UPTAKE OF Cd AND Pb BY NATURAL VEGETATION IN SOILS POLLUTED BY MINING ACTIVITIES

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ABSTRACT

The remediation of heavy metal-contaminated sites using hyperaccumulating plants represents a promising alternative to currently used methods. The potential of five species was examined to determine their tolerance and ability to accumulate metals for phytoremediation purposes, in polluted soils. The area studied is located in the SE of Spain and is heavily affected by the effects of mining activities. The total contents of heavy metals were determined in soil and plant samples and their soluble and bioavailable contents in soil samples. Average soil concentrations ranged from 2 mg kg^{-1} to 165 mg kg^{-1} for Cd and from 550 mg kg^{-1} to 3990 mg kg^{-1} for Pb. The concentration found in vegetable species studies ranged from 1.1 mg kg^{-1}(d.w) to 172 mg kg^{-1} (d.w) for Cd in roots and from 1.4 mg kg^{-1} (d.w) to 2.7 mg kg^{-1} (d.w) for Cd in leaves. Finally, lead content in plant samples ranged from 45 mg kg^{-1} (d.w) to 1089 mg kg^{-1} (d.w) in roots and from 52 mg kg^{-1} (d.w) to 960 mg kg^{-1} (d.w) in leaves.

Among the plant species collected, *Dittrichia viscosa* and *Arthrocnemum macrostachyum* were the best Cd and Pb accumulators from contaminated soils. The other plant species analyzed showed a lower heavy metal content and transfer factors values of less than 1, so that they can be considered as hypertolerant to heavy metals but not hyperaccumulators.

KEYWORDS: Cadmium; lead; hyperaccumulator plants; soil contamination; phytoremediation; transfer factor; bioavailability

INTRODUCTION

Trace element pollution is an important environmental problem. Mining activities, among others, are characterised by high waste generation, high concentrations of heavy metals and usually low pH values, a combination which often results in severe contamination problems [1, 2]. Although some metals are essential for life, high concentrations can produce negative effects. Many methods have been proposed to remediate heavy metal-contaminated soils, although most are very expensive or do not provide long-term solutions. The approaches include *ex situ* techniques such as soil washing, acid cleaning, ashing or soil replacement and electrokinetic techniques [3-6]. In contrast, *in situ* remediation techniques consist of adding chemicals to contaminated soils to reduce the solubility of metals through metal sorption and/or precipitation, thus, reducing metal transport from the contaminated soil to the surface and groundwater [7]. The aim of amendment addition is to reduce the bioaccessible fraction of metals, for which purpose, several materials have been used, including phosphate [8], natural zeolites [9], municipal biosolids [10], fly ashes [11, 12] red mud [13], cutting marble sludges [14] and iodide and citric acid [15].

Among more cost-effective solutions for remediating soils polluted by heavy metals, phytoremediation is one of the most environmentally friendly since it uses plants to remove pollutants from the environment or, at least, to render them harmless [16, 17].

Phytoremediation is considered as a potential solution for the remediation of contaminated soils. The success of phytoremediation technology is dependent on several factors such as the ability of the selected plant species for accumulating high concentrations of metal in the shoots and to produce high biomass [18]. Previous works have shown the possibilities of using *Brassicaceae* family [19], *Thlaspi* sp., *Alyssum* sp., *Phyla nodiflora* or *Gentiana pennelliana* [20] for phytoremediation of contaminated soils. Particularly, *Amaranthus blitoides* [21] has been used for phytoremediation of soils polluted by arsenic.

Some plant species can be grouped according to their trace element accumulation capability. For example, excluders have an avoidance (or restriction) mechanism which prevents element uptake, while accumulators have mechanisms of metal tolerance and accumulation in their above-ground biomass. A sub-group within the accumulators is represented by hyperaccumulators [22], which are plants commonly growing on metalliferous soils and able to complete their life cycle without any sign of metal phytotoxicity [23].

The aim of this work was to compare the trace element accumulation capacity of selected plant species growing on contaminated soils and to assess changes in the concentration of soil-available metals. Five plant species (*Limonium carthaginens*, *Arthrocnemum macrostachyum*, *Dittrichia viscosa*, *Glaucium flavum* and *Zygophyllum fabago*) were studied, and their transfer factors values were calculated.

MATERIALS AND METHODS

For this study, five soil samples and five plant species were taken from the surrounding area of Sierra Minera and Portman Bay (Murcia, SE Spain), close to the mining region of La Unión, which was subjected to mining activities since the times of the Roman Empire until 1991 [24].

Samples were air dried and sieved to < 2 mm for general analytical determinations. The pH was determined in a 1:1 suspension of soil in pure water and in 1M KCl [25]. Electrical conductivity (EC) was also determined. $CaCO_3$ content was determined by the Bernard calcimeter volumetric method, which consists of quantifying the CO_2 released when the sample is treated with HCl [26, 27]. The organic matter (O.M.) content was determined by sulfochromic oxidation [28, 29]. Particle size distribution was performed after dispersion of the fine soil and by combining extraction by Robinson pipette and sieving.

To determine the Cd and Pb content, soil samples were first ground to a fine powder using an agate ball mill and then 100 mg were placed in Teflon vessels to which 5 ml of concentrated HF acid solution, 200 µl of concentrated HNO_3 acid solution and 5 ml of water were added. Fresh vegetable samples, were separated into root and above-ground biomass and then lyophilized. 200 mg of lyophilized vegetal tissue were placed in Teflon vessels with 3 ml of water, 2 ml of concentrated H_2O_2 and 5 ml of concentrated HNO_3 acid solution. When digestion was complete (15 minutes at 1000 W in a Milestone ETHOS PLUS microwave), the samples were transferred to a volumetric flask and brought to 50 ml. Cd and Pb content were determined by electrothermal atomization atomic absorption spectrometry (ETAAS) using an Unicam 929 AASpectrometer. The reliability of the results was verified by analysing a standard reference material (SRM 2711 Montana Soil and SRM 1515 Apple leaves).

The bioaccessible concentrations of Cd and Pb in soil samples were measured using the DTPA soil test [30, 31]. For this purpose, 5g of air-dried soil (<2 mm) were mixed with 10ml of a solution containing 0.005 mol l^{-1} DTPA, 0.01 mol l^{-1} $CaCl_2$ and 0.1 mol l^{-1} TEA (pH=7.3). The mixture was shaken for 120 minutes at 3000 rpm. The suspension was then filtered immediately. Moreover, a 1:5 extraction was made to determine the water soluble metal content.

The mineralogical composition was studied by X-ray diffraction (XRD), using a Philips PW3040 diffractometer with Cu-Kα. X-powder software [32] was used to analyse the X-ray diffraction diagrams obtained by the crystalline powder method. The powder diffraction file (PDF2) database was used for peak identification, taking into account that the determination of minerals from soils by XRD analysis is not accurate below a limit of 5% of the total weight in a sample (depending on the crystallinity of individual minerals).

Finally, in order to evaluate the phytoextraction potential of the selected plants, the transfer factor was calculated. The term transfer factor (TF) has been defined as the ratio of metal concentration in plants to the total metal concentration in soil [33-35].

RESULTS AND DISCUSSION

The general soil characteristics are given in Table 1. Samples S2, S3 and S4 showed slightly alkaline pH and S1 and S5 acidic pH values. All the samples presented a very low organic matter percentage, low soluble salt content, low calcium carbonate percentage and sandy or sandy-clay textures (Table 2).

TABLE 1 - Chemical characteristics of soil samples

	pH H_20	EC (mS cm^{-1})	$CaCO_3$ (%)	O.M.(%)
S1	5.0	7.4	<0.1	0.3
S2	8.1	2.8	10.1	0.8
S3	7.5	0.2	0.4	0.8
S4	7.4	3.5	<0.1	1.9
S5	4.2	2.4	<0.1	1.0

TABLE 2 - Particle size distribution of soil samples (%)

	<2µm	2-20µm	20-50µm	50-100µm	100-250µm	250-500µm	500-1000µm	1000-2000µm
S1	0.9	4.5	4.3	7.2	17.1	11.9	16.2	37.9
S2	1.8	8.5	7.9	15.2	42.2	14.0	10.4	0.1
S3	0.4	1.1	0.6	0.7	7.7	11.7	24.2	53.6
S4	1.0	4.8	2.3	1.5	10.7	36.6	27.7	15.6
S5	2.9	7.4	5.0	2.0	19.8	14.2	48.3	0.4

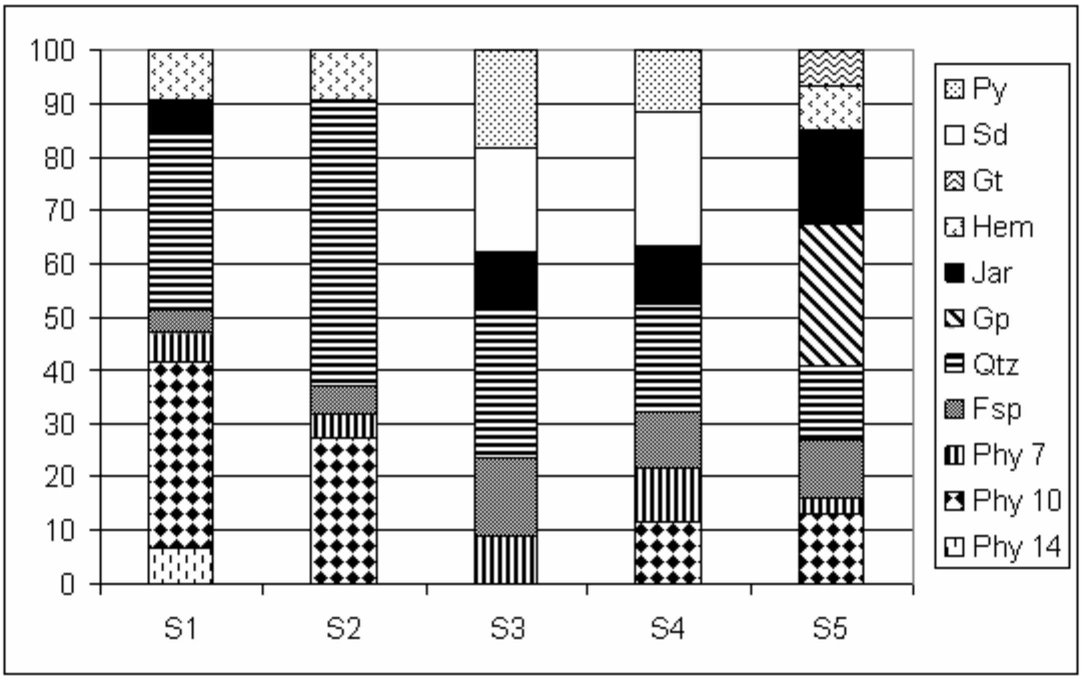

FIGURE 1 - Mineralogical composition of soil samples (%)

TABLE 3 - Total, bioavailable and soluble Cd and Pb content in soil samples.

	Total Cd (mg kg^{-1})	DTPA Cd (mg kg^{-1})	Soluble Cd (mg kg^{-1})	Total Pb (mg kg^{-1})	DTPA Pb (mg kg^{-1})	Soluble Pb (mg kg^{-1})
S1	90	8	11	3990	350	12
S2	120	2	<0.1 μg kg^{-1}	1240	120	2
S3	160	2	0.001	1700	150	0.04
S4	75	2	0.003	2330	230	0.08
S5	2	0.1	0.61	550	50	17
Mean	90	3	2.9	1960	180	6
Std. dev	58.6	3	4.8	1306	115	7.6

The plant species selected grew in poorly developed soils formed by materials from surrounding mining areas, carbonate materials and phillites. The mineralogical analysis showed that the main minerals were quartz, muscovite, kaolinite and illite while the minority minerals were products of the mining (Figure 1).

The total Cd and Pb contents are shown in Table 3. The total Cd concentration in soil samples collected from the site varied from 2 mg kg^{-1} in S5 to 160 mg kg^{-1} in S3, the mean Cd concentration being 90 mg kg^{-1}. Pb concentrations varied from 550 mg kg^{-1} in S5 and 3990 mg kg^{-1} in S1, with a mean value of 1960 mg kg^{-1}.

The DTPA and water soluble contents are also shown in Table 3. DTPA extractable amounts of Pb were much higher than DTPA extractable Cd. The mean values for DTPA-extracted Cd and Pb were 3 mg kg^{-1} and 180 mg kg^{-1}, while the corresponding values for the water soluble fractions were 2.9 mg kg^{-1} and 6 mg kg^{-1}.

The metal concentrations in plants varied with plant species [36]. Under normal growing conditions, plants can potentially accumulate certain metal ions up to one order of magnitude greater than the surrounding medium. The concentrations of Cd and Pb in plant biomass are shown in Table 4. The highest Cd value was that found in *Arthrocnemum macrostachyum* with 170 mg kg^{-1} (d.w) in roots and 4.5 mg kg^{-1} (d.w) in leaves. The same plant also showed the highest Pb values, 1090 mg kg^{-1} (d.w) in roots and 960 mg kg^{-1} (d.w) in leaves.

As mentioned above, the soil to plant transfer factor (TF) is an index for evaluating the transfer potential of a metal from soil to plant. The TF_{TOTAL} values for Cd and Pb in Table 5 show that the TF_{TOTAL} values ranged from 0.01 to 2.34, while values were lower for Pb, between 0.01 and 0.47.

It is well established that in order to predict the transfer of metals from soil to plants and to assess the risks associ-

TABLE 4 - Cd and Pb content in plant samples (d.w)

		Cd (mg kg^{-1})		Pb (mg kg^{-1})	
Scientific name		**Roots**	**Leaves**	**Roots**	**Leaves**
Glaucium flavum	S1	1.2	1.4	45	52
Zygophyllum fabago	S2	2.8	2.7	46	87
Limonium carthaginens.	S3	1.1	1.4	115	137
Arthrocnemum macrostachyum	S4	170	4.5	1090	960
Dittrichia viscosa	S5	4.6	2.6	121	510

TABLE 5 - TF values for plant species

		Cd		Pb	
Sample		TF_{TOTAL}	TF_{DTPA}	TF_{TOTAL}	TF_{DTPA}
Glaucium flavum	**Leaves**	0.01	0.18	0.01	0.15
	Roots	0.01	0.15	0.01	0.13
Zygophyllum fabago	**Leaves**	0.02	**1.35**	0.07	0.72
	Roots	0.02	**1.40**	0.03	0.38
Limonium carthaginens.	**Leaves**	0.008	0.70	0.08	0.89
	Roots	0.006	0.55	0.07	0.75
Arthrocnemum macrostachyum	**Leaves**	0.06	2.25	0.41	**4.17**
	Roots	**2.34**	**86**	0.47	**4.73**
Dittrichia viscosa	**Leaves**	**1.07**	**26**	0.26	**11**
	Roots	**1.92**	**46**	0.06	**2.52**

ated with the presence of metals in the food chain, the bioaccessible amount rather than the total amount should be used [37].

Therefore, the soil-to-plant transfer factor based on the DTPA-extractable Cd and Pb values (TF_{DTPA}) for all the five vegetable species was also calculated. These values ranged from 0.15 to 86 for Cd and from 0.13 to 11 for Pb.

The plant response to heavy metals in soil depends on the plant species, the total soil metal concentration, and the bioavailability of the metal. A comparison of our results with the criteria used to classify the hyperaccumulator plants [38] indicates that the three plant species collected from the mining sites under study are not hyperaccumulators, as clearly shown by their transfer factors lower than 1 (Table 5) and can thus be considered as hypertolerant. On the other hand, *Arthrocnemum macrostachyum* and *Dittrichia viscosa*, which have TF values generally much higher than 1, can be considered as Cd- and Pb-hyperaccumulators.

CONCLUSIONS

This study was conducted to screen plants growing on a contaminated site used for Cd and Pb phytoextraction. *Dittrichia viscosa* and *Arthrocnemum macrostachyum,* with their TF values greater than 1, have the potential to be used for phytoextraction. The other plant species collected from the mining sites were hypertolerant but not hyperaccumulators because their TF_{TOTAL} values were lower than 1. Although these plant species do not accumulate high concentrations of metals, they may be a good tool for reducing erosion effects and also for reducing the leaching of metals.

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REFERENCES

[1] Alloway, B.J. (1994) Toxic metals in soil-plant systems. Second edition, John Wiley and Sons (Eds.), Chichester, p.350.

[2] Wong, J.W.C., Ip, C.M. and Wong, M.H. (1998) Acid-forming capacity of Pb-Zn mine tailings and its implications for mine rehabilitation. Environmental Geochemistry and Health 20, 149-155.

[3] Mulligan, C.N., Yong, R.N. and Gibbs, B.F. (2001) Remediation technologies for metal-contaminated soils and groundwater, an evaluation. Engineering Geology 60,193-207.

[4] Hong, K.J., Tokunaga, S. and Kajiuchi, T. (2002) Evaluation of remediation process with plant-derived biosurfactant for recovery of heavy metals from contaminated soils. Chemosphere 49, 379-387.

[5] Ko, I., Lee, C.H., Lee, K.P., Lee, S.W. and Kim, K.W. (2006) Remediation of soil contaminated with arsenic, zinc, and nickel by pilot-scale soil washing. Environmental Progress 25, 29-48.

[6] Virkutyte, J., Sillanpää, M. and Latostenmaa, P. (2002) Electrokinetic soil remediation-critical overview. Science of the Total Environment 289, 97-121.

[7] McGowen, S.L., Basta, N.T. and Brown, G.O. (2001) Use of diammonium phosphate to reduce heavy metal solubility and transport in smelter-contaminated soil. Journal of Environmental Quality 30, 493-500.

[8] Matusik, J., Bajda, T. and Manecki, M. (2008) Immobilization of aqueous cadmium by addition of phosphates. Journal of Hazardous Materials 152, 1332-1339.

[9] Kosobucki, P., Kruk, M. and Buszewski, B. (2008) Immobilization of selected heavy metals in sewage sludge by natural zeolites. Bioresource Technology 99, 5972-5976.

[10] Brown, S.L., Henry, C.L., Chaney, R.L., Compton, H. and DeVolder, P. (2003) Using municipal biosolids in combination with other residuals to restore metal-contaminated mining areas. Plant and Soil 249, 203-215.

[11] Bertocchi, A.F., Ghiani, M., Peretti, R. and Zucca, A. (2006) Red mud and fly ash for remediation of mine sites contaminated with As, Cd, Cu, Pb and Zn. Journal of Hazardous Materials 134, 112-119.

[12] Terzano, R., Spagnuolo, M., Medici, L., Tateo, F. and Ruggiero, P. (2005) Characterization of different coal fly ashes for their application in the synthesis of zeolite x as cation exchanger for soil remediation. Fresenius Environmental Bulletin 14, 263-267.

[13] Garau, G., Castaldi, P., Santona, L., Deiana, P. and Melis, P. (2007) Influence of red mud, zeolite and lime on heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a contaminated soil. Geoderma 142, 47-57.

[14] Pérez-Sirvent, C., García-Lorenzo, M.L., Martínez-Sánchez, M.J., Navarro, M.C., Marimón, J. and Bech, J. (2007) Metal-contaminated soil remediation by using sludges of the marble industry: Toxicological evaluation. Environmental International 33, 502-504.

[15] Dirilgen, N., Atay, N.Z., Cagdas, M. and Tunc, H. (2008) Synergistic and antagonistic effects of SDS, iodide and citric acid on soil remediation. Fresenius Environmental Bulletin 17, 316-325.

[16] Cunningham, S.D. and Berti, W.R. (1993) Remediation of contaminated soils with green plants: an overview. In Vitro Cellular& Developmental Biology 29, 207-212.

[17] Raskin, I., Kumar, P.B.A.N., Dushenkov, S. and Salt, D.E. (1994) Bioconcentration of heavy metals by plants. Current Opinion Biotechnology 5, 285-290.

[18] R. Font, R., del Río, M. and de Haro, A. (2002) Use of near infrared spectroscopy to evaluate heavy metal content in *Brassica juncea* cultivated on the polluted soils of Guadiamar river area. Fresenius Environmental Bulletin 11, 777-781.

[19] Del Río, M., Font, R. and De Haro, A. (2004) Heavy metal uptake by *Brassica* species growing in the polluted soils of Aznalcóllar (Southern Spain). Fresenius Environmental Bulletin 13, 1439-1443.

[20] Yoon, J., Cao, X., Zhou, Q. and Ma, L.Q. (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Science of the Total Environment 368, 456-464.

[21] Del Río, M., Font, R., Vélez, D., Almela, C., Montoro, R. and De Haro, A. (2002) Uptake and distribution of arsenic in *Amaranthus blitoides* growing on contaminated soils. Freseniun Environmental Bulletin 11, 589-593.

[22] Adriano, D.C. (2001) Trace elements in terrestrial environments: Biogeochemistry, bioavailability and risks of metals. Second edition, Springer-Verlag, New York, p.866.

[23] Baker, A.J.M., McGrath, S.P., Reeves, R.D. and Smith, J.A.C. (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry, N., Banuelos, Q. (Eds.) Phytoremediation of contaminated soil and water. Lewis Publishers, Boca Raton, 85- 197.

[24] Navarro, M.C., Pérez-Sirvent , C., Martínez-Sánchez, M.J., Vidal, J. and Marimón, J. (2006) Lead, cadmium and arsenic bioavailability in the abandoned mine site of Cabezo Rajao (Murcia, SE Spain). Chemosphere 63, 484-489.

[25] Peech, M. (1965) Hydrogen-ion activity. In: Black, C.A. (Ed.) Methods of Soil Analysis. Part 2. Agronomy, American Society of Agronomy, Madison, Wisconsin, 922-923.

[26] Hulseman, J. (1966) An inventory of marine carbonate materials. Journal of Sedimentary Petrology 36, 622- 625.

[27] Muller, G. and Gatsner, M. (1971) Chemical analysis. Neues Jahrbuch fur Mineral Monatshefte 10, 466- 469.

[28] Nelson, D.W. and Sommers, L.E. (1982) Total carbon, organic carbon, and organic matter. In: Page, A.L. (Ed.) Methods of soil analysis. Part 2, American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, 539-580.

[29] Duchaufour, Ph. (1970) Précis de Pedologie. Masson and Cie (Eds), París, p.481.

[30] Lindsay, W.L. and Norvell, W.A. (1978) Development of DTPA soil test for Zn, Mn and Cu. Soil Science Society of America Journal 42, 421-428.

[31] Ruby, M. V., Davis, A., Link, T. E., Schoof, R., Chaney, R. L., Freeman, G. and Bergstrom, P. (1993) Development of an *in vitro* screening test to evaluate the *in vivo* bioaccessibility of ingested mine-waste lead. Environmental Science&Technology 27, 2870-2877.

[32] Martín D., (2004) Qualitative, quantitative and microtextural powder X-ray diffraction analysis. www.xpowder.com.

[33] Chumbley, C.G. and Unwin, R.J. (1982) Cadmium and lead content of vegetable crops grown on land with a history of sewage sludge application. Environmental Pollution 4, 231–237.

[34] Cui, Y.L., Zhu, Y.G., Zhai, R.H., Chen, D.Y., Huang, Y.Z. and Qiu, Y. (2004) Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. Environment International 30, 785-791.

[35] Harrison, R.M. and Chirgawi, M.B. (1989) The assessment of air and soil as contributors of some trace metals to vegetable plants. III-experiment with field-grown plants. Science of the Total Environment 83, 47–63.

[36] Alloway, B.J., Jackson, A.P. and Morgan, H. (1990) The accumulation of cadmium by vegetables grown on soils contaminated from a variety of sources. Science of the Total Environment 91,223-236.

[37] McLaughlin, M.J., Zarcinas, B.A., Stevens, D.P. and Cook, N. (2000) Soil testing for heavy metals. Communications in Soil Science and Plant Analysis 31, 1661-1700.

[38] Baker, A.J.M. and Brooks, R.R. (1989) Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery 1, 81-126.

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