World Academy of Science, Engineering and Technology
International Journal of Geological and Environmental Engineering
Vol:5, No:5, 2011

Introduction of Hyperaccumulator Plants with Phytoremediation Potential of a Lead- Zinc Mine in Iran

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***Abstract*—**Contamination of heavy metals represents one of the most pressing threats to water and soil resources as well as human health. Phytoremediation can be potentially used to remediate metal-contaminated sites. A major step towards the development of phytoremediation of heavy metal impacted soils is the discovery of the heavy metal hyperaccumulation in plants. In this study, the several established criteria to define a hyperaccumulator plant were applied. The case study was represented by a mining area in Hamedan province in the central west part of Iran. Obtained results showed that the most of sampled species were able to grow on heavily metal-contaminated soils and also were able to accumulate extraordinarily high concentrations of some metals such as Zn, Mn, Cu, Pb and Fe. Using the most common criteria, Euphorbia macroclada and Centaurea virgata can be classified as hyperaccumulators of some measured heavy metals and, therefore, they have suitable potential for phytoremediation of contaminated soils.

***Keywords*—**Enrichment factor, Heavy metals, Hyperaccumulator, Phytoremediation, Translocation factor

I. Introduction

THE contamination of soils due to the presence of toxic metals can result in serious negative consequences, such as damage of ecosystems and of agricultural productivity, deterioration of food chain, contamination of water resources, economic damage and, finally, serious human and animal health problems [18]. Therefore, the growing amounts of metals required in the world economy in terms of amount and extent of mining metals ores, of amount and diversity of finished products and by-products, and of amount and array of their disposal and containment methods cause new and increasing problems; this also because metals can affect environmental and human health in diverse settings- from the sites of mining to residential environments [1]. Many approaches have been developed, assessed and performed to cope with the soil pollution.

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Current available soil clean-up technologies are often high-priced and energy consuming, therefore, concurring with the CO2 emissions and, most of the time, soil disturbing so that the soil itself can rarely be utilized after the treatment. For these reasons the importance of soft and low-cost technologies, such as those afforded in ecologically engineered systems, is rising [16]. In these conditions, but overall for long- term projects, phytoremediation could be the cheapest and simplest option among the available soil clean-up strategies [23]. USEPA (2000) defines phytoremediation as “the use of plants for containment, degradation or extraction of xenobiotics from water or soil substrates” [6]. Plants that accumulate very high concentrations of metals in any aboveground tissue in their natural habitat are called hyperaccumulators [4]. The several established criteria to define a hyperaccumulator plant were applied. The most common definition of hyperaccumulator plant meets the following requirement; the concentration of the metal in the shoot must be higher than: 1000 mg kg− 1 of Cu, Co, Cr, Ni and Pb, or 10000 mg kg− 1 of Fe, Mn and Zn [4]-[14]. Other authors included besides de first previous requirement, three other ones: ii) the plant aboveground metal concentrations must be 10- 500 times higher than the same plant species from non-polluted environments [27]; iii) the shoot to root concentrations ratio must be invariably higher than 1 [15]-[27]. This indicates an efficient ability to transport metals from roots to shoots and, most likely, the existence of tolerance mechanisms to cope with high concentrations of metals; iv) the shoot to soil concentration ratio must be higher than 1, meaning higher metal concentrations in the plant than in the soil, which emphasizes the degree of plant metal uptake [15]-[27]. Hyperaccumulators are found in 45 different families, with the highest occurrence among the Brassicaceae [19]. These plants are quite varied, from perennial shrubs and trees to small annual herbs. The overall objectives of this research were: (1) to determine the concentrations of Zn, Mn, Cu, Pb and Fe in plant biomass growing on a contaminated site; (2) to identify hyperaccumulator plants with the several established criteria. Results of this study should provide insight for using native plants to remediate metal-contaminated sites.

II. Material And Methods

A. Site Characterization

The plant and soil samples used in this study were collected from a known metal-contaminated site located in an urban area, called Ahangaran lead- zinc mine of Malayer city, southwest of Hamedan (Fig. 1). The site has been vacant, occupied approximately 1600000 m2, and is covered mainly by grasses. Human activities such as mining have contributed to metal concentrations in this site. Contamination of heavy metals was mainly concentrated in the top 20 cm at the site.

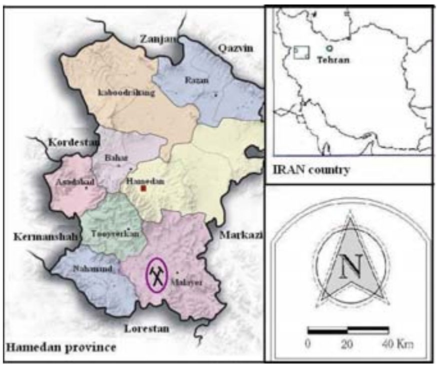

Fig. 1 Location of the study area

B. Soil Sampling and Analysis

Soils were sampled from the same sites and location points as the plants. The top 20 cm soil from between the plant roots was collected, air-dried for 3 weeks, and then sieved through a 2 mm mesh. Samples were then analyzed for total metals (Zn, Mn, Cu, Pb and Fe). Total metal contents were extracted by acid digestion. Metal contents were measured by Inductivity Couple Plasma (ICP- 710 Varian, Australia) according to reference [22]. Soil samples were also analyzed for pH, electrical conductivity (EC) and total phosphorus (P). The pH and EC values of soil samples were measured by a pH meter and an EC meter, according to references [25] and [20], respectively. Total P was measured using the method of Olsen [21] and soil texture, was determined according to reference [24].

C. Plant Sampling and Analysis

Twelve plant species were collected in the surrounding area of Ahangaran mine from May to June 2007. The studied species consisted of 11 genera and 8 families (Table I), of which 4 species belonged to Asteraceae, forming the most dominant component in studied site. Plant samples were thoroughly washed with running tap water and rinsed with deionized water to remove any soil particles attached to the plant surfaces, then oven dried (70ºC) to constant weight. The dried tissues were weighed and ground into powder for metal concentration analysis. Metal contents (Zn, Mn, Cu, Pb and Fe) of the plant samples were extracted by acid digestion followed by measurement of total concentrations of all elements of interest using Inductivity Couple Plasma (ICP-710 Varian, Australia) according to reference [26].

Table I
Species composition in the surrounding area of Ahangaran mine

Species No.	Scientific name	Family
1	Euphorbia macroclada Boiss.	Euphorbiaceae
2	Centaurea virgata Lam.	Asteraceae
3	Reseda lutea L.	Resedaceae
4	Echinophora platyloba DC.	Poaceae
5	Cichorium intybus L.	Asteraceae
6	Tamarix ramosissima Ledeb.	Tamaricaceae
7	Cirsium congestum Fisch. & C. A.	Asteraceae
8	Scrophularia scoparia Pennell	Scrophulariaceae
9	Chaerophyllum macropodum Boiss.	Apiaceae
10	Cardaria draba (L.) Desv.	Brassicaceae
11	Scariola orientalis (Boiss.) Sojak.	Asteraceae
12	Reseda alba L.	Resedaceae

D. Calculation of Hyperaccumulation Criteria

The definition of metal hyperaccumulation has to take in consideration not only the metal concentration in the above ground biomass, but also the metal concentration in the soil. Both enrichment factor (EF) and translocation factor (TF) have to be considered while evaluating whether a particular plant is a metal hyperaccumulator [12]. The enrichment factor is calculated as the ratio plant shoot concentration to soil concentration ([Metal]shoot/ [Metal]Soil) [7] and the translocation factor is the ratio of metal concentration in the shoot to the root ([Metal]Shoot/ [Metal]Root). Therefore, a hyperaccumulator plant should have EF or TF> 1.

III. Results

A. Soil Characterizations

Table II shows the values of the heavy metals and some characteristics in different localities on the mine activity areas. Here were detected the average values 12963.4 mg kg-1, 8917 mg kg-1, 120.1 mg kg-1, 8955.7 mg kg-1 and 16563.6 mg kg-1 for Zn, Mn, Cu, Pb and Fe, respectively. In addition, the average values for EC, pH, P and $CaCO_3$ were 0.83, 7.68, 12.67 and 22.23, respectively.

TABLE II
SOIL CHARACTERISTICS AND HEAVY METAL CONCENTRATIONS IN THE SURROUNDING AREA OF AHANGARAN MINE

Soils No.	Fe (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	CaCO3 (%)	P (mg/kg)	pH	EC (dS/m)
1	15704.8	9422.3	238.5	8411.8	12163.2	22.9	11.0	7.55	0.62
2	15512	8462	193	10928	14280.2	20.2	13.4	7.67	0.82
3	16235.2	7429	126.5	11034.6	12476.5	21.2	10.6	7.76	0.70
4	15632.5	10362	176.4	10633	14497.7	21.6	12.2	7.78	0.95
5	16907.3	12290.3	110	10682	16091.5	21.5	14.3	7.60	1.00
6	16451.5	10247.3	96.1	10153	14646.2	21.5	14.3	7.64	1.66
7	15565.6	6911.7	137.3	6013.7	11144	23.3	10.3	7.79	0.53
8	17883	7407	81	7860.3	13120	24.8	14.3	7.70	0.76
9	18410.3	8560.6	229	7468	15369.3	26.5	11.8	7.74	0.75
10	16469.6	10302.7	19	6463.2	11402.8	20.8	10.2	7.60	0.67
11	16050.6	9384.3	16.5	9917	10187.2	19.1	15.9	7.65	0.74
12	17940.6	6689	17.8	7440	10182.2	23.4	13.7	7.76	0.79

B. Metal Concentrations in Plants

Total Zn concentrations in the plant roots ranged from 63.8 mg kg-1 to 12003 mg kg-1 and plant shoots from 51 mg kg-1 to 8771.6 mg kg-1, with the maximum level in the roots of R. lutea and shoots of E. macroclada. Mn concentrations in plant roots differed among species at the polluted site from 157 mg kg-1 to 3976 mg kg-1 and in shoots from 94.6 to 12118 mg kg-1, with the maximum content in the roots of S. orientalis and shoots of C. virgata. Total Cu concentrations in the plant roots ranged from 8.6 mg kg-1 to as high as 261 mg kg-1 and plant shoots from 8.1 mg kg-1 to as high as 145.3 mg kg-1, with the maximum level in the roots of E. macroclada and shoots of E. platyloba. Total Pb concentrations in the plant roots ranged from 83.3 mg kg-1 to 7834.3 mg kg-1 and plant shoots from 114.6 mg kg-1 to 10126.3 mg kg-1, with the maximum level in the roots of S. orientalis and shoots of E. platyloba. Total concentrations of Fe in the plant roots ranged from 333.9 mg kg-1 to as high as 8943.3 mg kg-1 and plant shoots from 1113.6 mg kg-1 to as high as 69556.6 mg kg-1, with the maximum being in the roots and shoots of E. macroclada. Concentrations of Zn, Mn, Cu, Pb and Fe in collected plant species are provided in Figs 2- 6.

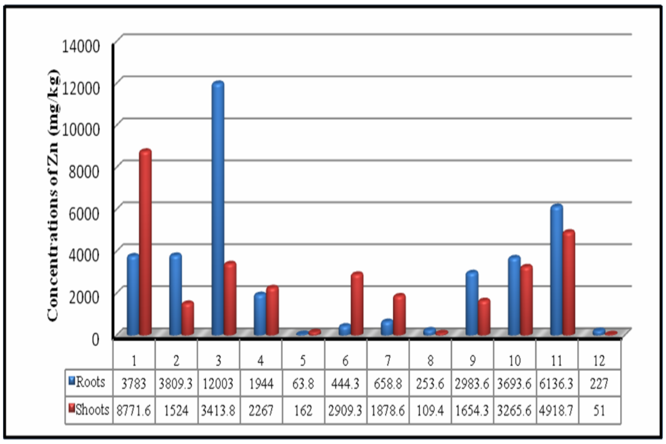

Fig. 2 Zinc concentrations in shoots and roots of plant samples

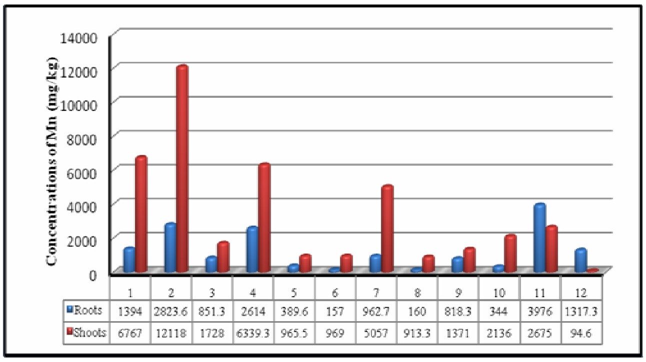

Fig. 3 Manganese concentrations in shoots and roots of plant amples

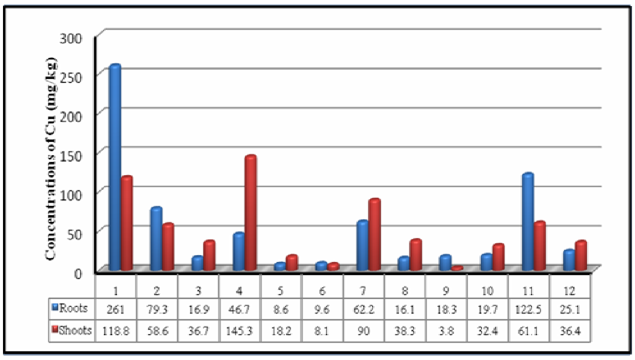

Fig. 4 Copper concentrations in shoots and roots of plant samples

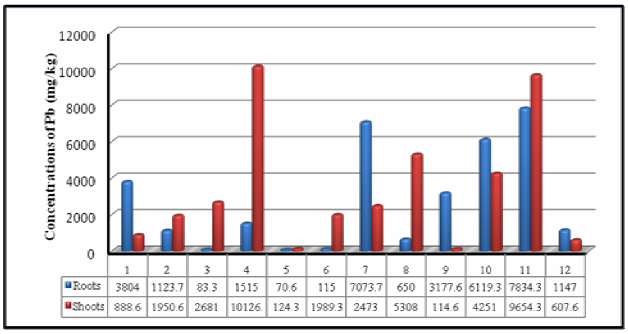

Fig. 5 Lead concentrations in shoots and roots of plant samples

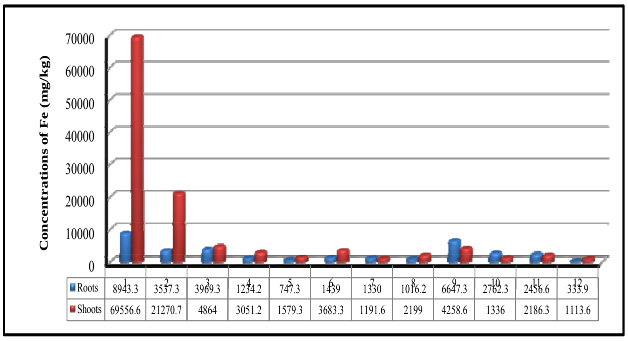

Fig. 6 Iron concentrations in shoots and roots of plant samples

IV. Discussion

A. Contamination of Heavy Metals in Soil and Plant Samples

The present study showed that some plants could colonize sites with a wide range of metal concentrations in the soils. According to references [9] and [10], 200 mg kg-1 Zn, 545 mg kg-1 Mn, 20 mg kg-1 Cu, 300 mg kg-1 Pb and 3800 mg kg-1 Fe, would be considered normal concentrations based on total fractions in soil. The metal (Zn, Mn, Cu, Pb and Fe) contents in the surrounding area of mine greatly exceed these ranges. Soil samples were classified non saline, limy and alkaline. Amount of soil phosphorous is sufficient for plant growth (Table 2).

Metal concentrations in plants vary with plant species [2]. Plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots, or through active transport crosses of the plasma membrane of root epidermal cells [11]. According to reference [9], toxic concentrations of heavy metals for various plant species are 100, 300, 20, 300 and 500 mg kg-1 for Zu, Mn, Cu, Pb and Fe, respectively; therefore in the most of the plant samples, heavy metal contents were higher than toxic levels.

B. Identification of Tolerant and Hyperaccumulator Plants in Study Area

When categorizing plants that can grow in the presence of toxic elements, the terms “tolerant” and “hyperaccumulator” are used. A tolerant species is one that can grow on soil with concentrations of a particular element that are toxic to most other plants [3]-[5]-[13], therefore the most of the plant species grown in the surrounding area of Ahangaram mine were tolerant to measured heavy metals.

Hyperaccumulator species accumulate appreciable quantities of metal in their tissue regardless of the concentration of metal in the soil [17]. The concept of phytoremediation that was first proposed by reference [8] involves the use of plant hyperaccumulators of heavy metals to remove pollutants from soils or waters.

Considering the hyperaccumulator definition of references [4] and [14], none of the plant species were hyperaccumulator for Cu, Zn and Mn. Except E. macroclada, C. intybus, C. macropodum and R. alba, all of the species were hyperaccumulators for Pb. C. virgata was a Mn hyperaccumulator because the values of 12118 mg kg-1 Mn found in the aboveground parts of this species, and finally C. virgata with 21270.7 mg kg-1 and E. Macroclada with 69556.6 mg kg-1 Fe were undoubtedly Fe hyperaccumulators. However, when applying the first requirements of references [15] and [27], it can be considered unusual number accumulators. There are 5 species that have been identified as Zn accumulators, 7 species as Cu and Pb accumulators, 10 species as Mn accumulators and 8 species as Fe accumulators based on this definition. In fact these plant species are able to accumulate unusually high concentrations of heavy metals in their aboveground parts. In this study, E. macroclada and C. virgata for Fe, C. virgata for Mn, C. draba, S. orientalis and R. alba for Cu and S. orientalis for Pb showed EFs> 1 in respect to total soil composition, i.e. were hyperaccumulators based on the second criteria of references [15] and [27] (Table 3), EF and TF values> 1 are in bold font at Table. Another requirement for classifying a hyperaccumulator plant is that, concentrations found in plants must be 10- 500 times higher than the ones growing in unpolluted environments [27], but in this study this requirement can not be tested due to lack of the sampled plants on other locations and soils.

V. Conclusion

Phytoremediation is the use of living plants to mop up pollution in the environment like metal contaminants in the soil, and restore ecological balance in a mining area. The autochthonous plants identified in this study are endemic of the semi-arid region from Hamedan, Iran. In general, sampled plants were almost annual and perennial herbaceous species that perennial specious with relatively large biomass were more suitable for phytoremediation purpose. According to the all mentioned criteria, E. macroclada and C. Virgata can be classified as hyperaccumulator for some measured heavy metals and they are good candidates to be used in phytoremediation of contaminated soils. Further investigations under controlled environmental conditions are required for evaluating the usefulness of these species in phytoremediation technologies.

TABLE. III
ENRICHMENT AND TRANSLOCATION FACTORS IN THE SELECTED PLANTS

Species No.	Translocation factor **					Enrichment factor *(EF)				
	Fe	Pb	Cu	Mn	Zn	Fe	Pb	Cu	Mn	Zn
1	**7.78**	0.23	0.45	**4.85**	**2.32**	**4.43**	0.09	0.50	0.80	0.72
2	**6.01**	**1.73**	0.74	**4.29**	0.40	**1.37**	0.23	0.30	**1.11**	0.11
3	**1.22**	**32.18**	**2.17**	**2.03**	0.28	0.30	0.36	0.29	0.16	0.27
4	**2.47**	**6.68**	**3.11**	**2.42**	**1.17**	0.19	0.98	0.82	0.60	0.16
5	**2.11**	**1.76**	**2.12**	**2.48**	**2.54**	0.09	0.01	0.16	0.09	0.01
6	**2.56**	**17.30**	0.84	**6.17**	**6.55**	0.22	0.19	0.08	0.09	0.20
7	0.89	0.35	**1.45**	**5.25**	**2.85**	0.08	0.36	0.65	0.84	0.17
8	**2.16**	**8.17**	**2.38**	**5.71**	0.43	0.12	0.72	0.47	0.12	0.00
9	0.64	0.04	0.21	**1.67**	0.55	0.23	0.01	0.02	0.18	0.11
10	0.48	0.69	**1.64**	**6.21**	0.88	0.08	0.41	**1.70**	0.33	0.29
11	0.89	**1.23**	0.50	0.67	0.80	0.14	**1.03**	**3.70**	0.27	0.48
12	**3.33**	0.53	**1.45**	0.07	0.22	0.06	0.09	**2.04**	0.01	0.00

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