JME

Journal of Mining & Environment,
Vol.8, No.3, 2017, 419-431.
DOI: 10.22044/jme.2017.897

Phytoextraction-based process of metal absorption from soil in mining areas (tailing dams) by Medicago sativa L. (Alfalfa) (Case study: Sarcheshmeh porphyry copper mine, SE of Iran)

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Received 16 January 2017; received in revised form 1 March 2017; accepted 5 March 2017
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Abstract

The present work was planned to evaluate the phytoextraction of metal mine tailings, Sarcheshmeh copper mine, SE of Iran, by the endemic plant species Medicago sativa L. (Alfalfa). In this pot experiment, we investigated the effects of seven amendments on the growth of alfalfa and uptaking metals from the mine tailings and stream sediment of tailing dam surface. The mean metal concentrations in both the tailing and stream sediment increased in the order of Hg < Te < Ag < Re < Ge < In < Ga <Zr < Cu. The results of this work showed that the concentration of these elements in the whole alfalfa plant increased in the order of Hg < Ag < Ge < Re < Zr < Ga < In < Te < Cu. The bioconcentration factor (BCF) for the metals, except for Cu, was calculated to be higher than one. The translocation factor (TF) was calculated to be greater than one for Re in all treatments (ranging from 858 to 3294), while this factor was mostly lower than one for other metals and metalloids. Leaching the tailings and sediment samples with water to significantly remove water-soluble salts has given rise to an increase in the translocation of metals from roots to shoots in alfalfa. Also adding silica sand improved substrate drainage to transfer metals to rhizosphere. It was found that all of the alfalfa grew well and produced a suitable biomass, showing a high tolerance to heavy metals and metalloids. Our experimental results implied that alfalfa showed a high phytoextraction potential for soils contaminated by heavy metals, while displayed a high uptake of Re. The results of this work should be further developed in order to confirm the potential of this species on the phytomining programs.

Keywords: *Sarcheshmeh Copper Mine, Mine Tailings, Phytoextraction, Bioconcentration Factor (BCF), Translocation Factor (TF).*

1. Introduction

In the past few decades, mining activities and production of metals have increased many folds due to a continuous increase in the world population. The increase in consumption of metals has led to the production of large amounts of tailings [1]. The waste materials of mineral processing plants have become the main form of mining waste, which is of the order of billions of tons and distributed around the world [2]. These tailings frequently contain a various range of metals not recovered in the mineral processing due to their low concentration [1]. On the other hand, they are often subjected to secondary movement that is facilitated by air and water (erosion) [2]. The environmental problems due to toxic concentration of heavy metals and metalloids such as arsenic [3], lead [4], copper [5], and mercury [6] are the results of the transport of these materials in the tailings.

The conventional clean-up technologies such as electro-dialysis, leaching, stabilization, and land-filling have been used to minimize the production, emission, and dispersion of pollutants from mine sites [7, 8]. All the methods may be efficient in the recovery of metal contaminated sites [9]; nevertheless, they are generally expensive due to the cost of off-site residual disposal, and may lead to other environmental problems stemming from the chemical agents added in the processes [10]. To the contrary, a series of techniques through green plants to stabilize or remove pollutants from soils has been named green technology. The best properties of green technology is an inexpensive run time performance, suitable to vast areas, environmentally friendly, and no change in the soil matrix [11]. This technology uses plants to extract elements from polluted or mineralized soils, and accumulates them in the harvestable parts of plants such as roots and above-ground shoots in order to remove the pollutants/contaminants from the field, known as phytoextraction [12]. Phytoextraction may be considered in two main scopes: (a) phytoremediation, where metal contaminants are stabilized or recovered for safe disposal [13], and (b) phytomining, where worth metals such as gold (Au), platinum (Pt), and thallium (Tl) are extracted via cropping for commercial purposes [14]. The feasibility of exploiting metals from low-grade ores, overburdens, mill tailings or mineralized soil that is uneconomic by conventional mining methods is proposed by phytomining [15]. Moreover, this technique not only provides a potential route for extracting precious metals from these areas but also does additionally increase levels of soil carbon, nutrients, and biological activity, therewith increasing the success rate of further native planting strategies [16].

Mine tailings are generally the fine-grained solid material residuals after the metals and minerals have been extracted from mined ore and processed water. However, the success of phytoextraction or phytomining depends on whether that plant can grow in this substrate. The physico-chemical characteristics of mine tailings are often not favorable for plant growth. The absence of oxygen supply to the roots, poor physico-chemical characteristics of the tailings, and lack of significant plant nutrients result in trying to grow plants in mine tailings having problems. There has been remarkable research works indicating that tailings can be improved as long as they would be mixed with other substrates [17]. The application of soil organic amendments such as composts, sewage sludge, manure, and plant cover is the base of cost-effective and environmentally supportable methods to manage landscapes in activated mining areas [18, 19]. Also inorganic amendments such as sand, porous ceramics, expanded shale, and calcined clay to poorly drained surface tailing is definitely a factor in increasing soil aeration. In a research work carried out by Moreno and Anderson [20], pumice was added to improve drainage of the fine-textured mine tailings. Silica sand would be a good alternative to use in amendment substrates because it has been defined resistant to a change in the pH value. As a result, in this work, the use of silica sand is as modification in soil applications to improve mine tailing drainage and aeration and influence plant growth. Amendment was avoided in silica sand by mixed particle size sand with lots of fine sands that can give rise to decrease in the substrate porosity by occupying microporous air space [21].

In this context, various plant species have been reported for phytoextraction of valuable metals such as Brassica juncea, Chilopsis linearis (Au) [22, 23], Alyssum bertolonii, Berkheya coddii (Ni) [24, 25], Haumaniastrum katangense, Crepidorhopalon perennis (Co) [26], Phytolacca acinosa (Mn) [27], and Hirschfeldia incana (Tl) [28] that may be used for the phytomining purpose after examining their performance in the fields.

In this way, some researches have focused on the potential of Alfalfa (Medicago sativa L.) to tolerate and accumulate trace metals. According to the previous works, this plant can take up Ni, Pb, Cu, Co, Cd, and Re [29-32].

In Iran, there are several metal mines, and the process of metal mining has led to intense heavy metal pollution [33]. Sarcheshmeh copper mine is the biggest metal mine in Iran located in SE of the country. A field survey has revealed that alfalfa can be grown well and it is widely distributed in this mining area with the highest heavy metal concentration of the rhizosphere soil.

The overall objectives of this research work are as follow:

1) To determine the concentration of trace metals in alfalfa biomass growing on a tailing substrate;
2) To compare the metal concentration in the above-ground biomass to those in roots and in substrate;
3) To make the first evaluation of the alfalfa potential usefulness in phytomining and phytoextraction.

2. Materials and methods

2.1. Site description

Sarcheshmeh porphyry copper deposit, the most important open-pit copper mine in Iran, is located 160 km SW of Kerman, SE of Iran. The geographical position of Sarcheshmeh mine is 56° 51′ 54″ E and 29° 57′ 31″ N, and its total area is 21 km^2 (Figure 1) [34].

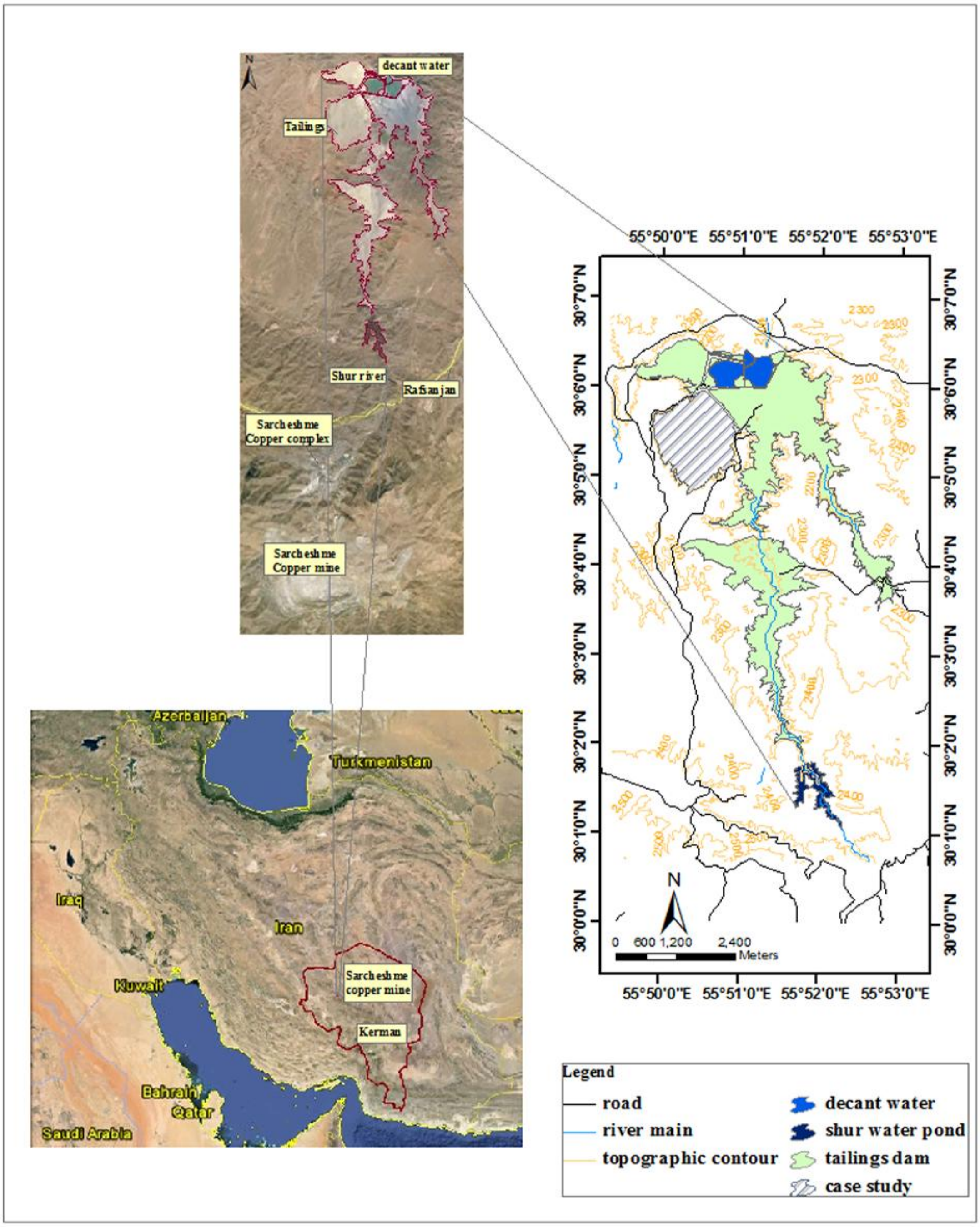

Figure 1. Satellite and topographic maps of Sarcheshmeh copper mine and tailing dam.

This ore body is located within the Urumieh-Dokhtar tectono-magmatic arc, the subduction of the Arabian platform formed by the NE-SW compression [35]. Consequently, the geology of the Sarcheshmeh mine is complex, with widely various rock properties. Mineralization in this ore body is related to a Late Tertiary granodiorite porphyry stock [36]. Chalcocite, chalcopyrite, covellite, bornite, and molybdenite are the main sulfide minerals of this deposit. The oxide zone of this deposit is mainly composed of cuprite, tenorite, malachite, and azurite [37]. This deposit has been assessed to be one of the greatest porphyry copper mines in the world, and is estimated to contain 1 billion tons of copper and about 30 times less for Mo [38].

Mining activities and mineral processing plants evermore produce many low-grade wastes and tailings that can give rise to environmental problems [39]. As a result, each year, almost 10,000 tons of reject waste, with an average grade of 0.6% Cu, and 1,215,000 tons of tailings, with an average grade of 0.1% Cu and 0.009% Mo, are produced in the Sarcheshmeh ore dressing plants [40]. More than 24 Mt of tailings has been produced and dumped by mineral processing operations in the Sarcheshmeh mine [34]. The tailing dam occupies an area of about 11 km^2, and its average depth was about 60 meters in 2015 (Figure 1).

The tailing site in the Sarcheshmeh copper mine is located about 25 km north of the mine in a mountainous area at 2300 m above the sea level. The annual average of the total precipitation is around 300-550 mm, and the average temperatures in this area in the summer and winter are about 35 C and −20 °C, respectively. The area is covered with snow about 3–4 months per year [41].

2.2. Collection and analysis of soil

The soil samples were collected from tailing sediments of the Sarcheshmeh copper mine. Two types of soils were sampled from the tailing, five samples entirely from basic materials of tailings and five other samples belonging to stream sediment affected by surrounding geological formation of tailing dam. Altogether, the samples were taken from depths of 10-50 cm. After sampling, the sediment was dried naturally and crushed into particles of diameters less than 0.3 cm. The soil samples were then analyzed for physico-chemical properties such as the pH value, electrical conductivity (EC), organic carbon (OC) concentration, and texture. The soil pH and EC values were measured in 1:2 soil:deionized water suspension triplicates using a glass electrode pH-meter and an EC-meter, respectively. The available N, P, K, and percent OC were analyzed by the standard methods [42, 43]. The soil texture was determined by the pipet method [44].

The soil digestion was carried out by transferring 1.0 g of dry soil samples to a beaker, where a mixture of 4 mL HNO_3 (63%) and 12 mL HCl (37%) was added. The solution was then heated at 80 °C for 2 h. The resulting solution was filtered and transferred to a 100-mL volumetric flask. The trace metal concentrations were determined by an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP/OES) using an AMETEK Spectro Arcos FHX ICP in Sharif University of Technology, Iran (http://nano.sharif.ir/en/).

On the other hand, the water-soluble salts of the substrates had to be decreased in advance since planting in high salinity conditions was expected to affect the plants adversely. Irrigation of mine tailings with water was done in order to realize how much water was in need to reduce the water-soluble salts of each substrate. This was carried out by stepwise addition of water to each potted substrate, followed by collection of the leachate and measurement of EC by an AZ-8301 multi-meter after each leaching [45]. The leaching experiment was carried out with irrigation of mine tailings until a satisfactory soil salinity was achieved; the mine tailings required 3 L of water to achieve an EC less than 5 mS cm^{-1}. The substrates were air-dried and prepared for the growth of plants after leaching.

The grown substrates consisted of a mixture of silica sand and mine tailings (m/m%), and salt leaching and no sign of salt leaching. Consequently, there were seven treatments setups in a fully randomized block design with three replications. The treatment details were as follow: 0% silica sand- 100% mine tailings (T1), 35% silica sand-65% mine tailings with water-soluble salt leaching (T2), 35% silica sand-65% mine tailings without water-soluble salt leaching (T3), 50% silica sand-50% mine tailings with water-soluble salt leaching (T4), 50% silica sand-50% mine tailings without water-soluble salt leaching (T5), stream sediment of tailings with water-soluble salt leaching (T6), and stream sediment of tailings without water-soluble salt leaching (T7).

2.3. Plant growth study

Alfalfa seeds were planted into the surface of 3-L PVC pots filled with different growth substrates, with 3 replicates per substrate type for alfalfa plant. Plants were allowed to grow for 4 weeks under natural light and ambient temperature in glasshouse conditions.

Plants were harvested by cutting the shoots at the soil surface, while the roots were carefully separated from the substrate. Shoots and roots were washed with tap water and distilled water to remove soil particles adhered to the plants. After washing, the plant samples were air-dried at room temperature (25 °C) for three days, and then the plant materials were separately oven-dried in paper bags at 50 °C for 24 h. After drying, each sample was weighed to determine the total dry biomass. The dried plant materials were grinded to less than 1 mm with a stainless steel mill for trace and heavy metal analysis. The plant samples were digested by weighing 1.0 g of a dry sample in a beaker. An acidic mixture of 3:1 conc. HCl:conc. HNO3 (aqua regia) was added, and the mixture was heated at 80 °C for 4 h. The solution was filtered and transferred to a 50-mL volumetric flask. Finally, they were analyzed for metalloid and heavy metal concentrations by the ICP-OES method (AMETEK Spectro Arcos FHX ICP) (http://nano.sharif.ir/en/).

2.4. Calculations and data analysis

One-way analysis of variance (ANOVA) was carried out followed by Duncan test to determine the significant differences among the different treatments. Differences at p-values < 0.05 were considered significant. The Spearman product moment correlation coefficients were used to express the associations of quantitative variables. All statistical analyses were performed using the SPSS (Statistic Program for Social Sciences) statistical program package, version 19 (http://www.ibm.com/us-en/).

The ability of plants to tolerate and accumulate heavy metals from soils can be estimated by the bioconcentration factor (BCF), defined as the ratio of the trace metal concentration in plant tissues to the trace metal concentration in the soil, and represents the relative concentration of the trace metals in the plants compared to that in the soil given in Equation 1 [46].

$$\mathrm{BCF} = \frac{\text{metal concentration in the plant; mgkg}^{-1}}{\text{metal concentration in the soil; mg kg}^{-1}} \quad (1)$$

The translocation factor (TF) is described as the ratio of the metal concentration in the shoots to that in the roots, and this was used to measure the ability of plants to translocate trace metals from roots to shoots. It was calculated using Equation 2 [47]:

$$\mathrm{TF} = \frac{\text{metal concentration in the shoots; mgkg}^{-1}}{\text{metal concentration in the roots; mg kg}^{-1}} \quad (2)$$

3. Result and disscution

3.1. Physico-chemical analysis of soil

The results of physico-chemical properties of the two types of soils tested are depicted in Table 1 prior to the treatments. The pH value of the tailings was estimated to be about 4.4±0.67, and the pH value for the stream sediment was recorded to be about 5.6±0.68. The stream sediment contained a lower EC value and a higher OC concentration rather than mine tailings. Also Table 1 provides information about the concentration of some trace elements of the different soils used in this work. The results obtained indicated that the concentrations of Ag and Cu (0.58±0.37 and 1217.76±500.95 mg kg^{-1}) in tailings were higher than their concentrations in stream sediment (0.12±0.093 and 710.19±478.49 mg kg^{-1}), whereas the Zr concentration (4.73±2.72 mg kg^{-1}) was lower in tailings related to stream sediment (7.28±3.24 mg kg^{-1}). Interestingly, the concentrations of Hg, In, Ge, Ga, and Re were equivalent or semi-equivalent in both substrates. Accordingly, the mean concentration of metals and metalloids in both the tailings and stream sediment increased in the order of Te < Hg < Ag < Re < Ge < In < Ga < Zr < Cu. In a previous work, Malakooti and Tonkaboni [34] have reported that the concentration of metals and metalloids in the Sarcheshmeh tailing increase in the order of Hg < In < Te < Re < Ag < Zr < Ga < Cu. Furthermore, in another study, Lak [48] has found that the metal and metalloid concentrations in this area follow the order of Te < In < Re < Ag < Ge < Ga < Zr < Cu.

Table 1. Physico-chemical properties of tailing and stream sediment.

Parameters	Tailing (n=5)	stream sediment (n=5)
pH	4.4±0.67	5.6±0.68
Electrical conductivity (mS cm^{-1})	15.26±2.08	11.9±2.35
Organic carbon (wt.%)	0.26±0.11	0.37±0.12
Available nitrogen (mg/kg)	22.08±10.08	33.55±10.71
Available phosphorous (mg/kg)	11.05±6.64	9.37±1.95
Available potassium (mg/kg)	200.4±78.48	248.7±120.28
Texture	Silty Loam	Silty Loam
Ca (me/L)	19.19±9.18	23.65±4.1
Mg (me/L)	50.33±11.63	21.77±13.63
Cl (me/L)	93.33±27.45	38.83±4.33
SO_4 (me/L)	79.73±29.2	49.69±12.67
HCO_3 (me/L)	0	1.24±0.29
Trace element (mg/kg)		
Ag	0.58±0.37	0.12±0.093
Cu	1217.76±500.95	710.19±478.49
Hg	0.047±0	0.047±0
Zr	4.73±2.72	7.28±3.24
In	1.53±0.93	1.85±1.01
Te	0.01±0	0.01±0
Ge	1.13±0.45	1.30±0.56
Ga	2.30±0.6	2.08±1.00
Re	1.05±0.47	0.95±0.4

Both substrates had poor physical textures, and were likely to be problematic substrates for plants. The fine texture and the nutrient availability of the soil are beneficially affected by silica sand in soil restoration. The plant grown on metalliferous soils is affected by many factors such as poor physical texture, low amount of water and nutrient holding capacity, deficiency of major nutrients (N, P, K), acidity and alkalinity, water supply, toxic metals, salinity, stability, and surface temperature [49]. The primary purpose of this work was to investigate the ability of the alfalfa plant species to tolerate and extract trace metals within 4 weeks.

3.2. Metal and metalloid accumulation characteristics

With the objective of evaluating the possibility of alfalfa metal phytoextraction, seven soil treatments were employed to assess the capacity of this plant to extract and accumulate trace metals. Plant tissues from alfalfa were analyzed 4 weeks after sowing. Concentrations of nine trace metals and metalloids including Ag, Cu, Hg, Zr, In, Te, Ge, Ga, and Re were studied separately in different plant tissues. Determining outliers were performed as a primary step of the statistical study (on metal concentration) in the plant tissues associated with amending them by substituting with maximum values after them [50]. As a result, a box plot of metal concentrations was drawn for the whole tissues of the plants in seven treatments (Figure 2).

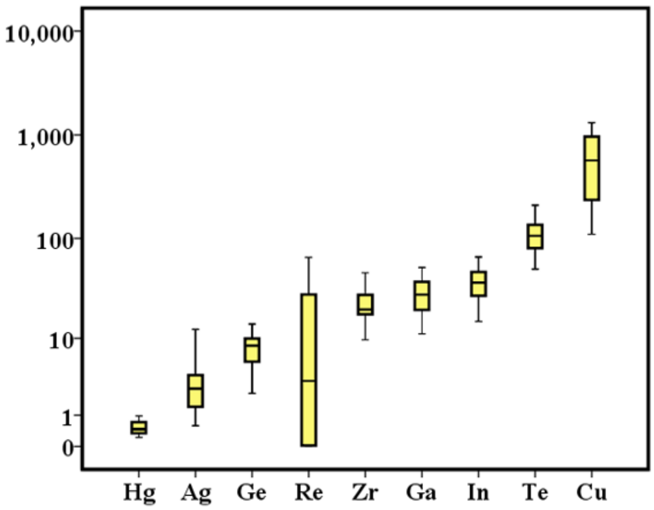

Figure 2. Box plot of metal and metalloid concentrations in whole alfalfa plants of treatments.

Moreover, this plot illustrates the data range for all metals and metalloids in the plant tissues. The data shows that the accumulation patterns for the metals and metalloids in the whole plants increase in the order of Hg < Ag < Ge < Re < Zr < Ga < In < Te < Cu. The accumulation results for metals and metalloids, except for Te and partly Zr, in the alfalfa tissues approximately follows their concentration in substrate media. The widest accumulation range was observed for the Re uptake.

Trace metal and metalloid concentrations in different tissues of alfalfa are shown in Figure 3. Each bar chart was recorded by the mean ±SD (standard division) of the metal concentrations in the root and shoot tissues at seven treatments, and all values were calculated from 3 replicates.

The highest Ag concentration in both the root and shoot tissues were 12.4±4.6 and 2.5±0.4 mg kg^{-1} in T3, respectively. The lowest values were recorded in T6 and T1 (2.5±0.4 and 1±0.5 mg kg^{-1}) in the root and shoot tissues, respectively. According to this result, Ag levels in the roots were in an order of magnitude higher than shoots.

In both the root and shoot tissues, T4 was allocated to the highest Cu concentration with 1222.2±91.5 and 490.8±91.3 mg kg^{-1}, and also T6 and T1 represented the lowest values of Cu among all the tissues.

Hg, Zr, In, Te, and Ga showed similar patterns in T6 treated soils. The highest concentrations of these metals were recorded in this treatment for both the root and shoot tissues. The concentrations of Hg, Zr, In, Te, and Ga in the root and shoot tissues were 1±0, 39.6±0, 63.5±2.3, 193.7±15.9, 50.3±1.6, and.8±.08, 30.9±3, 50.8±2.4, 140.1±9.6, 36.8±3.2 mg kg^{-1}, respectively. Also in the root tissues, T3 showed a concentration closed to T6 for these metals.

The T4 treatment presented a high Ge concentration in the root tissue (11.2±0.4 mg kg^{-1}) and a low metal concentration in the shoot tissue (4.1±1.8 mg kg^{-1}). Similar results were found in the case of the stream sediment with salt leaching application (T6) with the lowest and highest concentration of Ge in the root (8.3±0 mg kg^{-1}) and shoot (9.1±0.5 mg kg^{-1}).

The root and shoot tissues in alfalfa have accumulated higher concentrations of Ag, Cu, Hg, Zr, In, Te, Ge, and Ga with greater amounts of these metals in the substrate. Interestingly, the uptake potential of plant was found to increase for Ag, Cu, Hg, Zr, In, Te, Ga, and Ge in the root tissues compared with the shoot tissues. Most of these metals concentrated in the root tissue of alfalfa were not moved to the upper part of the plant.

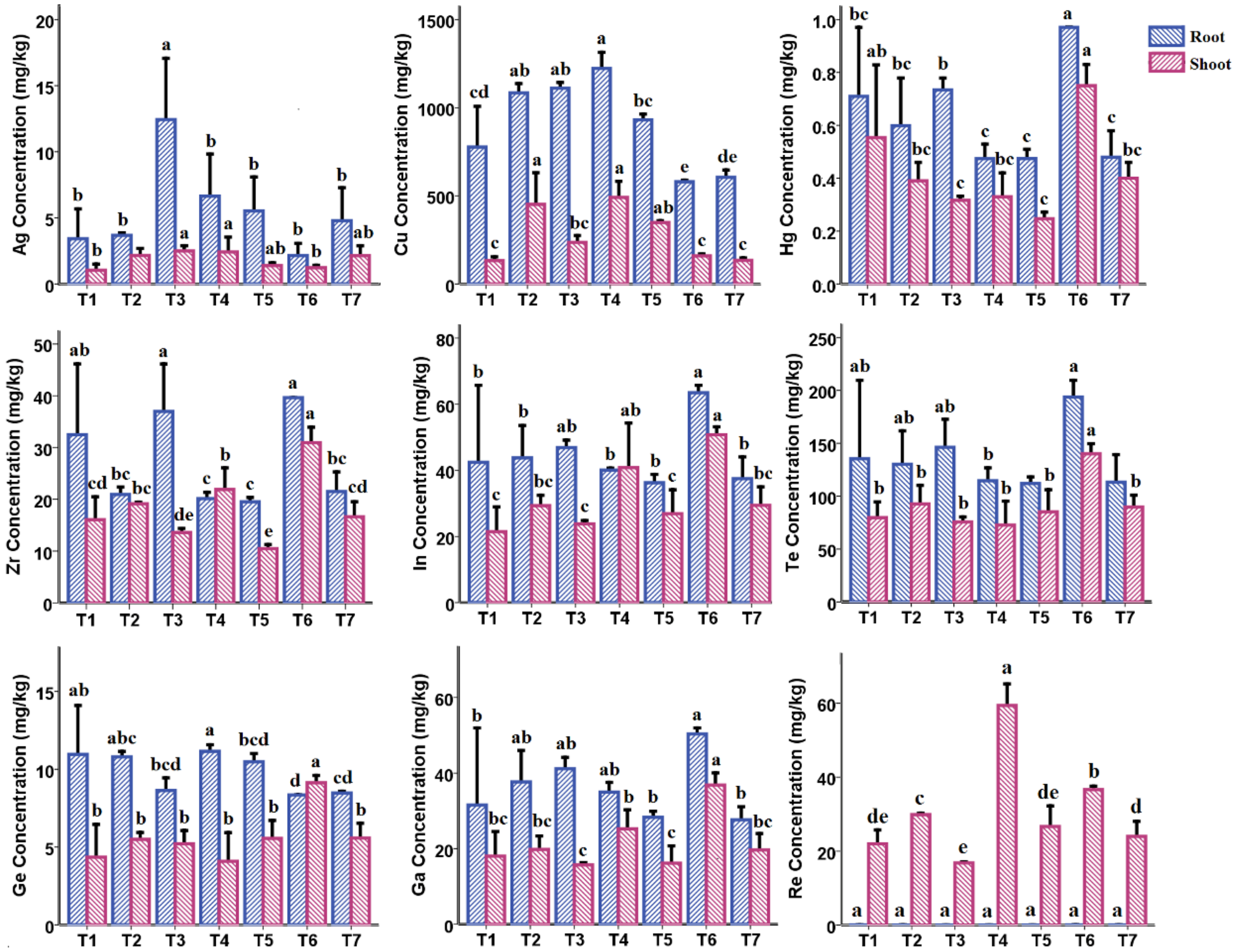

Figure 3. Metal and metalloid concentrations of alfalfa in different treatments. Values in each column represent the mean ±SD (Standard Deviation). Error bars show the standard deviations. The different letters in each treatment represent significant differences ($p < 0.05$) using different amendments as analyzed by Duncan test on a Statistical Analysis System.

The alfalfa shoot tissue exhibited a remarkable capacity to uptake Re, with the concentrations within the different treatments spanning from 16.8±0.3 to 59.4±5.8 mg kg^{-1} at T3 to T4. Interestingly, Re concentrations in root tissues of alfalfa were almost similar for all treatments but their relationship to the shoot concentrations differed by the most order of magnitude. It is notable that alfalfa was unable to uptake Re in its root, and its concentration did not increase over 0.03 mg kg^{-1}.

In a previous work, Ha and Sakakibara [51] have reported the concentration ranges of Ag, Cu, In, and Ga to be 0.71-35.3, 18.7-160, 0.1-5.66, and 0.34-8.7 mg kg^{-1}, respectively, in the root tissue of the plants growing in an area surrounding a lead-zinc mine site. In contrast, they have reported the concentration ranges of 0.22-13.5, 8.56-87.5, 0.01-4.28, and 0.13-6.75 mg kg^{-1} for Ag, Cu, In, and Ga, respectively, in the shoot tissue of these plants. Desjardins and Pitre [52] have reported the concentration ranges of Ag and Cu to be 4.8-34.68 and 60.5-277.00 mg kg^{-1} in the root tissue, respectively, and 4.6-0.8 mg kg^{-1} for Ag and unrecorded data for Cu in the shoot tissue of plants cultivated in brownfield soils contaminated with Ag and Cu. Wiche and Heilmeier [53] have investigated Ge accumulation in herbs and grasses. The Ge concentration in shoot was significantly higher in grasses than in herbs (358 and 3 mg kg^{-1}, respectively). Lominchar and Sierra [54] have reported the concentration ranges of 7.64–94.32 and 0.23–1.00 mg kg^{-1}, respectively, in the root and shoot tissues of Typha domingensis grown in an area affected by mercury mine, while a maximum Hg concentration has been recorded to be 394 mg kg^{-1} in sediment samples of the studied area. Bozhkov and Tzvetkova [55] have recorded a concentration of 593 mg kg^{-1} for Re in the alfalfa plant cultivated on soils from copper mine regions with concentration of 5 mg kg^{-1} for Re. In a research of two plant species, evaluated the feasibility of Re phytomining, Novo and Mahler [32] have reported the concentrations of 23396 and 161.9 mg kg^{-1} for Re in the shoot and root tissues, respectively, for Indian mustard growing on soils spiked with 80 mg kg^{-1} of Re. In a previous work, concentrations of Ag, Cu, In, Ga, and Ge in the root tissue were higher than the shoot tissue, while the highest Re accumulation occurred in the shoot tissues of plants.

In this work, in root tissues, the highest accumulation of most metals and metalloids occurred when the stream sediment with salt leaching application (T6) followed by tailings combined with silica sand in 2:1 ratio without leaching (T3), and the lowest concentration was recorded when no leach tailings combined with silica sand in a 1:1 ratio (T5) was applied. Also in shoot tissues, the stream sediment with salt leaching application (T6) was allocated to the highest concentration of most them followed by leach tailings combined with silica sand at 1:1 ratio (T4), while pure tailings without amendment (T1) and tailings combined with silica sand in a 2:1 ratio (T3) was observed lowest metal uptake. This result showed that the treatment of water-soluble salt leaching has high concentration of metals and metalloids in both the root and shoot tissues, while treatments with no removed water-solution salts have different manner in the high absorbent of metals and metalloid in root.

The Pearson correlation of trace metal accumulations are presented in Table 2 for the root and shoot species separately. In the root tissue, significant positive correlations were found between the Hg, Zr, In, Te, Ga, and Re contents in the alfalfa plant at all substrate treatments. However, low or non-significant correlations were found from the data for the other metals. Positive correlations were observed between In, Te, Ga, Ge, and Re content in the shoot tissue, whereas no correlation was found between Cu, Ag, and Hg contents in the shoot tissue.

Table 2. Pearson correlation matrix of metals and metalloids in root and shoot tissues of alfalfa plant.

		Ag	Cu	Hg	Zr	In	Te	Ge	Ga	Re
Root	Ag	1								
	Cu	.670**	1							
	Hg	0.054	-0.014	1						
	Zr	0.216	0.06	.938**	1					
	In	0.138	0.144	.938**	.909**	1				
	Te	0.107	0.077	.963**	.935**	.958**	1			
	Ge	0.269	.641**	-0.115	-0.095	0.008	0.025	1		
	Ga	0.135	0.266	.879**	.837**	.968**	.898**	0.085	1	
	Re	-0.089	-0.116	.671**	.656**	.694**	.726**	0.128	.574**	1
Shoot	Ag	1								
	Cu	0.37	1							
	Hg	-0.026	-0.317	1						
	Zr	0.141	0.049	.807**	1					
	In	-0.079	0.175	0.115	.500*	1				
	Te	-0.089	-0.091	.464*	0.4	.554**	1			
	Ge	-0.096	-0.129	0.27	0.241	.531*	.918**	1		
	Ga	-0.196	0.059	0.283	.581**	.926**	.649**	.571**	1	
	Re	0.063	.460*	0.233	.662**	.713**	0.347	0.203	.689**	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

3.3. Bioconcentration and translocation of metals in Alfalfa

The capacity of metal accumulation in relation to plant tissues is the bioconcentration factor (BCF) [56]. The bioaccumulation of these trace metals and metalloids in the root and shoot tissues of alfalfa is depicted in Figure 4. In this work, the bioconcentarion of Cu was 0.6-1 and 0.1-0.4 in the root and shoot tissues, respectively. The results obtained suggest that the alfalfa plant was unable to uptake Cu in underground tissue higher than its concentration in soil, and then translocated this metal to the aerial parts at all of the treatment soils.

A BCF higher than one was observed for Ag, Hg, Zr, In, Te, Ga, and Ge in both the root and shoot tissues of alfalfa grown in substrates. The results obtained suggest that the bioconcentration of these metals in the root tissue was mostly an order of magnitude higher than those of the shoot tissue. The application of stream sediment (T7) allocated the highest amount of BCF for Ag in the root and shoot tissues (53.7 and 24), whereas the main tailing soil (T1) demonstrated the lowest BCF for Ag in the root and shoot tissues with 5.9 and 1.8 values, respectively. On the other hand, the bioconcentration of Hg, In, Te and, Ga was significantly higher in both the root and shoot tissues of plant grown on T6 than other amended soils.

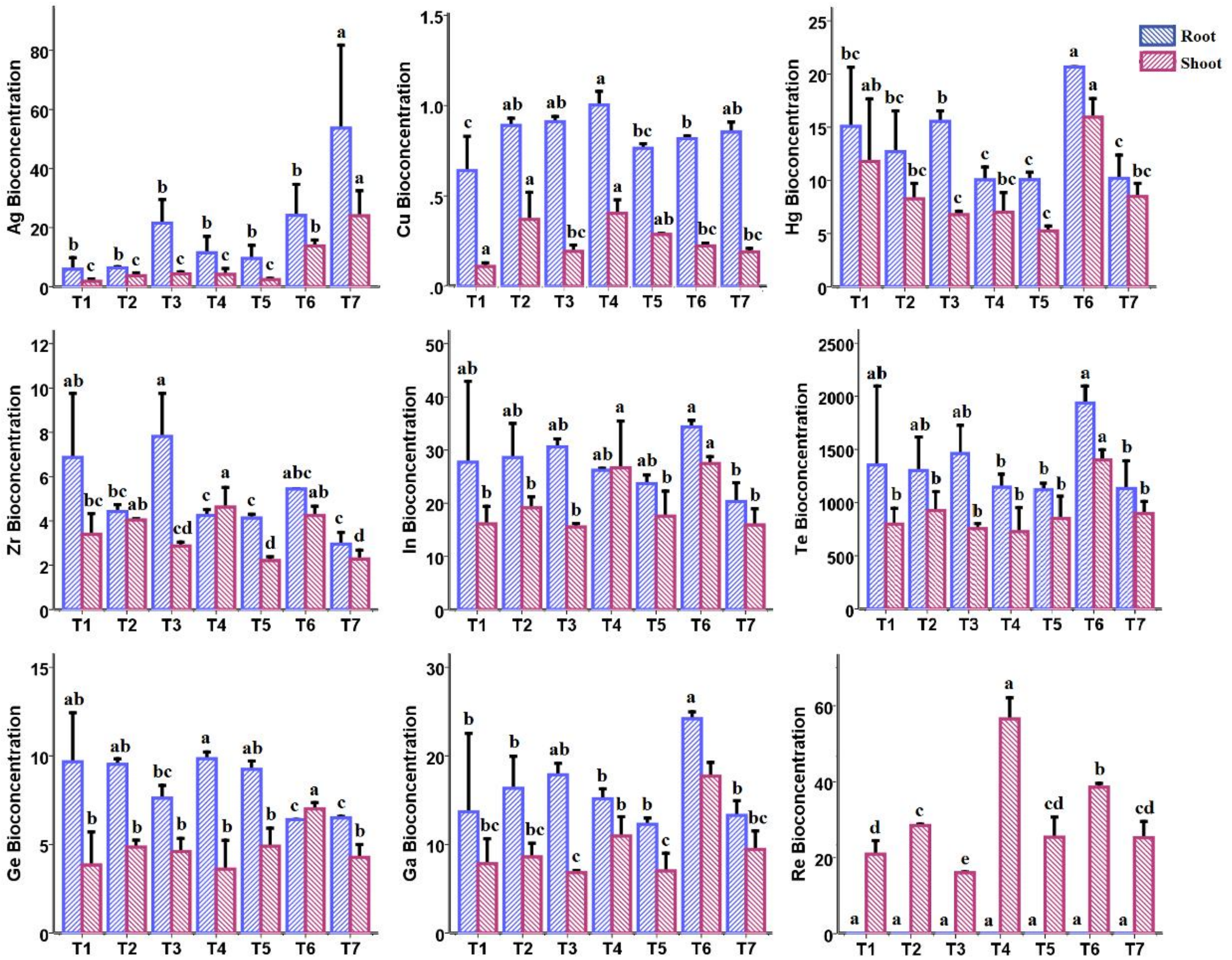

Figure 4. Metal and metalloid bioconcentrations of alfalfa in different treatments. Values in each column represent the mean ±SD (standard deviation). Error bars show standard deviations. Mean values of metals and metalloids at treatments in each column denoted by the same letters are not significantly different at $p < 0.05$, as analyzed by Duncan test.

The results obtained show an increase in BCF for Te in each treatment most order of magnitude rather to other metals BCF, therefore, BCF of Te fluctuates between 1145 and 1936 in root, and 726 and 1401 in shoot, denoting the plant fitness to extract Te from the Substrate. As regards, the highest BCF for Te was observed in T6 treatment, the stream sediment with water-soluble salt leaching application.

In the root tissue, BCF for Re was less than 1 in all treatments. Still, the values for the alfalfa shoot were 800- to 1886-fold higher, oscillating from 16 to 56.6 in T3 and T4. These values indicate the capacity of each treatment, especially T4 and T6, to translocate Re to their aerial parts. Generally, it suggests the aptitude of these species to endure Re during their growth.

It was observed that there was a marked different behavior of Ge on shoot and root tissues. Therefore, the highest concentration in root (11.2±0.4) and the lowest in shoot (4.1±1.8) belonged to T4 treated soil.

On the other hand, the capacity to translocate metals and metalloids to above-ground tissues was confirmed by calculating the translocation factors (TFs). A TF value higher than 1 indicates the translocation of a metal from the root to an above-ground tissue [47].

TFs for Cu and Ag from alfalfa root to shoot indicate that the lowest range between these metals and metalloids were 0.2-0.4 and 0.2-0.7, respectively, whereas not only in Hg, Zr, In, Te, Ge, and Ga, TF was observed to be close to one but also they were recorded to be equal one in T4, T4, T6, and T1 for Zr, In, Ge, and Ga respectively. Also this result suggests that most of the metals Ag, Cu, Hg, Zr, In, Te, Ge, and Ga were restricted to the root tissue of alfalfa, and therefore, they were not moved to the upper parts of the plant (Table 3).

Moreover, TF for Re ranged from 858 to 3294 at T3 and T4, respectively. Consequently, these values indicated the extraordinary ability of this species to absorb Re from the substrate; eventually, alfalfa translocates Re to its aerial parts [31, 57]. It is known that rhenium is freely accumulated in the green part of plants, while in the root tissues, Re is actually absent. It has been suggested that uptake of ReO_4^- (the most stable chemical form of Re) [58] by plants occur with uptake of nutrient cations such as K^+. Then ReO_4^- can move up through the xylem as a substitute for Cl^- and be translocated to the shoot. However, Re that is not an essential element, through these uptake mechanisms, could be highly accumulated in plants [59, 60].

According to the TF values in all treatments, alfalfa was most efficient in translocating Re followed by Zr, In, Ge, Ga, Te, and Hg. Low translocation of Ag and Cu indicates that the plant was unwilling to transfer these metals from its root to its shoot.

Based on these results, the highest TF for most elements belonged to the T6 treatment followed by the T4 and T2 treatments, while the lowest was the T3 treatment. Finally, the accumulation and translocation results revealed that the application of water-soluble salt leaching in substrate media is a contributing factor in the metals transferred from root to shoot. The silica sand amendment of tailings played a significant part in the stabilization of soil and root absorbency of metals from substrate.

Table 3. Translocation factor for metals and metalloids of alfalfa in different treatments. The values are expressed as the mean ±SD (standard deviation). Different letters indicate significant differences between treatments of the substrate harvest at $p < 0.05$ as analyzed by Duncan's multiple range test.

	Ag	Cu	Hg	Zr	In	Te	Ge	Ga	Re
T1	0.4 ± 0.1^{ab}	0.2 ± 0^{c}	0.8 ± 0.1^{ab}	0.5 ± 0.1^{c}	0.8 ± 0.7^{a}	0.8 ± 0.6^{a}	0.5 ± 0.3^{b}	1 ± 1.1^{a}	1068.7 ± 555.4^{bc}
T2	0.6 ± 0.1^{ab}	0.4 ± 0.1^{a}	0.7 ± 0.1^{b}	0.9 ± 0^{b}	0.7 ± 0.1^{a}	0.7 ± 0^{a}	0.5 ± 0^{b}	0.5 ± 0^{a}	1483 ± 380.7^{bc}
T3	0.2 ± 0.1^{b}	0.2 ± 0.1^{c}	0.4 ± 0^{c}	0.4 ± 0.1^{d}	0.5 ± 0^{a}	0.5 ± 0.1^{a}	0.6 ± 0^{b}	0.4 ± 0^{a}	858.1 ± 9.1^{c}
T4	0.4 ± 0^{ab}	0.4 ± 0^{a}	0.7 ± 0.1^{b}	1.1 ± 0.1^{a}	1 ± 0.4^{a}	0.6 ± 0.1^{a}	0.4 ± 0.2^{b}	0.7 ± 0.2^{a}	3294 ± 196.4^{a}
T5	0.3 ± 0.2^{ab}	0.4 ± 0^{ab}	0.5 ± 0^{c}	0.5 ± 0^{c}	0.8 ± 0.3^{a}	0.8 ± 0.2^{a}	0.5 ± 0.1^{b}	0.6 ± 0.2^{a}	1606 ± 414.5^{b}
T6	0.7 ± 0.4^{a}	0.3 ± 0^{bc}	0.8 ± 0.1^{ab}	0.8 ± 0.1^{b}	0.8 ± 0.1^{a}	0.7 ± 0^{a}	1.1 ± 0.1^{a}	0.7 ± 0.1^{a}	1244.6 ± 33.4^{bc}
T7	0.5 ± 0.1^{ab}	0.2 ± 0^{c}	0.8 ± 0.1^{a}	0.8 ± 0^{b}	0.8 ± 0^{a}	0.8 ± 0.1^{a}	0.7 ± 0.1^{b}	0.7 ± 0.1^{a}	1219 ± 554.9^{bc}

4. Conclusions

This study was conducted to probe the alfalfa plant growing on mine tailings to determine its potential for metal accumulation. The results of this work demonstrated that alfalfa could successfully extract metals and metalloids amended with silica sand and along with water-soluble salt leaching process on mine tailings. Overall, the most suitable silica sand-mine tailing combination was determined to be 50% silica sand – 50% mine tailings, whereas the leaching of water-soluble salts affected the translocation of metals from root to shoot. It is likely that adding silica sand improved substrate drainage to transfer metals and metalloids to rhizosphere. Stream sediment of tailing dam demonstrated higher accumulation and translocation of metals and metalloids than mine tailing sediment.

The experimental results illustrated that none of the alfalfa tissues showed BCF greater than one for Cu because the soil Cu concentration in this site was relatively high. On the other hand, Ag, Hg, Zr, In, Te, Ge, and Ga had high accumulations in the root and shoot tissues of alfalfa. Therefore, their bioaccumulation factor for the whole tissues of alfalfa was greater than one, while the translocation factor for them was mostly lower than one or close to it. According to other research works, only plant species with both BCF and TF greater than one had the potential to be used for phytoextraction. Based on the present work, the high TF related to water-soluble salt leaching treatments is responsible for applying similar amendments to improve translocation of Ag, Hg, Zr, In, Te, Ge, and Ga from root to shoot. These results are considered as indicative of the ability of alfalfa plant to take up metal ions from a soil matrix contaminated with heavy metals and metalloids. It can be concluded that the alfalfa plant can effectively remove Ag, Hg, Zr, In, Te, Ge, and Ga from the tailing dam. It also actively accumulates heavy metals and metalloids in the root tissue, and slightly translocates them from the roots to the shoots. Therefore, it is proposed that alfalfa could be a suitable candidate for phytoextraction, especially phytomining approach of these metals.

Therefore, further studies should be carried out, and supervised experiments (with different conditions of soil, amendment uses, and other influencing factors) are required to define the conditions in which this plant is applicable, and also it is important to evaluate the possibility of enhancing its metal uptake rates to improve TF.

Furthermore, the accumulation and translocation results disclosed that alfalfa had a tendency to extensively accumulate Re in its shoot. This high translocation could be exploited in the cultivation of the local ecotype in mine tailings to phytomining Re located to the soil. Moreover, the financial outcomes out of Re phytomining may be of particular interest for developing countries, not only because of its simplicity, low cost, and potential economic impact, but also due to the broad districts of tailings from commercial and artisanal mining found in these nations [2]. Finally, it may be concluded that alfalfa has the potential to recover metalloid and metal mine tailing sites and reclamation purposes.

Acknowledgments

This work was entirely supported by the National Iranian Copper Industries Company through a doctoral funding to the first author, S. Tabasi, who is a PhD candidate in mining exploration engineering at the Amirkabir University of Technology (Tehran Polytechnic). The authors are grateful to Dr. M. Jalali for his help in the final refinement of the paper.

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فرآیند استخراج گیاهی فلزات از خاک مناطق معدنی به وسیله گیاه یونجه (مطالعه موردی: سد رسوب‌گیر معدن پورفیری مس سرچشمه، جنوب شرق ایران)

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ارسال ۲۰۱۷/۱/۱۶، پذیرش ۲۰۱۷/۳/۵

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چکیده:

هدف این مطالعه ارزیابی استخراج گیاهی فلزات موجود در باطله‌های فرآوری معدن مس سرچشمه، جنوب شرقی ایران، با بهره‌گیری از گیاه یونجه یکی از گونه‌های گیاهی بومی این منطقه است. در آزمایش‌های گلدانی، تأثیر هفت تیمار متفاوت بر روی رشد گیاه یونجه و میزان جذب فلزات از باطله‌های فرآوری و رسوبات آبراهه‌ای سطح سد باطله مورد پژوهش قرار گرفت. متوسط غلظت فلزات در هر دو بستر رشد به ترتیب $Hg < Te < Ag < Re < Ge < In < Ga < Zr < Cu$ به دست آمد. همچنین غلظت این عناصر در گیاه یونجه به ترتیب $Hg < Ag < Ge < Re < Zr < Ga < In < Te < Cu$ اندازه‌گیری شد. فاکتور تجمع زیستی (BCF) تمام فلزات به جز مس بیشتر از یک محاسبه شد. در حالی که فاکتور انتقال (TF) تنها برای عنصر رنیوم در تمامی تیمارها بیشتر از یک به دست آمد. آبشویی نمونه‌های باطله و رسوبات آبراهه‌ای در جهت زدودن نمک‌های محلول در آب موجود در بافت خاک منجر به افزایش انتقال فلزات از ریشه به اندام هوایی در این گونه گیاهی به کارگیری شد. از طرف دیگر، افزودن ماسه‌سنگ به بهبود زهکشی بستر خاک و در نتیجه انتقال بیشتر فلزات به ریزوسفر منجر شد. در مجموع رشد شایسته گیاه یونجه همراه با تولید زیست توده مناسب نشان‌دهنده تحمل‌پذیری بالا این گیاه در مواجهه با غلظت بالای فلزات و شبه فلزات سنگین است. نتایج این پژوهش حاکی از پتانسیل بالای گیاه یونجه در استخراج گیاهی فلزات سنگین از خاک‌های آلوده و قدرت جذب بالای رنیوم است. بهره‌گیری از این گونه گیاهی در اجرای تکنولوژی گیاه معدنی (phytomining) نیازمند انجام مطالعات تکمیلی بیشتر است.

کلمات کلیدی: معدن مس سرچشمه، باطله فرآوری، استخراج گیاهی، فاکتور تجمع زیستی، فاکتور انتقال.