2.56	2.80	0.90 cb	0.14 c
Bromus tectorum	34.40	17.50	1.96 b	0.87b
Lactuca orientalis	16.14	7.55	2.13 b	0.37 c
Poa bulbosa	3.30	4.70	0.70 b	0.23 c
Taraxacum officinale	25.80	22.60	1.14 b	0.86 b
Euphorbia spp	25.95	18.60	1.39 b	0.91b

Means with the same letter in each column are not significantly different (p < 0.05)

Table 7. Chromium concentrations (mgkg⁻¹) and coefficient in plant samples

Species	Shoot	Root	TF	BCF
Phragmites australis	50.40	63.90	0.78 c	4.26 a
Scirpus maritimus	85.00	296.0	0.28 d	19.70 b
Polygonum lapathifolium	21.03	8.50	2.47 b	0.56 c
Cardaria draba	12.39	10.03	1.23 b	0.66 c
Alisma plantago-aquatica	18.50	6.60	2.80 b	0.44 c
Hordeum glaucum	21.30	4.58	4.60 a	0.30 c
Bromus tectorum	11.70	8.27	1.41 b	0.55 c
Lactuca orientalis	2.90	5.80	0.50 c	0.38 c
Poa bulbosa	4.70	12.30	0.38 d	0.82 c
Taraxacum officinale	7.70	3.44	2.23 b	0.22 c
Euphorbia spp	12.50	9.81	1.27 b	0.65 c

Means with the same letter in each column are not significantly different (p<0.05)

Discussion and Conclusion

Contaminated soil with toxic heavy metals is a serious environmental problem, which may be solved with phytoaccumulation. In this study, analyzed metal contents in soils (100–400 mgkg⁻¹ Zn, Cu 60–125 mgkg⁻¹ and 75–100mgkg⁻¹ Cr, Alloway, 1990) and wastewater (Zn>10 mgLi⁻¹, Cr > 0.5 mgLi⁻¹ and Cu>1 mgLi⁻¹, U.S. EPA, 1993) were found to be high (Table 3).

ANOVA results of soil wastewater samples showed that there were no significant differences among samples at three sites (p>0.05); it might be due to the monotonous distribution of heavy metals at the profile of soil and relatively high availability in the sediments and wastewater. The results demonstrated that some plants could a wide range of tolerate concentrations in the soils. According to Alloway (1990), Zn, Cu and Cr at high threshold limits would be considered toxic to plants based on total fractions in

soil. The metals contents in the study area of Lia greatly exceeded these ranges (Tables 5, 6, 7); therefore, the 11 plant species grown in these contaminated sites exhibited high metals tolerance. The species with the highest heavy metals concentration for shoots and roots were *S. maritimus* and *P. australis*, respectively and metal concentrations were above the phytotoxic range (100-400 mgkg⁻¹ Zn, 20-100 mgkg⁻¹ Cu and 5-30 mgkg⁻¹ Cr) in both species (Kabata-Pendias, 2001).

Metal concentrations in plants vary with plant species (Alloway et al., 1990). BCFs were calculated to assess concentrations in roots to environmental loading. In this study, metals in S. maritimus and P. australis were accumulated in roots with concentrations greater than was found in adjacent sediments with BCF of >1. The dominant uptake pathway of metals from the sediment was via the rhizosphere system. It is generally known that most metals tend to accumulate in the roots rather

than in shoots (Fitzgerald et al., 2003), which suggests that the plants adopt either external or internal exclusion mechanisms to hinder translocation of metals to the aerial tissues (Hansel et al., 2001). Khan *et al.*, (2009) in their study for the purification of industrial wastewater by macrophyte reported the highest bioconcentrations factor of chromium (3.5) for the P. australis. These researchers introduced species with BCF of >1 suitable for the purification of contaminated soils. TFs were calculated to enable the assessment of transport of accumulated metals from root to shoot and the ability of phytoremediation has commonly been characterized by TF (Yoon et al., 2006; Usman and Mohamed 2009), in this study metals in shoots of S. maritimus and P. australis were lower than half that of root comparing with other plants. relatively low accumulation of heavy metals in shoot tissues was probably due to the need of plants to prevent toxicity to the photosynthetic apparatus as suggested by other authors (Stoltz and Greger, 2002; Bragato et al., 2006). The root of plant species are mainly responsible for heavy metal phytoextraction and plants species with TF<1 have the potential for phytostabilization (Yoon et al., 2006; Usman et al., 2012), because in this process the metal tolerant plant species heavy metals immobilize absorption and accumulation by roots, adsorption onto roots or precipitation within the rhizosphere (Wong, 2003). This process also decreases metal mobility and reduce the likelihood of metals entering into the food chain. Therefore, the use of metal tolerant native flora represents an inexpensive long-term solution (Ashraf et al., 2011).

Nouri *et al.* (2011) in their study showed plant species with TF<1 are useful for phytoremediation of contaminated soil with magnesium, iron, copper and zinc. *P. australis* and *S. maritimus* among the 11 plant species

investigated showed BCF values >1 and TF values <1, indicating strong potential for use in phytostabilization. P. australis and S. maritimus having the highest BCF and the lowest TF were the most promising plants species for Zn, Cu and Cr phytostabilization. Conversely, plants which have low metal bioaccumulation in their roots and high TF, might be useful for phytoextraction because it would likely increase metal bioaccumulation in its shoots. Overall, we found that P. australis and S. maritimus were the best for phytostabilization of Zn, Cu and Cr and 9 other plant species could be useful for phytoextraction of metals.

Results of this research work indicated that among the 11 sampled plant species, metal translocation into shoots appears to be very restricted in S. maritimus and P. australis so that these plant species could be used in order to stabilize the metals and reduce the metal dispersion through wind erosion and grazing animals. Further studies would be needed on possible investigating deeply the translocation of metals to tissues of plants and investigate the phytoremediation efficacy of identified plant species against various concentrations of Zn, Cu and Cr.

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ارزیابی تجمع فلزات سنگین در گیاهان رشد یافته در خاکهای آلوده (مطالعه موردی: قزوین، ایران)

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چکیده. آلودگی محیطزیست به فلزات سنگین نگرانی جدی است که مربوط به فعالیتها انسانی است. مطالعه حاضر به منظور تعیین میزان تجمع فلزات در گونههای گیاهی در منطقه صنعتی لیا (قزوین، ایران) و ارزیابی قابلیت پالایندگی گونههای بومی رشد یافته در خاکهای آلوده انجام گرفت. نمونهبرداری از خاک و پساب در سه سایت به صورت شعاعی به فواصل ۳۰۰ متر از نقطه خروجی پساب انجام شد. در هر نقطه در طول ترانسکهای ۱۰۰ متری در پلاتهای به ابعاد ۵ در ۲متر، نمونههای گیاه و نمونههای خاک از عمق ۲۰-۰ و ۴۰-۲۰ سانتیمتری از منطقه ریزوسفری ریشه برداشت شد. غلظت مس، روی و کروم در ریشه و اندام هوایی ۱۱ گونه **گیاهی، خاک و پساب** در سه سایت اندازه گیری شد. فاکتور تجمع و فاکتور انتقال جهت تعیین پتانسیل گیاه پالایی اندازه گیری شد. نتایج نشان داد که غلظت فلزات در خاک و پساب بیشتر از آستانه تعیین شده است. غلظت فلزات در نمونههای خاک بهترتیب Cr>Zn>Cu و در يساب به ترتيب Zn>Cr>Cu بود. . Zn>Cr>Cu وScirpus maritimus L بود. حداکثر مقدار روی، مس و کروم را در ریشه داشتند که مناسب برای گیاه تثبیتی (با حداکثر BCF و حداقل TF) بودند. حداقل مقدار روی (۷/۲۴ و ۳/۲۹ میلی گرم در کیلوگرم بهترتیب برای اندام هوایی و ریشه، BCF=۰/۰۷) و مس قابل استخراج (۲/۵۶ و ۲/۸۰ میلی گرم در کیلوگرم بهترتیب برای اندام هوایی و ریشه، ۱۴-۱۹۲۶ مربوط به ... Hordeum glaucum L. بود. همچنین، حداقل مقدار کروم در officinale L. تعیین شد. نتایج نشان داد که گیاهانی که حداقل فاکتور تجمع در ریشه و حداکثر دارند نقش مهمی در برداشت فلزات در فرآیند گیاه استخراجی دارند.

كلمات كليدى: گياه پالايى، فلزات سنگين، TF، BCF، منطقه صنعتى ليا

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