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Heavy metal Phytoremediation potentials of *Lepidum sativum* L., *Lactuca sativa* L., *Spinacia oleracea* L. and *Raphanus sativus* L

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This study assessed the potential phytoremedial use of *Lepidum sativum* L. spp. *sativum* (cress), *Lactuca sativa* L. convar. *sativa* (lettuce), *Spinacia oleracea* L. (spinach) and *Raphanus sativus* L. var. *niger* (radish). These plants are commonly grown agricultural crops near the Cyprus Mining Corporation (CMC) area in Cyprus. Potted plants of these species were irrigated with water from three CMC tailing ponds. Plants of each species were transplanted into plastic pots filled with 5 kg of sandy soils. Higher concentrations of four heavy metals were found in plant tissues: arsenic (As), cadmium (Cd), iron (Fe), and lead (Pb). The most suitable plant species for the remediation of As and Pb was found to be *L. sativa* with Rf of 15.30% and 12.92%, respectively. *L. sativum*, *R. sativus* and *S. oleracea* are less suitable for the remediation of those elements with smaller Rf. For other tested elements: Cd and Fe, none of the test plants' Rf were found to be above 1.61% which is very low and makes the test plants un-suitable for the remediation of Cd and Fe.

Keywords: Cyprus Mining Corporation (CMC), *Lactuca sativa* L., *Lepidium sativum* L., phytoremediation, *Raphanus sativus* L., *Spinacia oleracea* L.

INTRODUCTION

Phytoremediation, the process of using plants to remove pollutants from the environment, has emerged as a potential cost-effective solution to remove contaminats from soils (Salt et al., 1995). Although arsenic (As), (Cd), and lead (Pb) are not essential elements such as copper (Cu), iron (Fe), manganese (Mn) or zinc (Zn), they are uptaken by some plants and are potentially toxic to humans and animals (Wallace, 1989). Plants that accumulate elements in higher concentrations than other plants are termed hyperaccumulators (Baker and Proctor, 1990). Boyd and Martens (1992) attributed hyperaccumulation to a tolerance for elements and drought, uncontrollable metal uptake and self-protection from herbivores and parhogens.

According to Watanbe (1997), hyperaccumulators used for phytoextraction should have high rates of accumulation and translocation, fast growth and a high production of biomass. Brooks et al. (1997) specified hyperaccumulators as having uptake and accumulate elements above-ground at a rate of 100 times greater than normal. This amount is at least 10 ppm for Hg; 100 ppm for Cd; 1000 ppm for Co, Cr, Cu and Pb; and 10000 ppm for Zn and Ni (Baker et al., 2000). Heavy metals in the soil that were not a problem for thousands of years are becoming a problem resulting from negative actions by humans. These metals potentially threaten life in cities, agricultural areas and natural environments. On the other hand, there are approximately 400 hyperaccumulators for every heavy metal (Robinson, 1997). *Thlaspi* is a well known hyperaccumulator species where it accumulates heavy metals in its shoots at astoundingly high levels. A typical plant may accumulate about 100 ppm Zn and 1 ppm Cd. Thlaspi can accumulate up to 30,000 ppm Zn and 1,500 ppm Cd in its

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shoots, while exhibiting few or no toxicity symptoms (Brown et al., 1994). Some more plants, including canola (*Brassica napus* L.), oat (*Avena sativa* L.), and barley (*Hordeum vulgare* L.), tolerate and accumulate metals such as selenium, Cu, Ca and Zn (Banuelos et al., 1997; Ebbs et al., 1997; Brown et al., 1994).

Phytoremediation techniques of pollutants removal from the environment has been successfully tested in many locations, in the US and Europe although, full-scale applications are still limited. (Agbontalor, 2007). According to researcher, phytoremediation is not only a feasible environmental remediation option but also presents many advantages, as compared to other remediation techniques. For example Alfalfa and Juniper are used for soil and ground water phtoextraction in Ogden, Utah USA. The other good example for rhizofiltration is the sunflowers; they are used to clean radionuclides ground water in Chernobyl, Ukraine (EPA, 1998).

Mining, an important industry, results in creating potential problems and hazards for humans and environments. Mining in Cyprus began during in Copper Age (3000 B.C.E) and was important for Phoenicians, Greeks, and Romans. The name *Cyprus* originated from the word *copper* (Kurusakız and Uğur 1999). Modern mining operations in Cyprus were established in Gemikonağı by mine researches Charles Gunther and Phillip Wiseman in 1913 (Lavendar, 1962; Bear, 1963). Gemikonağı is located at western north of Cyrpus. Cyprus Mining Corporation (CMC) was established in 1916 and continued operations until 1974 (Kurusakız and Uğur, 1999). During this period, heavy metals accumulated in tailing ponds as mineral waste flowed into and polluted the Mediterranean Sea, changing the color of the seashore for 5 km.

Twelve tailing ponds near the CMC house include toxic waste from mining Cu, gold (Au), silver (Ag) and Fe (Cohen 2002). Eight tailing ponds store principally Cu; four ponds store mainly Fe pyrite. The ponds, which also include Fe, sulphur (S), and other heavy metals, are generally muddy and appear black, brown, or reddish brown. During winters, these ponds fill with water; during summers, water evaporates and ponds are covered with a hard covering. The pyrite in the ponds increases the pH of waste water and contaminates the atmosphere with SO_2 (Cohen, 2002). This study sought to determine the phytoremediation potential of *Lepidum sativum* L. spp. *sativum* (cress), *Lactuca sativa* L. convar. *sativa* (lettuce), *Spinacia oleracea* L. (spinach) and *Raphanus sativus* L. var. *niger* (radish) to accumulate heavy metals.

MATERIALS AND METHODS

Selection and production of test plants

The plants tested in this study were selected among the most frequently grown plants near the Cyprus Mining Corporation areas (CMC): *Lepidium sativum* L. ssp. *sativum* (cress), *Spinacia oleracea* L. (spinach), *Raphanus sativus* L. var. *niger* (radish) and *Lactuca sativa* convar. *sativa* (lettuce). Two families include hyperaccumulators: *Raphanus sativus* L. (Brown et al., 1994; Gáspár and Anton, 2002) and *Lactuca sativa* convar. *sativa* (Tu et al., 2002).

Seeds of these plant species were sowed into violas filled with sandy-soils (Pinto et al., 1998). The water of the 3 tailing ponds (TP) of CMC (TP 12, TP 14 and TP 17) was diluted to 10%. Tailing pond #17 is a pond of waste cupper where #12 and #14 are ponds of waste pyrite. #14 pond includes the highest heavy metal concentration where #17 pond includes the lowest. The pH of these ponds is 2.8. One viola was irrigated with tap water as a control treatment (Hinchman and Negri, 1994). Six healthy plants were selected to represent each plant species and were transplanted into plastic pots filled with 5 kg (Vysloužilová et al., 2003) of sandy soils (Küpper et al., 1999).

Data collection and analysis

Following 60 days of development (Pinto et al., 1998), the plants were uprooted from the pots, washed with pure water, placed into bags and taken to the laboratory for analysis. Plants were designated as below-ground and above-ground and decomposed by Method 7300 (NIOSH 2003) using nitric acid and perchloric acid. Soils were air-dried for 48 hours and decomposed using Method SW-846, 3050B (USEPA 1996). Element concentrations in the digests were determined by Inductively Coupled Plasma (Fassel and Kniseley, 1974; NIOSH 2003). The remediation factor (Rf) of the plants was calculated using the formula of Vysloužilová et al. (2003) by dividing mg of element removed from pot (by both shoots and roots) to the mg of element found in pot.

A check of the assumptions required for ANOVA analysis indicated that the Rf percentage of the dependent variable was normally distributed and the variances of the remediation factors were equal across groups. Thus, a two-way between-groups analysis of variance (ANOVA) was performed to explore the effects of species as measured by the Rf. Post hoc testing of group pairs for significance was performed using Duncan's multiple range test at $P < 0.05$.

RESULTS

A low pH in the tailing ponds increased the amount of heavy metal uptake in the test plants. Among the four plants and four water treatments, only *L. sativum* watered with a 10% concentration from tailing pond 14 failed to grow. This is because tailing pond 14 includes the

Table 1. Remediation Factors (Rf%) of *L. sativa, L. sativum, R. sativus* and *S. oleracea* and Post-Hoc Comparisons for Dependent Variable of Rf% for Water Treatments and Elements

Plant species	Treatments	Remediation factor (Rf%)			
		As	Cd	Fe	Pb
L. sativa	Control	0.21 (c)	0.37 (d)	0.19 (a)	0.14 (d)
	17 TP	13.49 (b)	1.29 (c)	0.04 (d)	15.31 (c)
	14 TP	23.68 (a)	2.26 (a)	0.07 (c)	17.14 (b)
	12 TP	23.83 (a)	1.81 (b)	0.12 (b)	19.09 (a)
L. sativum	Control	0.12 (c)	0.07 (c)	0.08 (a)	0.07 (b)
	17 TP	4.44 (a)	0.86 (a)	0.01 (b)	22.03 (a)
	14 TP	0.00 (c)	0.00 (c)	0.00 (c)	0.00 (b)
	12 TP	2.28 (b)	0.55 (b)	0.01 (b)	22.03 (a)
R. sativus	Control	0.55 (d)	1.27 (c)	0.61 (a)	0.48 (d)
	17 TP	13.37 (b)	1.77 (b)	0.06 (c)	14.33 (b)
	14 TP	6.52 (c)	0.70 (d)	0.02 (d)	5.89 (c)
	12 TP	27.65 (a)	2.72 (a)	0.20 (b)	24.92 (a)
S. oleracea	Control	0.18 (d)	0.23 (d)	0.10 (a)	0.11 (d)
	17 TP	5.00 (c)	0.43 (c)	0.01 (d)	10.49 (c)
	14 TP	22.03 (a)	2.09 (a)	0.08 (b)	22.69 (a)
	12 TP	13.45 (b)	0.99 (b)	0.05 (c)	15.89 (b)
L. sativa	**Average of all treatments**	**15.30 (a)**	**1.43 (b)**	**0.11 (b)**	**12.92 (a)**
L. sativum		**1.71 (d)**	**0.37 (d)**	**0.02 (d)**	**11.03 (d)**
R. sativus		**12.02 (b)**	**1.61 (a)**	**0.22 (a)**	**11.40 (c)**
S. oleracea		**10.16 (c)**	**0.93 (c)**	**0.06 (c)**	**12.29 (b)**

Duncan's multiple range test applied to the elements (As, Cd, Fe and Pb) seperately and values followed by the same letter or letters are not significantly different at a 5% level.

highest heavy metal concentration. None of the test plants, however, fulfilled the criterion to qualify as a hyperaccumulator; however, more accumulations of As, Cd, Fe, and Pb were found in test plants than would be found under normal conditions. Within the water treatment groups, the control treatments had a significantly lower remediation factor (Rf) than the three water treatments from tailing ponds 17, 14, and 12 for As, Cd and Pb (Table 1). However for Fe, the Rf of control treatment found to be more than the three treatments. This is because Fe is an essential element for plants and control water treatment had no other heavy metals which means no interaction among elements. Thus, control plants found suitable soil conditions to accumulate Fe in their body. The Rf of *L. sativa* was significantly higher ($P < 0.05$) than three other plant species for As and Pb. Therefore, most suitable plant species for the remediation of As an Pb was found to be *L. sativa* with Rf of 15.30% and 12.92%, respectively. Althouh, there are significant differencess between the Rf of *L. sativa* and the three other plant species, it can also be concluded from the results that the use of *R. sativus* and *S. oleracea* for the remediation of As and Pb, can give good results where their Rf are above 10%. On the other hand, *L. sativum*'s Rf for Pb is 11.03% which is only 1.89% less than *L. sativa*. However, *L. sativum* is not suitable for the remediation of other elements. When the problem heavy metals are Cd and Fe, the Rf of all test plants were found to be below 1.61% which is very low and makes the test plants un-suitable for the remediation of Cd and Fe. For this elements, the Rf of *R. sativus* was significantly higher ($P < 0.05$) than three other plant species.

The concentration of As removed from the soils by *L. sativum*, *L. sativa*, *S. oleracea* and *R. sativus* differed from each other and differed between water treatments (Table 2). In this study, the lowest arsenic accumulation was obtained from *R. sativus*; however, because of the high biomass, this species had the greatest Rf: 27.65% in TP 12. No significant difference was found in Rf of *L. sativa* for As among tailing ponds 12 and 14.

R. sativus accumulated the least amount of Cd, however it had the highest Rf among the test plants. The mean Cd Rf of *L. sativa*, *L. sativum*, *R. sativus* and *S. oleracea* was 1.43%, 0.37%, 1.61% and 0.93% respectively. Thus, none of the tests plants were hyperaccumulators and are not suitable for the phyroremediation of soils contamined with Cd.

Fe was the highest accumulated element found in test

Table 2. mg pot^{-1} elements removed from soil by *L. sativa, L. sativum, R. sativus* and *S. oleracea* and Post-Hoc Comparisons for Dependent Variable of mg/pot for Water Treatments and Elements

Plant species	Treatments	mg pot^{-1} elements removed by plants			
		As	Cd	Fe	Pb
L. sativa	Control	0,0001 (c)	0,0002 (c)	0,46 (d)	0,00007 (c)
	17 TP	0,008 (b)	0,0005 (b)	15,40 (c)	0,009 (b)
	14 TP	0,01 (a)	0,001 (a)	28,82 (b)	0,01 (a)
	12 TP	0,01 (a)	0,001 (a)	39,60 (a)	0,01 (a)
L. sativum	Control	0,00006 (b)	0,00004 (c)	0,18 (c)	0,00003 (b)
	17 TP	0,002 (a)	0,0004 (b)	3,79 (a)	0,01 (a)
	14 TP	0,00 (c)	0,00 (d)	0,00 (d)	0,00 (c)
	12 TP	0,01 (a)	0,0002 (a)	2,60 (b)	0,01 (a)
R. sativus	Control	0,0003 (d)	0,0009 (d)	1,45 (d)	0,0002 (d)
	17 TP	0,008 (c)	0,0006 (c)	20,89 (b)	0,008 (c)
	14 TP	0,003 (b)	0,0004 (b)	8,22 (c)	0,003 (b)
	12 TP	0,02 (a)	0,001 (a)	58,01 (a)	0,01 (a)
S. oleracea	Control	0,00009 (d)	0,0001 (d)	0,25 (d)	0,00005 (c)
	17 TP	0,003 (b)	0,0002 (c)	5,65 (c)	0,006 (b)
	14 TP	0,01 (a)	0,001 (a)	33,10 (a)	0,01 (a)
	12 TP	0,008 (c)	0,0005 (b)	15,35 (b)	0,009 (b)

Duncan's multiple range test applied to the elements (As, Cd, Fe and Pb) seperately and values followed by the same letter or letters are not significantly different at a 5% level.

plants, but its accumulation is also high in soils, and the Rf of all test plants was low. Accepting this criterion, no test plant qualified as a hyperaccumulator for Fe. The highest accumulation for Fe was 7550 ppm in above-ground parts and 18631 ppm in below-ground parts. The mean Rf for Fe in *L. sativa*, *L. sativum*, *R. sativus* and *S. oleracea* was 0.11, 0.02, 0.22 and 0.06 respectively.

Pb accumulation found in *L. sativa L. sativum*, *R. sativus* and *S. oleracea* was also moderate as in As. *L. sativum*, which did not develop when irrigated by water from TP 14, had a high Rf for Pb, thus the effectiveness of this plant to accumulate toxic materials would decrease in more contaminated soils. Although the Pb content of soils was not high, the Rf for test plants was 11.03–12.92%, qualifying the test plants as suitable for the phytoremediation of soils contaminated with Pb.

DISCUSSIONS

Concentrations of As, Cd, and Pb negatively affect plants (Chen, 1999). The presence of these elements explains why test plants developed (with one exception) but produced low biomasses. A low pH in the tailing ponds increased the amount of heavy metal uptake in the test plants. Some researchers (Wang et al., 1999) reported that soil acid concentrations also increase heavy metal uptake. The majority of hyperaccumulators contain less biomass than other plants (Baker et al., 2000; Barceló and Poschenrieder 2003). Although none of the plants qualified as hyperaccumulators, the total biomass of the plants was used to determine the amount of removed elements (mg/pot) and the Rf of each test plant. The concentrations of As, Cd, Fe and Pb removed from the soils by *L. sativum*, *L. sativa*, *S. oleracea* and *R. sativus* differed from each other and differed between water treatments. Similarly, Vysloužilová et al. (2003) found that the amount of Cd and Zn removed from seven different plants and soils varied. Stoltz and Greger (2002) also observed differences in uptake and translocation properties of the same plant species between field-grown plants and plants grown in hydroponics.

In this study, the lowest arsenic accumulation was obtained from *R. sativus*; however, because of the high biomass, this species had the greatest Rf: 27.65% in TP 12. This result supported the results of (Baker et al. 2000) where they reported that most suitable plants for phytoremediation is a hyperaccumulator producing a high biomass, but it is difficult to find both characteristics in the same plant species. The average Rf of test plants for Cd was determined as 1.09%. This percentage is too low when compared with the results of Vysloužilová et al. (2003). They reported that *Salix* spp. Species have around 83% Rf. This is because of the high biomass of *Salix* spp. species.

REFERENCES

Agbontalor EA (2007). Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries. Educational Research and Review Vol. 2 (7),151-156.

Baker AJM, Mcgrath SP, Reeves RD, Smith JAC (2000). Metal hyper accumulator plants: A review of the ecology and physiology of a biological resource for phyto remediation of metal-polluted soils. In: Phytoremediation of contaminated soil and water. N. Terry, G. Banuelos (eds). Lewis Publishers, London, New York, Washington, 85-107.

Baker AJM, Proctor J (1990). The influence of cadmium, copper, lead, and zinc on the distribution and evolution of metallophytes in the British Isles. Plant Syst Evol, 173: 91-108.

Banuelos GS, Ajwa HA, Mackey B, Wu L, Cook C, Akohoue S, Zambruzuski S (1997). Evaluation of different plant species used for phytoremediation of high soil selenium. Journal of Environmental Quality. 26: 639-646.

Barceló J, Poschenrieder C (2003). Phytoremediaiton: Principles and perspectives. Contributions to Science, 2: 1-4.

Bear LM (1963). The mineral resources and mining industry of Cyprus. Ministry of Commerce and Industry, Geological Survey Department: Bulletion No. 1, Cyprus.

Boyd RS, Martens SN (1992). The *raison d'être* of metal hyperaccumulation by plants. In The vegetation of ultramafic (serpentine) soils. Baker AJM, Proctor J, Reeves RD (Eds). Intercept, Andover pp. 279-281

Brooks RR, Lee J, Reeves RD, Jaffré T (1977). Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. Journal of Geochemical Exploration, 7: 49-77.

Brown SL, Chaney RL, Angle JS, Baker AJM (1994). Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc and cadmium-contaminated soil. J. Envirol Qual 23: 1151-1157.

Cohen HA (2002). Phase I environmental assessment of the Lefka-Xeros area of northern Cyprus. USAID Project, Funded by USAID and UNDP. Environmental Society of Lefka, Cyprus. 147p.

Chen ZS (1999). Relationship between Heavy Metal Concentration in Soils of the Taiwan and Uptake by Crops. Taiwan ROC, 15p.

Ebbs SD, Lasat MM, Brady DJ, Cornish J, Gordon R, Kochian LV (1997). Phytoextraction of cadmium and zinc from a contaminated soil. J. Environmental Quality 26 : 1424-1430.

EPA (1998). A Citizen's Guide to Phytoremediation. EPA 542-F-98-011. United States Environmental Protection Agency. p. 6. Available at: http://www.bugsatwork.com/XYCLONYX/EPA_GUIDES/PHYTO.pdf

Fassel VA, Kniseley RN (1974). Inductively coupled plasma optical emission spectroscopy. Anal. Chem, 46(13): 1110A-1120A.

Gáspár GM, Anton A (2002). Heavy metal uptake by two radish varieties. Proceeding of the 7th Hungarian Congress on Plant Physiology, 46: 113-114.

Hinchman R, Negri C (1994). The grass can be cleaner on the other side of the fence. Argonne National Laboratory, 12(2): 8-11.

Küpper H, Zhao FJ, McGrath SP (1999). Cellular compartmentalization of Zinc in leaves of the hyperaccumulator *Thlaspi* caerulescens. Plant Physiology, 119: 305-311.

Kurusakiz K, Uğur H (1999). Determination of the Environmental Problems Caused by CMC (Cyprus Mining Corporation) and Pollution in the Gemikonağı Lake. Republic of Turkey, Ministry of Environment pp.19.

Lavendar D (1962). The story of Cyprus Mines Corporation: San Marino, California, Henry E. Huntington Library and Art Gallery p.387

NIOSH (2003). Elements by ICP (Nitric/Perchloric acid ashing). Manuel of Analysis Methods, Method 7300, 4th ed.

Pinto AP, Vilar MT, Pinto FC, Mota AM (1998). *Sorghum* cultures in Cadmium contaminated soils. Influence of Organic Matter: 1-23.

Robinson BH (1997). The phytoextraction of heavy metals from metalliferous soils. PhD Thesis, Massy University, New Zealand (unpublished).

Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet I, Raskin I (1995). Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Biotechnol, 13: 468-475.

Stoltz E, Greger M (2002). Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environ. Exp. Bot., 47: 271–280.

Tu C, Ma LQ, Bondada B (2002). Plant and environment interactions. Arsenic accumulation in the hyperaccumulator Chinese brake and its utilization potential for phytoremediaiton. J. Environ Qual. 31: 1671-1675.

USEPA (1996). Acid digestion of sediments, sludges and soils, SW-846, Method 3050B, Revision 2.

Vysloužilová M, Tlustoš P, Száková J (2003). Cadmium and zinc phytoextraction potential of seven clones of *Salix* spp. planted on heavy metal contaminated soils. Plant Soil Environ 49(12): 542-547.

Wallace A (1989). Plant responses to some hardly known trace elements and trace element composition and distribution in plants. Soil Sci 147: 461-464.

Wang J, Huang CP, Allen HE, Poesponegoro I, Poesponegoro H, Taikiyama LR (1999). Effects of dissolved organic matter (DOM) and pH on heavy metal uptake by sludge particles exemplified by Cu(II) and Ni(II): There-variable model. Water Environmental Research 71: 139-147.

Watanbe ME (1997). Phytoremediation on the brink of commercialization. Environ. Sci. Technol. 31: 182–186.