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Phytoremediation Potential of Native Plants Growing on a Heavy Metals Contaminated Soil of Copper mine in Iran

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Abstract**—**A research project dealing with the phytoremediation of a soil polluted by some heavy metals is currently running. The case study is represented by a mining area in Hamedan province in the central west part of Iran. The potential of phytoextraction and phytostabilization of plants was evaluated considering the concentration of heavy metals in the plant tissues and also the bioconcentration factor (BCF) and the translocation factor (TF). Also the several established criteria were applied to define hyperaccumulator plants in the studied area. Results showed that none of the collected plant species were suitable for phytoextraction of Cu, Zn, Fe and Mn, but among the plants, Euphorbia macroclada was the most efficient in phytostabilization of Cu and Fe, while, Ziziphora clinopodioides, Cousinia sp. and Chenopodium botrys were the most suitable for phytostabilization of Zn and Chondrila juncea and Stipa barbata had the potential for phytostabilization of Mn. Using the most common criterion, Euphorbia macroclada and Verbascum speciosum were Fe hyperaccumulator plants. Present study showed that native plant species growing on contaminated sites may have the potential for phytoremediation.

Keywords**—**Bioconcentration 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 [15]. 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 [2].

Many approaches have been developed, assessed and performed to cope with the soil pollution. Current available soil clean-up technologies are often high-priced, 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 [14]. In these conditions, but overall for long-term projects, phytoremediation could be the cheapest and simplest option among the available soil clean-up strategies [21]. USEPA (2000) defines phytoremediation as “the use of plants for containment, degradation or extraction of xenobiotics from water or soil substrates” [5]. Remediation of heavy metals by plant species can be divided into three groups: phytoextraction, metal accumulating plants are planted on contaminated soil and later harvested in order to remove metals from the soil; rhizofiltration, roots of metal accumulating plants absorb metals from polluted effluents and are later harvested to diminish the metals in the effluent; and phytostabilisation, metal-tolerant plants are used to reduce the mobility of metals, thus, the metals are stabilized in the substrate [18]-[1]. Plants with both bioconcentration factor and translocation factor greater than one (TF and BCF> 1) have the potential to be used in phytoextraction. Besides, plants with bioconcentration factor greater than one and translocation factor less than one (BCF> 1 and TF< 1) have the potential for phytostabilization [26].

Plants that accumulate very high concentrations of metals in any aboveground tissue in their natural habitat are called hyperaccumulators [4]. Based on references [4] and [12], hyperaccumulators are defined as plants that accumulate> 1000 mg kg− 1 of Cu, Co, Cr, Ni or Pb, or> 10000 mg kg− 1 of Fe, Mn or Zn. Other authors included besides de first previous requirement, two other ones: (ii) the translocation factors must be invariably higher than one (TF> 1). 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 [13] and (iii) the enrichment factor must be higher than one (EF> 1), meaning higher metal concentrations in the plant than in the soil, which emphasizes the degree of plant metal uptake [13]-[25]. There has been a continuing interest in searching for native plants that are tolerant towards heavy metals [19]-[13].

The overall objectives of this research were: (1) to

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determine the concentrations of Cu, Zn, Fe and Mn in plant biomass growing on a contaminated site; (2) to assess the feasibility of using of these plants for phytoremediation purpose and (3) 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 a square (N 34º 57' 16" and E 48º 8' 26", N 34º 56' 14" and E 48º 8' 22", N 34º 55' 58" and E 48º 11' 34" and N 34º 54' 53" and E 48º 10' 25") in the surrounding area of Hame Kasi mine of northwest Hamadan province in Iran. The site has occupied approximately 10000 m2 and is covered mainly by grasses. Human activities such as mining have contributed to elevated metal concentrations in this site. Contamination of heavy metals was mainly concentrated in the top 20 cm at the site (Fig. 1).

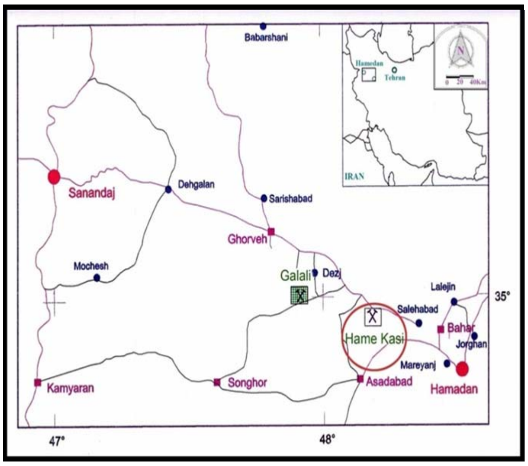

Fig. 1 Location of the study area

B. Plant Sampling and Analysis

Seventeen plant species were collected in the surrounding area of Hame Kasi mine from January to June 2007. The voucher specimens were deposited at Bu-Ali Sina University Herbarium (ICN 10998) and were labeled as follows: Iran, prov. Hamadan, 35 km from Hamadan to Kordestan, Alt. 2386 m. The studied species consisted of 15 genera and 9 families (Table 1), of which 8 species belonged to Asteraceae, forming the most dominant component in the 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 (Cu, Zn, Fe and Mn) of the plant samples were extracted by acid digestion followed by measurement of total concentrations of all elements of interest using atomic absorption spectrophotometer (GBC Avanta, Australia) according to reference [24].

TABLE I
SPECIES COMPOSITION IN SURROUNDING AREA OF HAME KASI MINE

Species No.	Scientific name	Family
1	Euphorbia macroclada Boiss	Euphorbiaceae
2	Centaurea virgata Lam.	Asteraceae
3	Ziziphora clinopodioides Lam..	Lamiaceae
4	Cousinia bijarensis Rech. F.	Asteraceae
5	Biebersteinia multifida DC.	Geraniaceae
6	Stachys inflate Benth.	Lamiaceae
7	Cousinia robustus L.	Asteraceae
8	Eryngium billardieri F. Delaroche	Umbelliferae
9	Chondrila juncea L.	Asteraceae
10	Cousinia sp.	Asteraceae
11	Scariola orientalis (Boiss.) Sojak.	Asteraceae
12	Cirsium congestum Fisch. and C. A.	Asteraceae
13	Chenopodium botrys L.	Chenopodiaceae
14	Stipa barbata Desf.	Poaceae
15	Astragalus verus L.	Fabaceae
16	Heliochrysum armenium DC.	Asteraceae
17	Verbascum speciosum Schard.	Scrophulariaceae

C. 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 (Cu, Zn, Fe and Mn). Total metal contents were extracted by acid digestion. Metal contents were measured by atomic absorption spectrophotometer (GBC Avanta, Australia) according to reference [20]. Soil samples were also analyzed for pH, electrical conductivity (EC) and total P. The pH and EC values of soil samples were measured by a pH meter and an EC meter, according to references [23] and [16] methods, respectively. Total phosphorus was measured using the method of Olsen [17] and soil texture, was determined according to reference [22].

D. Data Analysis

The mobility of the heavy metals from the polluted substrate into the roots of the plants and the ability to translocate the metals from roots to the harvestable aerial part were evaluated respectively by means of the bioconcentration factor (BCF) and the translocation factor (TF). BCF is defined as the ratio of metal concentration in the roots to that in soil ([Metal]Root/ [Metal]Soil), TF is the ratio of metal concentration in the shoots to the roots ([Metal]Shoot/

[Metal]Root). The ability of plants to tolerate and accumulate heavy metals is useful for phytoextraction and phytostabilization purpose (Yoon et al., 2006). Plants with both bioconcentration factors and translocation factors greater than one (TF and BCF> 1) have the potential to be used in phytoextraction. Besides, plants with bioconcentration factor greater than one and translocation factor less than one (BCF> 1 and TF< 1) have the potential for phytostabilization [26].

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 [11]. The enrichment factor is calculated as the ratio plant shoot concentration to soil concentration ([Metal] shoot/ [Metal] Soil) [6]. Therefore, a hyperaccumulator plant should have EF> 1 or TF> 1, as well as total accumulation> 1000 mg kg-1 of Cu, Co, Cr, Ni or Pb, or> 10000 mg kg− 1 of Fe, Mn or Zn.

III. RESULTS AND DISCUSSION

A. Soil Characteristics

Selected characteristics of the soil samples collected from this study are shown in Table 2.

TABLE. II
SOIL CHARACTERISTICS OF THE SURROUNDING AREA OF HAME KASI MINE (MEAN± SE)

Parameter	Value
EC (dS/m)	1.28± 0.1
pH	7.70± 0.01
P (mg/kg)	26.3± 2.5
CaCO3 (%)	6.8± 0.2
Clay (g/kg)	112± 0.9
Silt (g/kg)	181± 1.6
Sand (g/kg)	707± 1.9

B. Metal Concentrations in Soil and Plants

The present study shows that some plants can colonize sites with a wide range of metal concentrations in the soils. According to references [8] and [9], 20 mg kg-1 Cu, 200 mg kg-1 Zn, 545 mg kg-1 Mn and 3800 mg kg-1 Fe, would be considered normal concentrations based on total fractions in soil. The metal (Cu, Zn, Fe and Mn) contents in the surrounding area of mine greatly exceed these ranges (Tables 3, 4, 5, 6).

Metal concentrations in plants vary with plant species [3]. 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 [10]

Total Cu concentrations in the plant roots ranged from 18.8 mg kg-1 to as high as 475 mg kg-1 and plant shoots from 0.67 mg kg-1 to as high as 265.4 mg kg-1, with the maximum level in the roots of E. macroclada and shoots of S. orientalis. Total Zn concentrations in the plant roots ranged from 23.1 mg kg-1 to 1177 mg kg-1 and plant shoots from 38.3 mg kg-1 to 1037 mg kg-1, with the maximum level in the roots of C. botrys and shoots of C. juncea. Total concentrations of Fe in the plant roots ranged from 182 mg kg-1 to as high as 30437.7 mg kg-1 and plant shoots from 144.3 mg kg-1 to as high as 27278.7 mg kg-1, with the maximum being in the roots of E. macroclada and shoots of V. speciosum. Mn concentrations in plant roots differed among species at the polluted site from 25 mg kg-1 to 723 mg kg-1 and in shoots from non-detectable to 568 mg kg-1, with the maximum content in the roots of C. juncea and shoots of E. macroclada. According to reference [8], toxic concentrations of heavy metals for various plant species are 20, 300, 100 and 500 mg kg-1 for Cu, Mn, Zn and Fe, respectively; therefore in the most of the plant samples, heavy metal contents were higher than toxic levels.

Concentrations of Cu, Zn, Fe and Mn in collected plant species are provided in Tables 3, 4, 5 and 6.

TABLE. III
COPPER CONCENTRATIONS IN SOIL AND PLANT SAMPLES (MG KG− 1)

Scientific name	Roots	Shoots	Soil
E. macroclada	475	164	355.7
C. virgata	159.3	56	472.7
Z. clinopodioides	115.3	72	444.7
C. bijarensis	84	97	325.7
B. multifida	138.9	206.7	330.8
S. inflate	18.8	0.6	352.3
C. robustus	46.6	101.3	414
E. billardieri	30.7	1.8	446
C. juncea	210.3	61.3	436.7
Cousinia sp.	166.3	232.7	368.1
S. orientalis	314.7	265.4	528.3
C. congestum	111.3	95.7	487.7
C. botrys	134	92	521
S. barbata	94.7	63	471.3
A. verus	119.3	74.4	575.7
H. armenium	237	171.7	554.2
V. speciosum	260.7	246.2	477.7

TABLE. IV
ZINC CONCENTRATIONS IN SOIL AND PLANT SAMPLES (MG KG− 1)

Scientific name	Roots	Shoots	Soil
E. macroclada	23.1	12021	27718.2
C. virgata	550.3	3754.3	32699.8
Z. clinopodioides	1049	2056.3	33181.5
C. bijarensis	43.7	1061.2	34728.8
B. multifida	47.7	471.3	38283.5
S. inflate	67.3	376.4	37131.7
C. robustus	112.3	1814.3	33047.7
E. billardieri	273.3	1265.3	33039
C. juncea	820	144.3	33257.2
Cousinia sp.	1014	6582	31617.5
S. orientalis	972	4640.7	37252.5
C. congestum	843.7	3175.2	28604
C. botrys	1177.7	1516.8	37715.3
S. barbata	285	6161.3	34975
A. verus	605.3	2054.7	28753.2
H. armenium	35.3	1421.7	34030.9
V. speciosum	1000	27278.7	27933.8

TABLE. V
IRON CONCENTRATIONS IN SOIL AND PLANT SAMPLES (MG KG− 1)

Scientific name	Roots	Shoots	Soil
E. macroclada	30437.7	12021	27718.2
C. virgata	2240.7	3754.3	32699.8
Z. clinopodioides	2680	2056.3	33181.5
C. bijarensis	1007	1061.2	34728.8
B. multifida	1462	471.3	38283.5
S. inflate	491	376.4	37131.7
C. robustus	2618.5	1814.3	33047.7
E. billardieri	727	1265.3	33039
C. juncea	242	144.3	33257.2
Cousinia sp.	8774.7	6582	31617.5
S. orientalis	10084	4640.7	37252.5
C. congestum	4850.7	3175.2	28604
C. botrys	411	1516.8	37715.3
S. barbata	4460.3	6161.3	34975
A. verus	3587.4	2054.7	28753.2
H. armenium	182	1421.7	34030.9
V. speciosum	11754	27278.7	27933.8

TABLE. VI
MANGANESE CONCENTRATIONS IN SOIL AND PLANT SAMPLES (MG KG− 1)

Scientific name	Roots	Shoots	Soil
E. macroclada	150	568	962.2
C. virgata	78.7	147.7	679.5
Z. clinopodioides	76.7	109.3	816.8
C. bijarensis	142.7	776	688.6
B. multifida	117.3	43	694.7
S. inflate	95	29.5	848.2
C. robustus	136.3	213.7	636.2
E. billardieri	61.7	2.3	673.9
C. juncea	723	94.1	701.5
Cousinia sp.	69.7	184.3	850.5
S. orientalis	151.3	144.6	750.3
C. congestum	245.7	23.3	824.2
C. botrys	346.7	166.7	860.5
S. barbata	497.1	191	495.2
A. verus	30.3	46.3	780.5
H. armenium	140.3	338.6	828.3
V. speciosum	25	*nd	831.7

*nd stands for not detected

C. *Accumulation and Translocation of Metals in Plants*

A plant's ability to accumulate metals from soils can be estimated using the BCF and a plant's ability to translocate metals from the roots to the shoots is measured using the TF. The process of phytoextraction generally requires the translocation of heavy metals to the easily harvestable plant parts, i.e., shoots [26], while phytostabilization process requires the strong ability to reduce metal translocation from roots to shoots [7]. By comparing BCF and TF, the ability of different plants in taking up metals from soils and translocating them to the shoots can be compared [26].

As shown in Table 7, among the sampled plants, none of them were suitable for phytoextraction of Cu, Zn, Fe and Mn but E. macroclada was the most suitable for phytostabilization of Cu and Fe (BCF = 1.33 and 1.10 and TF = 0.34 and 0.39, respectively), Z. clinopodioides, Cousinia sp. and C. botrys were the most efficient for phytostabilization of Zn (BCF = 1.06, 1.00 and 1.03 and TF = 0.12, 0.14 and 0.11, respectively) and C. juncea and S. barbata had the potential for phytostabilization of Mn (BCF = 1.03 and 1.00 and TF = 0.13 and 0.38, respectively).

Considering the hyperaccumulator definition of references [4] and [12], none of the plant species accumulated Cu above 1000 mg kg− 1 and Zn and Mn above 10000 mg kg− 1 in the shoots i.e. none of them are Cu, Zn and Mn hyperaccumulators while E. macroclada and V. speciosum (12021 mg kg− 1 and 27278.7 mg kg− 1, respectively) undoubtedly are Fe hyperaccumulators (Tables 3-6). However, when applying the requirements of reference [13], it can be considered unusual number accumulators. According to this criterion C. bijarensis is a hyperaccumulator of Cu, Zn, Fe and Mn (TF = 1.15, 1.44, 1.50 and 5.44, respectively), C. robustus is a hyperaccumulator of Cu, Zn and Mn (TF = 2.17, 1.82 and 1.57, respectively), H. armenium is a hyperaccumulator of Zn, Fe and Mn (TF = 1.43, 7.81 and 2.41, respectively), E. macroclada is a hyperaccumulator of Zn and Mn (TF = 8.40 and 3.79, respectively), S. barbata is a hyperaccumulator of Zn and Fe (TF = 1.12 and 1.38, respectively), Cousinia sp. is a hyperaccumulator of Cu and Mn (TF = 1.40 and 2.64, respectively), C. virgata is a hyperaccumulator of Fe and Mn (TF = 1.54 and 1.88, respectively), V. speciosum, C. botrys and E. billardieri are hyperaccumulators of Fe (TF = 2.32, 3.70 and 1.74, respectively), Z. clinopodioides and A. verus are hyperaccumulators of Mn (TF = 1.42 and 1.53, respectively), S. inflate and C. juncea are hyperaccumulators of Zn (TF = 1.17 and 1.26, respectively) and B. multifida is a hyperaccumulator of Cu (TF = 1.49). In this study, only C. bijarensis showed EF> 1 (EF = 1.13) i.e. is a Mn hyperaccumulator based on references [13] and [25] (Table 7).

TABLE. VII
ACCUMULATION AND TRANSLOCATION OF CU, ZN, FE AND MN IN SELECTED PLANTS

Scientific name	Bioconcentration factor *(BCF)				Translocation factor **(TF)				Enrichment factor ***(EF)			
	Mn	*Fe*	*Zn*	*Cu*	*Mn*	*Fe*	*Zn*	*Cu*	*Mn*	*Fe*	*Zn*	*Cu*
E. macroclada	**1.33**	0.02	**1.10**	0.15	0.34	**8.40**	0.39	**3.79**	0.46	0.21	0.43	0.59
C. virgata	0.34	0.63	0.07	0.11	0.35	0.11	**1.54**	**1.88**	0.12	0.07	0.11	0.22
Z. clinopodioides	0.26	**1.06**	0.08	0.09	0.62	0.12	0.77	**1.42**	0.16	0.13	0.06	0.13
C. bijarensis	0.26	0.04	0.03	0.21	**1.15**	**1.44**	**1.05**	**5.44**	0.30	0.06	0.03	**1.13**
B. multifida	0.42	0.05	0.04	0.17	**1.49**	0.34	0.32	0.37	0.62	0.02	0.01	0.06
S. inflate	0.05	0.07	0.01	0.11	0.03	**1.17**	0.77	0.31	0.002	0.09	0.01	0.03
C. robustus	0.11	0.10	0.08	0.21	**2.17**	**1.82**	0.70	**1.57**	0.24	0.19	0.05	0.33
E. billardieri	0.07	0.18	0.02	0.09	0.06	0.88	**1.74**	0.04	0.004	0.16	0.04	0.003
C. juncea	0.48	0.56	0.007	**1.03**	0.29	**1.26**	0.60	0.13	0.14	0.74	0.004	0.13
Cousinia sp.	0.45	**1.00**	0.28	0.08	**1.40**	0.14	0.75	**2.64**	0.63	0.14	0.21	0.22
S. orientalis	0.59	0.96	0.27	0.20	0.84	0.05	0.46	0.95	0.50	0.05	0.12	0.19
C. congestum	0.23	0.69	0.17	0.30	0.86	0.12	0.65	0.09	0.20	0.08	0.11	0.03
C. botrys	0.26	**1.03**	0.01	0.40	0.69	0.11	**3.70**	0.48	0.18	0.12	0.04	0.19
S. barbata	0.20	0.23	0.13	**1.00**	0.66	**1.12**	**1.38**	0.38	0.13	0.26	0.18	0.38
A. verus	0.23	0.63	0.12	0.04	0.62	0.06	0.57	**1.53**	0.13	0.04	0.07	0.06
H. armenium	0.43	0.03	0.005	0.17	0.72	**1.43**	**7.81**	**2.41**	0.31	0.05	0.04	0.41
V. speciosum	0.54	0.95	0.42	0.03	0.94	0.19	**2.32**	-	0.51	0.18	0.98	-

**BCF = metal concentration ratio of plant roots to soil, **TF = metal concentration ratio of plant shoots to roots, ***EF = metal concentration ratio of plant shoots to soil. Values> 1 are in bold font.

IV. CONCLUSION

This study was conducted to screen plants growing on a contaminated site to determine their potential for metal accumulation. Based on among the 17 sampled plant species, metal translocation into shoots appears to be very restricted in the most plant species so that harvesting plants will not be an effective source of metal removal in this site. However, in the view of toxicology, this could be a desirable property, as metals would not pass into the food chain via herbivores, and thus avoid potential risk to the environment. Therefore, plants with low shoot accumulation should be used in order to stabilize the metals and reduce the metal dispersion through grazing animals or at leaf senescence. In general, sampled plants are almost annual and perennial herbaceous species that perennial specious with relatively large biomass are more suitable for phytoremediation purpose.

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