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In vitro phytoremediation potential of heavy metals by duck weed *Lemna polyrrhiza* L. (Lemnaceae) and its combustion process as manure value

M.N. Abubacker,[1,*] C. Sathya[2]

[1] PG & Research Department of Biotechnology, National College (Autonomous), Tiruchirapalli- 620001, Tamilnadu, India
[2] Department of Botany, Seethalakshmi Ramaswami College, Tiruchirapalli- 620002, TamilNadu, India

ORIGINAL RESEARCH ARTICLE

ABSTRACT

In vitro experiments on chromium, copper, lead and zinc bioaccumulation using duck weed *Lemna polyrrhiza* L. (Lemnaceae) was conducted with 5, 10 and 20 mg/100 mL concentrations for a period of 20 days. The SEM-EDX elemental analysis was used to characterize the interaction between the metal and plant. The results revealed the bioaccumulation of lead was high as 20.91%, followed by copper 9.71%, zinc 5.66% and chromium 1.86% was observed. The combustion process of metal–loaded *L. polyrrhiza* biomass shows the total reduction of lead 1.72%, zinc 1.47%, chromium 0.93% and copper 0.86%. The combusted biomass in the form of ash 10% + river sand passed through 1 mm sieve, sterilized was supplemented to *Brassica juncea* pot culture, which revealed the healthy growth and ensured the manure value of metal-loaded biomass.

KEYWORDS
combustion process; heavy metals; *Lemna polyrrhiza*; manure value; metal loaded; phytoremediation

1. INTRODUCTION

Accumulation of heavy metals caused due to anthropogenic activities often results in nutrient imbalance and productivity loss in land and aquatic ecosystem (Pergent and Pergent-Martini, 1999). Related researches in bioaccumulation of essential and non-essential metals using aquatic macrophytes (Singh and Ghosh, 2005; Vesk and Allaway, 1997) was found useful in monitoring and ameliorating the heavy metals in water bodies (Vajpayee et al. 1995; Whitton and Kelley, 1995). Usually the plants have the ability to accumulate heavy metals such as Cr, Fe, Mn, Pb, Zn, Cu and Ni which are utilized for the growth. Certain aquatic plants also have the tendency to absorb and accumulate heavy metals with no known specific biological function. However, excessive accumulation of heavy metals will be toxic to plants. The ability to tolerate elevated levels of heavy metals and accumulation in high concentration has evolved independently or in combination of both in different plant species (Cheng, 2003; Ernst et al.1992) The emphasis of most studies gradually shifted towards the use of aquatic plants as monitors for heavy metal water pollution. Soil and water contaminated with metals pose a major environmental and human health hazard that needs an effective and affordable technological solution. Microbial bioremediation has been successful in degradation of specific organic contaminants, but is ineffective at addressing the challenge of certain toxic heavy metal contamination (Raskin et al. 1997). In recent years, there has been a lot of interest in the study of heavy metal accumulating plants which are used for environmental remediation as well as for application, termed as phytoremediation. Phytoextraction is one method of phytoremediation in which the metal accumulating plants are used to remove pollutants from contaminated sites by concentrating in the harvestable form from the plant (Salt et al. 1995; Zhuang et al. 2007). This is a cost-effective 'green' technology which can be employed to remove toxic metals from soil and water (Chen and Cutright, 2002; Huang et al. 2011).

Corresponding author: **M. N. Abubacker**
Tel: +91 9894058524
Fax: +91 431 2458169
E. mail: abubacker_nct@yahoo.com

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In the present study the aquatic duck weed *L. polyrrhiza* L. (Lemnaceae) was subjected to heavy metal concentrations in in vitro conditions to examine the bioaccumulation potential and subsequently, its combustion process as manure value was carried out to highlight the possibility of using accumulated heavy metals as manure for plant growth.

2. MATERIALS AND METHODS

2.1. Collection of material

Lemna polyrrhiza, the duck weed employed in the present study was collected from a polluted water body in Tiruchirapalli, Tamil Nadu, India. The plant has a small round and thick leafy structure. Flat green upper and slightly convex purple lower structure contains several rootlets (Gamble, 2008). The plants were acclimatized for 5 days in tap water in 250 mL flask and then subjected to in vitro studies.

2.2. Methods

2.2.1 Phytoremediation procedures

After acclimatization, the plants were tested in in vitro condition for 3 different concentration of chromium (potassium dichromate, Merck), copper (copper(II) sulphate, Himedia), lead (lead acetate, Merck), and zinc (zinc sulphate, Himedia) at 5, 10, and 20 mg/100 mL, respectively. The respective heavy metal concentrations were added to each of the Petridish and were exposed to normal sun-light for detention time of 20 days. The Petridishes were shaken at regular interval for uniform distribution of metals in aqueous medium.

2.2.2. Anatomical studies

Control and bioaccumulated chromium, copper, lead and zinc leaves of *L. polyrrhiza* was washed with running deionised water, and were subjected to anatomical studies. The cross sections were taken with a thickness of 200-300 µm using a clean stainless-steel razor. The unstained sections were mounted in microscopic slides using a drop of glycerine covered with a cover slip and photographed by light microscopy with 100 X magnification (Olympus CH20i).

2.2.3. SEM EDX elemental analysis

SEM-EDX elemental analyses were carried out for bioaccumulation of Cr, Cu, Pb and Zn. The combustion process for manure value of all the metals was also evaluated in this study. To study the nature of *L. polyrrhiza* after bioaccumulation, the leaves were collected on 20th day after exposure to respective heavy metals. They were initially dried in shade, followed by hot air oven (at 50 ^{o}C for 1 h). Using mortar and pestle, the dried material were powdered and placed in steel stub with carbon tape and sputter coated with gold particle for 50 sec in high vacuum conditions for SEM-EDX analysis. The images of *L. polyrrhiza* biomass after phytoremediation was captured using scanning electron microscope coupled with energy dispersive X-ray consisting 3.5 nm and 2.5 nm resolution for tungsten filament (LaB6) and EDX detector resolution 133 eV. (TESCON, Czechoslovakia) (Jamari et al., 2014).

2.2.4. Pot culture studies

Pot culture studies were conducted using *Brassica juncea* at three different formulations to analyze the growth conditions. Each pot incorporated with 20

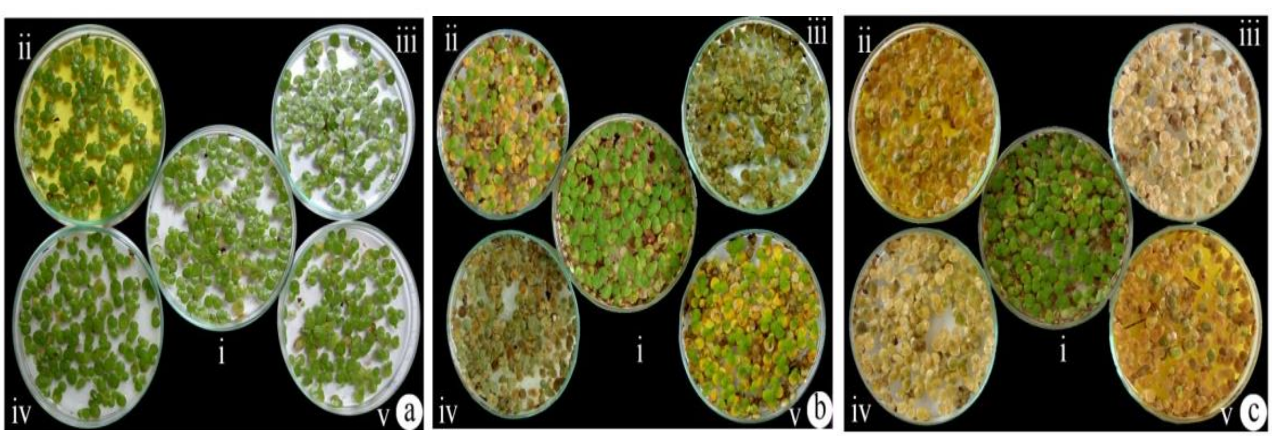

Figure 1. Bioaccumulation of heavy metals by *L. polyrrhiza*. (i) control (water), (ii) chromium, (iii) copper, (iv) lead, and (v) zinc; (a) initial stage of bioaccumulation (day 1), (b) second stage of bioaccumulation (day 10), and (c) third stage of bioaccumulation (day 20).

mg/100 mL of (i) heavy metals (Cr, Cu, Pb, Zn) (ii) dried *L. polyrrhiza* biomass after phytoremediation and (iii) combusted metal-loaded biomass as manure. The controls were maintained with tap water and were carried out for a period of 20 days.

3. RESULTS AND DISCUSSION

3.1. Bioaccumulation analysis of heavy metals

Studies on bioaccumulation of heavy metals such as Cr, Cu, Pb and Zn were conducted on *L. polyrrhiza* (aquatic weed) at 5, 10 and 20 mg/100 mL concentrations for a period of 20 days. The results indicated that *L. polyrrhiza* was able to accumulate the heavy metals and there were no morphological changes observed and remain healthy till 9th day of experimental condition. Subsequently, observation on the 10th day indicated that the plant morphology has changed due to the accumulation of heavy metals and the survival percentage was found different. Cr and Zn accumulated plants both showed 50% survival, Cu accumulated plants showed 40% whereas Pb accumulated plants showed 30% survival. On the 20th day of observation, there was further reduction in survival percentage. It was observed that Cr, Zn accumulated plants showed 20% survival, whereas Cu and Pb accumulated plants exhibited 10% survival (Figure 1).

Accumulation of heavy metals in plant causes negative growth effects and also reduces their photosynthetic process (Sandalio et al. 2001). The metal accumulation in *L. polyrrhiza* and its subsequent anatomical studies have shown that the heavy metals were accumulated in the mesophyll tissues and more profusely on the cell wall in accordance with study of *Thlaspi caerulescens* (Wojcik et al. 2005). The bio accumulated leaf of *L. polyrrhiza* was sectioned, examined and micro-photographed as indicated in section 2.2. The micro-photography of unstained leaf anatomy revealed the bioaccumulation of Cr and Cu in the mesophyll tissue (Figure. 2). Since Pb and Zn are colorless heavy metals; leaf anatomy appears similar to control.

3.2. SEM - EDX elemental analysis of heavy metals

Scanning electron microscopy equipped with Energy Dispersive X-Ray (SEM-EDX) analysis was conducted to detect the bioaccumulation of heavy metals at cellular and sub-cellular levels in *L. polyrrhiza* biomass in single analysis revealed 1.86% for chromium, 9.71% for copper, 20.91% for lead and 5.65% for zinc. In control sample, these metals were not detected (Table 1, Figure 3).

SEM analysis of *L. polyrrhiza* biomass samples clearly reveals the surface texture and pores in the materials along with the morphological changes with respect to shape and size of the materials after accumulation of heavy metal ions. A clear difference in the surface of control compared to metal-loaded biomass samples was visualized. It was also observed that the surface of materials has changed into new particles ensure the metal sorption as reported by Giri and Patel (2012).

3.3. Pot culture studies

In final stage of experiments, the metal-loaded biomass was subjected to combustion process and the results indicated significant reduction in the percentage of heavy metals 0.93% for Cr, 0.86% for Cu, 1.47% for Zn and 1.72% for Pb than that of metal- loaded biomass before combustion process (Figure 3). The product after combustion (ash) was supplemented to *Brassica*

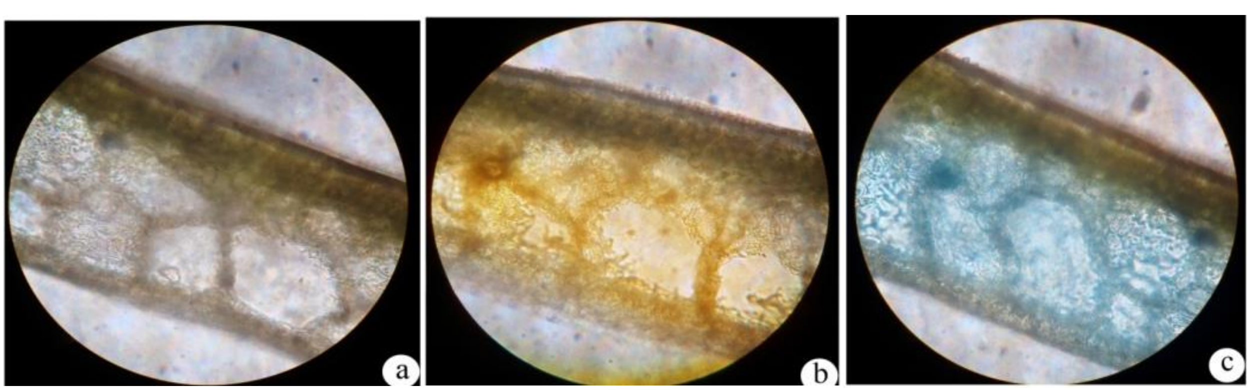

Figure 2. Leaf anatomical studies of heavy metals by *L. polyrrhiza*. (a) control, (b) bioaccumulation of chromium in mesophyll tissue, and (c) bioaccumulation of copper in mesophyll tissue

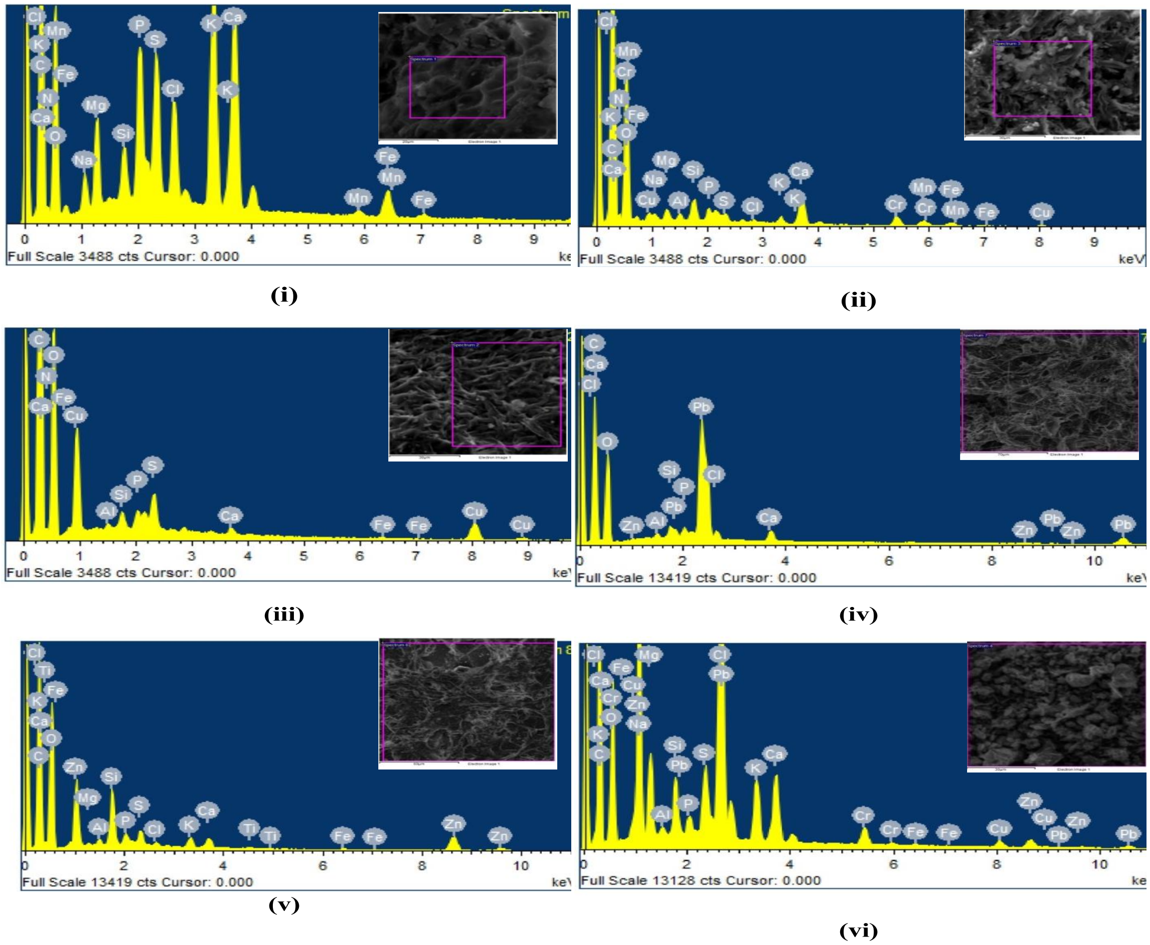

Figure 3. Elemental analysis of *L. polyrrhiza* L. using SEM-EDX. (i) control (ii) bioaccumulation of chromium (iii) copper (iv) lead (v) zinc and (vi) bioaccumulation of metals and its product after combustion

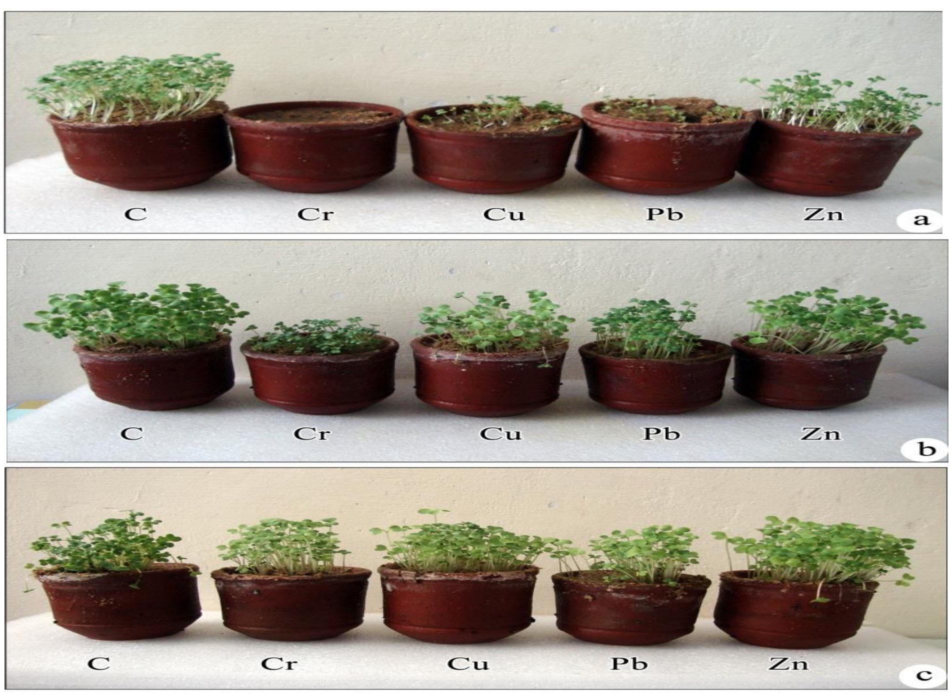

Figure 4. Pot culture Studies of *Brassica juncea*. C - control, Pot cultures supplemented with 20 mg/100 mL of (a) heavy metals (Cr, Cu, Pb, Zn) (b) Dried metal-loaded *L. polyrrhiza* biomass (c) Combusted metal-loaded biomass as manure.

Table 1. SEM-EDX analysis of bioaccumulation of metals and its combustion process in *L. polyrrhiza* biomass as manure value

Metals	Control (%)	Bioaccumulation 20 mg/100 mL				
		Cr	Cu	Pb	Zn	Combustion process (manure)
C	45.28	52.56	44.60	44.01	50.52	48.71
N	5.26	6.28	12.79	–	–	–
O	28.65	31.20	30.10	31.92	38.94	23.21
Na	0.80	0.25	–	–	–	6.23
Mg	1.38	0.36	–	–	0.12	1.66
Si	0.67	0.82	0.43	0.54	2.01	0.93
P	2.25	0.32	0.38	0.45	0.42	0.34
S	2.17	0.28	1.18	–	0.54	0.95
Cl	1.98	0.18	–	0.35	0.21	9.22
K	4.93	0.48	–	–	0.53	1.61
Ca	4.59	2.23	0.39	1.00	0.58	1.92
Mn	0.29	1.11	–	–	–	–
Fe	1.75	0.97	0.28	–	0.17	0.13
Al	–	0.20	0.13	0.20	0.20	0.14
Cr	–	1.86*	–	–	–	0.93
Cu	–	0.89	9.71*	–	–	0.86
Pb	–	–	–	20.91*	–	1.72
Zn	–	–	–	0.62	5.65*	1.47
Ti	–	–	–	–	0.11	–

*maximum sorption of respective heavy metals

juncia pot culture and it was observed that plants were found to grow healthy (Figure 4), which ensures the manure value of metal-loaded *L. polyrrhiza* biomass. This result reveals the possibility of further application of metal-loaded products in detoxification of heavy metals as reported earlier by Lassat (2002).

4. CONCLUSIONS

Contamination of the aquatic bodies by various pollutants like heavy metals and poly-aromatic hydrocarbons have caused imbalance in the natural functioning of the aquatic ecosystem. Phytoremediation works best at sites by reducing the pollutant concentration through bioaccumulation onto biomass. SEM-EDX analysis confirms the bioaccumulation of heavy metals by *L. polyrrhiza* biomass. Due to this special characteristic feature, this aquatic plant can be employed easily for cost effective and eco-friendly green technology for heavy metal reduction from the polluted aquatic ecosystem and also recycle these heavy metal pollutant as manure through combustion process.

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REFERENCES

Chen, H. and Cutright, T.J. (2002) The interactive effects of chelator, fertilizer, and rhizobacteria for enhancing phytoremediation of heavy metal contaminated soil. Journal of Soils and Sediments, 2, 203-210.

Cheng, S. (2003) Heavy metals in plants and phytoremediation. Environmental Science and Pollution Research, 10, 335-340.

Ernst, W.H.O., Vekleji, J.A.C. and Schat, H. (1992) Metal tolerance in plants. Acta Botanica Neerlandica, 41, 229-248.

Gamble, J.S. (2008) Flora of the Presidency of Madras. Vol. III. Bishen Singh Mahendra Pal Singh Publishers, Dehra Dun, pp. 1110-1111.

Giri, A.K. and Patel, R.K. (2012) Phytoaccumulation potential and toxicity of arsenic ions by *Eichhornia crassipes* in hydroponic system. Journal of Bioremediation and Biodegradation, 3, 137-142.

Huang, H., Yu, N., Wang, L. and Yang, X. (2011) The phytoremediation potential of bioenergy crop *Ricinus communis* for DDTs and cadmium co-contamination soil. Bioresource Technology, 102, 11034-11038.

Jamari, S., Embong, Z. and Bakar, I. (2014) Elemental composition study of heavy metal (Ni, Cu, Zn) in riverbank soil by electrokinetic-assisted phytoremediation using XRF and SEM/EDX. AIP Conference proceedings, 1584, 221-227.

Lassat, M. (2002). Phytoextraction of toxic metals: A review of biological mechanisms. Journal of Environmental Quality, 31, 109-120.

Pergent, C. and Pergent-Martini, C. (1999) Mercury levels and fluxes in *Podosonia oceanica* meadows. Environmental Pollution, 106, 33-37.

Raskin, I., Smith, R.D. and Salt, D.E. (1997) Phytoremediation of metals: Using plants to remove pollutants from the environment. Current Opinion in Biotechnology, 8, 221-226.

Sandalio, L.M., Dalurzo, H.C., Gomes, M., Romero-Puertas, M.C. and Del-Rio, L.A. (2001) Cadmium induced changes in the growth and oxidative metabolism of pea plants. Journal of Experimental Botany, 52, 2115-2126.

Salt, D.E., Blaylock, M., Kumar, P.B.A.N., Dushenkov, V., Ensley, B.D., Chet, L. and Raskin, L. (1995) Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Biotechnology, 13, 468-474.

Singh, S.P. and Ghosh, M. (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Applied Ecology and Environmental Research, 3, 1-18.

Vajpayee, P., Rai, U.N., Sinha, S., Tripathi, R.D. and Chandra, P. (1995) Bioremediation of tannery effluent by aquatic macrophytes. Bulletin of Environmental Contamination and Toxicology, 55, 546-553.

Vesk, P.A. and Allaway, W.G. (1997) Spatial variation of copper and lead concentrations of water hyacinth plants in a wetland receiving urban run-off. Aquatic Botany, 59, 34-44.

Whitton, B.A. and Kelley, M.G. (1995) Use of algae and other plants for monitoring rivers. Australian Journal of Ecology, 20, 45-56.

Wojcik, M., Vangronsveld, J., D'Haen, J. and Yukiendorf, A. (2005) Cadmium tolerance in *Thlaspi caerulescens*. Environmental and Experimental Botany, 53, 163-171.

Zhuang, P., Yang, Q.W., Wang, H.B. and Shu, W.S. (2007) Phytoextraction of heavy metals by eight plant species in the field. Water Air and Soil Pollution, 184, 235-242.