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Technical Note

Using Trifolium Alexanderium for Phytoremediation of some heavy metals in tailings dam in Anjir-Tange coal washing plant, Mazandaran, Iran

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Abstract

Phytoremediation is a technology that uses plants for the remediation of the contaminated soils, sediments, tailings, and groundwaters. In this work, the ability of *TrifoliumAlexanderium* for the phytoremediation of the tailings soil in the Anjir-Tange coal washing plant was investigated. For this purpose, *Trifolium* sp. was cultivated in three soils consisting of the tailings dam, an agricultural soil, and a mixed soil. The concentrations of Fe, Cr, Cd, and P, and the factorsTF (translocation factor), BCF (bio-concentration factor), and BAF (bio-accumulation factor) were measured in the soils and plants after the harvest of *Trifolium* sp. The results obtained showed that BCFs in the agricultural soil, tailings dam, and mixed soil were 10.4, 12.24, and 7.23, respectively. These results also showed that *TrifoliumAlexanderium* was able to accumulate Cd in the root tissues and stabilize it, and thus it can be regarded as an appropriate species for the stabilization of the Cd ions in the contaminants and soils**.** The results obtained suggest that this plant can be a good candidate for use in the revegetation and phytostabilization of the Cd-contaminated lands in the region.

Keywords: *Phytoremediation, Tailings, Coal Washing Plant, Trifolium Alexanderium.*

1. Introduction

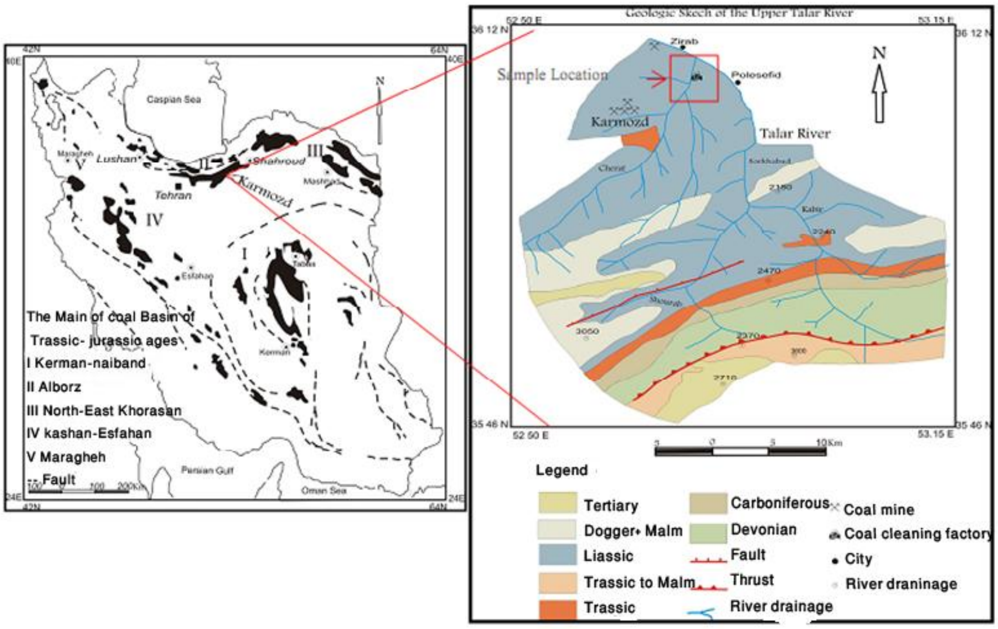
One of the main permanent environmental problems raising critical concerns to human health and ecosystems is a soil contaminated with heavy metals [1] due to their carcinogenic and mutagenic effects [2]. In contrast with organic pollutants, metals do not degrade, and are usually not mobile in a calcareous soil. The residence time for the heavy metals present in a soil can be of thousands of years [3]. Thus it is very important to remove heavy metals from the lands near the mining areas [4]. The phytoextraction of metals from soils has been the major scientific and technological progress in the past years [5-7]. Phytoremediation is defined as the use of plant-based processes to remove, decrease or render harmless environmental pollutants from soils [8]. This method of soil remediation has many advantages compared with other remediation procedures including low-cost, in situ development, and minimum environmental impact [9-12].

In coal-mining wastes, the elements Cd, Cr, Cu, Pb, Ni, and Zn are often found in large amounts, and are potentially toxic to plants. The amount of any metal taken up by plants from metal-contaminated soils depends on the soil properties, plant type, growth stage, and climatic factors [13].

The Anjir-tangeh coal washing plant is one of the largest and oldest coal-processing centers situated in the central Alborz coal basin, Mazandaran province, Iran [14]. It lies between the 53˚ 1΄ 20″ E and 53˚ 1΄ 0″ E longitudes, and the 36˚ 8΄ 40″ N and 36˚ 7΄ 55″ N latitudes. The central Alborz coal mine is located on the Shemshak formation with the age of upper Triassic-middle Jurassic (lias) (Figure 1).

The Zirab coal washing plant is located on the bank of the Cherat stream in the Mazandaran province, in the north of Iran. The wastes are produced and dumped by a jig machine. The flotation processes leave a large quantity of liquid and solid wastes that accumulate in the waste dump and tailings dam. The excess amount of the waste is sent to a pool that has been constructed near the factory. At present, more than 1.5 million tons of wastes are piled up in the dumping areas and sedimentary basins near the coal washing plant (Figure 2).

In this work, the capability of a trifolium species in the growth and phytoremediation of metals in the tailings dam was studied. Previous studies have shown that the impact of the tailings dump on the Cd concentration in native plants is significant but plants can grow on these wastes without any difficulty [16]. The main objective of this study was to use one of the native plants in this region, *Trifoliumalexanderium*,for the phytoremediation of soils.



(a) **(b)**

Figure 1. a: Situation of Zirab coal washing plant located in north of Iran; b: geological map of Talar watershed [15].

Figure 2. Accumulation of a large amount of waste in Zirab coal washing plant.

2. Materials and method

2.1. Sampling and experimental method

A compound waste sample was collected randomly from 5 to 10 locations from the top layer (5-30 cm) of the spoil of the Zirab coal washing plant sites located in the Mazandaran Province in Iran. The mean annual value precipitation and temperature were 700 mm and 13 ˚C, respectively. Three types of soil were used for cultivation of Trifoliumalexanderium under the glasshouse conditions. These soils were the tailings dam, an agricultural soil, and a mixed soil (one waste of tailings dam:one agricultural soil). Each soil was planted by Trifoliumalexanderium in three replications. Irrigation was carried out daily to maintain a soil moisture similar to that for the Zirab coal washing plant site content near the field capacity. Due to the favorable conditions for the trifolium growth, all the optimizing systems for the glasshouse were switched-off.

The plants were harvested in 60 days. The soil samples were air-dried, and sieved through 2-mm diameter sieves for analysis. The harvested plants were removed carefully from the soils in the experimental pots, and then the shoot and root samples were washed separately using tap water, HCl (0.01 N), and distilled water, respectively, to remove all the soil and organic particles from the plant organs. Then the shoot and root samples were dried in an oven at 80 °C for 48 h. The dry weights were measured separately, and then the plant shoot and root samples were grounded and stored for further chemical analysis.

This experiment was considered as a completely randomized design in three replicates. Analysis of variance (ANOVA) was used to determine the significance of the effects of soil type on the plant growth index and plant capability for the remediation of the coal washing wastes.

2.2. Analytical method

The organic carbon (OC) contents of all samples were analyzed by the dichromate oxidation and titration with ferrous ammonium sulfate. The total phosphorus contents were extracted using $NaHCO_3$ (0.5 M) (pH 8.5), and determined spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid. The total nitrogen contents were determined by the Kjeldahl method. The P, Fe, Cr, and Cd contents were extracted by HNO_3-H_2O_2-HCl [17] extracted using DTPA (diethylenetriaminepentaacetic acid), and measured by ICP-OES [18]. The chlorophyll (a, b) and carotenoid contents of the leaves were measured by the Hiscoxand-Israelstam method [19].

3. Results and discussion

3.1. Soil analysis

To test the impact of the tailings dam on the total P and metal concentrations s in the soils, ANOVA was carried out (Table 1). The results obtained showed that the impact of coal washing waste was not significant on the total concentrations of P, Cr, and Cd in the soil, although it was significant ($P \leq 0.05$) on the total Fe concentration.

The highest Fe concentration was measured in the agricultural soil with 2.109 (%). It was significantly higher than those in the mixed soil and coal washing waste gathered from the tailings dump. Although the total Fe concentration was relatively higher than that in the mixed soil, this difference was not significant (Table 2).

ANOVA carried out on the elements available in the soils showed that the impact of waste application was not significant on the concentrations of Cr and Cd in the soils, although it was significant on the Fe ($P \leq 0.05$) and P ($P \leq 0.01$) availabilities in the soils (Table 3).

Table 1. Impact of application of coal washing waste on total concentrations of P, Fe, Cr, and Cd in agricultural soil.

	Mean square			
	P	**Fe**	**Cr**	**Cd**
Waste application	0.000099^{ns}	$^{*}0.123$	20.534^{ns}	0.0538^{ns}
Error	0.00123	0.014	15.44	0.028

* indicates a statistically significant effect in level of 5 percent, and ^{ns} indicates no statistically significant effect.

Table 2. Duncan's tests of means of total Fe in soils after Trifolium harvest.

Soil type	Meanconcentration of total iron (%)
Agricultural soil	2.109^{a}
Mixed soil	1.769^{b}
Coal washing waste	1.748^{b}

Table 3. Impact of application of coal washing waste on P, Fe, Cr, and Cd contents of agricultural soil.

	Mean square			
	P	Fe	Cr	Cd
Waste application	6.35^{**}	11.08^{*}	$1.9\times10^{-6\ ns}$	0.00047^{ns}
Error	0.354	1.06	7.36×10^{-7}	0.0000011

* and ** indicate statistically significant effects in levels of 5 and 1 percents, respectively, and ^{ns} indicates no statistically significant effect.

The phosphorous availability was higher in the agricultural soil compared with that in the mixed soil and coal washing waste. The means of the available P were 4.13 and 1.22 ppm in the agricultural soil and coal washing waste, respectively (Table 4). These soils had a considerably low available P for the plant growth, and the plants showed P-deficiency symptoms. This will be discussed later in this paper.

In contrast, the highest concentration of the available Fe was measured in the coal washing waste; it was 5.01 ppm. The availability of Fe in the agricultural soil was significantly low (1.2 ppm), and the addition of coal washing waste (1:1 w/w) improved it, although not significantly. The available Fe in the mixed soil was 2.5 ppm. Although the total Fe content of the agricultural soil was significantly high, its available form was significantly low compared with that in the coal washing waste. The addition of washing waste increased the available Fe in the agricultural soil (Table 4). The waste materials related to the washing processes contained an abundance of sulfide minerals, mainly pyrite. Exposure of pyrite to atmospheric oxygen and soil moisture may favor pyrite oxidation, and ultimately cause an increase in the soil and drainage water acidity and an improvement in the soil heavy metal availability [20-23]. Continuous oxidation of pyrite in the dump of coal washing waste may release many elements from the waste dump. They may be transported downstream as dissolved ions. Some other metals such as Fe and Al are rapidly removed from the aqueous system by precipitation as solid phases [24, 25]. DoulatiArdejani et al. (2011) have reported that the Fe concentration in the stream sediments decrease gradually downstream with rise in the pH value. There were small differences in the Fe and SO_4^{2-} concentrations and the pH values in the sediments at a distance of 1,000 m from the waste dump towards further downstream [26]. Here, the oxidation of sulfide minerals in waste may increase the Fe availability in the coal washing waste and the soil mixed with it.

ANOVA showed that the impact of waste application was significant on the OC and total nitrogen contents of the soils ($p \leq 0.01$) (Table 5).

The highest contents of OC (6.08%) and total nitrogen (0.418%) were measured in the coal washing waste. The lowest contents were measured in the agricultural soil; they were 0.45% and 0.076% in the agricultural soil, respectively (Table 6). The application of coal washing waste in the mixed soil improved its OC and total nitrogen contents. Thus this may be a useful soil treatment, especially in arid and semi-arid soils with low levels of organic matter. However, the lowest C/N ratio (5.92) was calculated in the agricultural soil. The organic matter available in the coal washing waste was mainly carbonated.

Table 4. Duncan's tests of means of available concentrations of P and Fe in soils after Trifolium harvest.

Soil type	P (ppm)	Fe (ppm)
Agricultural soil	4.13^{a}	1.2^{b}
Mixed soil	2.65^{b}	2.5^{b}
Coal washing waste	1.22^{c}	5.0^{a}

Table 5. Impact of application of coal washing waste on OC and total nitrogen contents of agricultural soil.

	Mean square	
	OC	**Total nitrogen**
Waste application	24.18^{**}	0.089^{**}
Error	0.2	0.0001

** indicates a statistically significant effect in level of 1 percent, and ^{ns} indicates no statistically significant effect.

Table 6. Duncan's tests of means of OC and total nitrogen contents of soils after *Trifolium* harvest.

Soil type	Organic carbon (%)	Total nitrogen (%)
Agricultural	0.45[c]	0.076[c]
Mixed soil	3.88[b]	0.199[b]
Coal washing waste	6.08[a]	0.418[a]

3.2. Plant growth indices

In this part of the work, to investigate the impact of the tailings dump on the growth of *Trifoliumalexanderium*, the plant pigments and the root and shoot dry weights were analyzed. Although plant growth was better in the agricultural soil, ANOVA showed that the impact of waste application was not significant on the root and shoot dry weights of *Trifoliumalexanderium* (Table 7). This result is not in accordance with the result obtained by Roy et al. (2010) [27]. According to a previous study, the concentrations of heavy metals in the tailings dump are lower than the critical range [28].

ANOVA showed that the coal washing waste application had no significant impact on the chlorophyll (a, and b) and carotenoid contents of the leaves in *Trifoliumalexanderium* (Table 8). This result was not in accordance with the findings of Roy et al. (2010) [27]. They have investigated the extent of accumulation of some heavy metals in the root and aerial plant parts and the total chlorophyll content of *Cajanascajan* exposed to mine spoil. They have found that the levels of heavy metals available seem to be phytotoxic to *C. cajan*, causing growth inhibition and reduction in the pigment and photo-pigment contents (chlorophylls a and b). The maximum reduction was about 42%. The toxic effect of heavy metals on photosynthesis has also been reported by Morita and Wang [29, 30].

Heavy metals affect the gradual inhibition of chlorophylls (a and b) and their synthesis after flowering due to their increased accumulation in the leaves of mine plants. This results in the loss of chlorophylls and decrease in the photosynthetic activity [31]. However, disturbance of plant metabolism by the presence of excess heavy metals appears to happen in multiple ways like respiration inhibition due to reduction in the chlorophyll contents and inhibition of some photosynthetic functions in leaves [32]. The reduction in the chlorophyll content may be due to the alteration of the chloroplast structure and thylakoid membrane composition under such growth conditions [33]. Our results were different to their results because, here, the concentrations of heavy metals in the tailings dump of the coal washing plant were not too high to cause a significant decrease in the pigment content in trifolium (Table 8).

Table 7. Impact of application of coal washing waste on root and shoot dry weights of plants.

	Mean square	
	Root	**Shoot**
Waste application	0.0012[ns]	0.027[ns]
Error	0.0044	0.0059

[ns] indicates no statistically significant effect.

Table 8. Impact of application of coal washing waste on carotenoid and chlorophylls a and b (Cl) concentrations in plants.

	Mean square			
	Carotenoid	**Cl (total)**	**Cl (b)**	**Cl (a)**
Waste application	0.042[ns]	0.431[ns]	0.069[ns]	0.154[ns]
Error	0.0117	0.268	0.02	0.15

[ns] indicates no statistically significant effect.

3.3. Heavy metal concentration and phytoremediation factors

In the mine waste, most of the plant species grow well but some of them show abnormal growth because of the nutrient deficiency and the presence of heavy metals [34, 35]. Reduction in the yield has been suggested to result from phytotoxicity in relation to the uptake of heavy metals [1]. In order to investigate the impact of coal tailings dump on plants, and to study the capability of trifolium in the phytoremedition of the soil present in the region, the concentrations of Cd, Cr, P, and Fe in the shoots and roots of the plants were measured. ANOVA showed that the impact of the application of coal washing waste in the soil was significant on the Cd ($P \leq 0.05$) and P

($P \leq 0.01$) contents of the shoots of *Trifolium* (Table 9).

The results obtained for the mean test of concentrations of P and Cd in the plant shoots were tabulated in Table 10. The lowest Cd concentration was obtained in the plants grown in the agricultural soil sample (0.641 ppm), and the highest level was measured in the plants grown in the coal washing waste (1.265 ppm). In contrast, the highest P concentration was measured in the plants cultured in the agricultural soil sample (0.144%), and the lowest P concentration was measured in the plants grown in the coal washing waste. The results obtained from this study showed that P available in the coal washing waste was not available for use in the plants, and that the plants were not able to uptake it, as the plants stricken to P-deficiency, increasing livid color in leaves (Figure 3).

Table 9. Impact of application of coal washing waste on concentrations of P, Fe, Cr, and Cd in plant shoots.

	Mean square			
	P	**Fe**	**Cr**	**Cd**
Waste application	0.0047^{**}	0.002^{ns}	0.538^{ns}	$^{*}0.307$
Error	0.0002	0.000	0.768	0.033

* and ** indicate statistically significant effects in levels of 5 and 1 percents, respectively, and ^{ns} indicates no statistically significant effect.

Table 10. Duncan's tests of means of P and Cd contents in shoots of *Trifolium*.

Soil type	P (%)	Cd (ppm)
Agricultural soil	0.144^{a}	0.641^{b}
Mixed soil	0.09^{b}	1.079^{a}
Coal washing waste	0.066^{b}	1.265^{a}

(a)

(b)

Figure 3. a: Difference between colors of trifolium leaves grown in agricultural soil; b: in tailings dump (b).

ANOVA showed that application of the coal washing waste had significant effects on the concentrations of Cr ($P \leq 0.01$) and P ($P \leq 0.05$) in the roots of *Trifolium* (Table 11).

The highest and the lowest P concentrations were measured in the root organs of the plants grown in the agricultural soil (0.179%) and in the coal washing waste (0.062%), respectively. The highest Cr concentration was measured in the plants grown in the agricultural soil (4.72 ppm) (Table 12).

In this work, to evaluate the capability of Trifolium for the phytoremediation of heavy metals, the bio-concentration factor (BCF, the ratio of metal concentration in plant roots to that in soil), translocation factor (TF, the ratio of metal concentration in shoots to that in plant roots), and bio-accumulation factor (BAF, the ratio of metal concentration in plant shoots to that in soil) were calculated. Yoon et al. (2006) have found that the plants with a high BCF and low TF have the potential for phytostabilization [36]. In addition to the metal concentrations suggested by Baker and Brooks (1989) to qualify a hyper-accumulator, it is meaningful in the screening of the potential metal hyper-accumulators if both BAF and TF are larger than 1 [37, 38].

ANOVA for TF showed that the impact of waste application was not significant on this factor for Cd, Cr, and P, although it was effective in TF for Fe ($P \leq 0.01$) (Table 13).

The lowest TF for Fe was measured in the agricultural soil (Table 14).

Table 11. Impact of application of coal washing waste on concentrations of P, Fe, Cr, and Cd in plant roots.

	Mean square			
	P	Fe	Cr	Cd
Waste application	0.0104*	0.016^{ns}	3.997**	0.062^{ns}
Error	0.0014	0.001	0.233	0.126

* and ** indicate statistically significant effects in levels of 5 and 1 percents, respectively, and ^{ns} indicates no statistically significant effect.

Table 12. Duncan's tests for means of P and Cr in roots of Trifolium.

Soil type	P (%)	Cr (ppm)
Agricultural soil	0.179^{a}	4.72^{a}
Mixed soil	0.104^{ab}	3.712^{b}
Coal washing waste	0.062^{b}	2.42^{c}

Table 13. Impact of application of coal washing waste on TF for P, Fe, Cr, and Cd in trifolium.

	Means square			
	P	Fe	Cr	Cd
Waste application	0.0346^{ns}	0.027**	0.122^{ns}	0.102^{ns}
Error	0.035	0.002	0.111	0.042

** indicates a statistically significant effect in level of 1 percent, and ^{ns} indicates no statistically significant effect.

Table 14. Duncan's tests for means of TF for P, Fe, Cr, and Cd in *Trifolium*.

	Means of TF			
Soil type	P	Fe	Cr	Cd
Agricultural soil	0.873	0.194^{b}	0.32	0.429
Mixed soil	0.867	0.306^{a}	0.62	0.726
Coal washing waste	1.056	0.386^{a}	0.705	0.76

BAF equals a heavy metal concentration above the ground part (mainly leaves or leaves plus shoots were appropriate) divided by the same metal content in the soil. BAF shows the ability of a plant to uptake elements from the soil. ANOVA showed that the impact of waste application was not significant on BAF for Cr and Cd in the soil, although it showed a significant difference between BAFs for P and Fe ($P \leq 0.01$) (Table 15).

Means of BAF for Fe, P, Cd, and Cr were tabulated in Table 16. The lowest BAF for Fe was measured in the plants grown in the agricultural soil (Table 16), which is due to a high Fe concentration in this soil (Table 2). The highest means of BAF for P was measured in the agricultural soil (Table 16), which is due to a high P concentration in the shoots of the plants grown in this soil (Table 10).

BCF equals a heavy metal concentration under the ground part divided by the same metal content in soil. This factor shows the ability to uptake the elements in the soil and accumulation in the root. ANOVA showed that the impact of application of the waste coal washing in the soil was significant on BCFs for P and Cr ($P \leq 0.05$) (Table 17).

The highest BCF for P was obtained for the plants grown in the agricultural soil. The highest and the lowest BCFs for Cr were obtained for the plants grown in the agricultural soil and tailings dump, respectively (Table 18).

The results obtained from this work showed that due to the low BAF, BCF, and TF for Cr, *Trifolium* was not suitable for the phytostabilization or phytoextraction of this metal from the soil, whereas *Trifolium* had a high BCF, and a low TF for Cd had the potential for phytostabilization of this metal in the soil.

Table 15. Impact of application of coal washing waste on BAF for P, Fe, Cr, and Cd.

	Mean square			
	P	Fe	Cr	Cd
Waste application	2.6301**	0.00014**	0.00068^{ns}	15.83^{ns}
Error	0.15	0.000	0.0015	13.564

** indicates a statistically significant effect in level of 1 percent, and ^{ns} indicates no statistically significant effect.

Table 16. Duncan's tests for means of BAF for P, Fe, Cr, and Cd in *Trifolium*.

	Means of BAF			
Soil type	**P**	**Fe**	**Cr**	**Cd**
Agricultural soil	2.95[a]	0.015[b]	0.071	4.28
Mixed soil	1.57[b]	0.025[a]	0.098	8.858
Coal washing waste	1.17[b]	0.028[a]	0.073	6.175

Table 17. Impact of application of coal washing waste on BAF for P, Fe, Cr, and Cd.

	Means square			
	P	**Fe**	**Cr**	**Cd**
Waste application	5.408*	0.000087[ns]	0.0134*	19.21[ns]
Error	7.8	0.00037	0.0017	10.303

* indicates a statistically significant effect in level of percent, and [ns] indicates no statistically significant effect.

Table 18. Duncan's tests for means of BCF for P, Fe, Cr, and Cd in *Trifolium*.

	Means of BCF			
Soil types	**P**	**Fe**	**Cr**	**Cd**
Agricultural soil	3.7[a]	0.082	0.277[a]	10.387
Mixed	1.8[b]	0.081	0.156[ab]	12.236
Coal washing waste	1.09[b]	0.073	0.093[b]	7.231

4. Conclusions

Based on this work, the level of heavy metals does not seem to be phytotoxic to *Trifoliumalexanderium*, and it does not cause growth inhibition and effect on the plant pigment content. The greenhouse experiment carried out showed that the bio-availability of P was considerably low in the tailings soil. Trifolium sp. showed a low TF for Cd, which is important in phytoremediation. The plants were able to accumulate the Cd ions in the roots, and the shoots had a lower Cd concentration. The results obtained may be of importance for the stabilization of Cd ions in the tailings dump. Trifolium sp. can decrease the mobility of metals in the soil by absorption, immobilization, and precipitation in the rhizosphere. When phytoextraction is not a feasible remediation procedure for metal contaminated soils, alternatives such as phytostabilization have to be considered. Trifolium may be a desired species for revegetating the coal washing waste because of its capability to grow on these poor and harsh wastes.

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چکیده:

گیاه بهسازی تکنیکی برای استفاده از گیاهان برای بهسازی خاک‌های آلوده، رسوبات، باطله‌ها و آب‌های زیرزمینی است. در این کار توانایی گیاه شبدر برسیم برای بهسازی باطله‌های کارخانه زغال شویی انجیر تنگه مورد بررسی قرار گرفته است. برای این منظور گیاه شبدر در سه نوع خاک شامل باطله کارخانه، خاک کشاورزی و مخلوط دو خاک کاشته شد. غلظت آهن، کروم، کادمیوم و فسفر و فاکتور ترابری (TF)، فاکتور گردآوری (BAF) و فاکتور تغلیظ زیستی (BCF) در خاک‌ها و گیاهان پس از برداشت اندازه‌گیری شدند. نتایج به‌دست‌آمده نشان می‌دهد که فاکتور تغلیظ زیستی در خاک کشاورزی، دمپ باطله و خاک مخلوط به ترتیب برابر ۱۰/۴، ۱۲/۲۴ و ۷/۲۳ است. این نتایج همچنین نشان می‌دهد که این گیاه می‌تواند کادمیوم را در بافت ریشه انباشته کند؛ بنابراین می‌تواند گونه گیاهی مناسبی برای تثبیت آلودگی کادمیوم در خاک باشد. نتایج به‌دست‌آمده نشان می‌دهد که این گیاه می‌تواند گزینه مناسبی برای بازسازی گیاهی و پایدارسازی زمین‌های آلوده به کادمیوم در منطقه باشد.

کلمات کلیدی: گیاه بهسازی، دمپ باطله، کارخانه زغال شویی، شبدر برسیم.