Trakia Journal of Sciences, No 1, pp 60-65, 2013

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

PRESENTATION OF BROOKLIME (*VERONICA BECCABUNGA* L. SSP. *ABSCONDITA* M.A. FISCHER) AS A NEW HYPERACCUMULATOR OF LEAD AND CADMIUM

N. Hosseini [1]*, B. Asghari [2], A. Hossein K. Abadi Frahani [3], M. Ghorbanpour[1]

[1]Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran
[2] Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran
[3]Department of Animal Science, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran

ABSTARCT

As a part of our screening program for aquatic plants of the flora of Iran with the high capacity of heavy metals accumulation, in current study effectiveness of brooklime (*Veronica Beccabunga* L. ssp. *abscondita* M.A. Fischer) was tested for the removal of lead (Pb) and cadmium (Cd) from the solutions containing these metals.

For this purpose, wild growing plant seedlings after adaptation to new conditions were planted in pots with different Pb (0, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 200, 300 mg L^{-1}) and Cd concentrations (0, 0.025, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100 mg L^{-1}). Results showed that there was a linear relationship between heavy metals concentrations and accumulation of these elements in plant tissues. Maximum values of Pb (34.0 mgg^{-1}) and Cd (123.1 mgg^{-1}) were detected in plants grown in 300 and 50 mg L^{-1} of these chemicals, respectively. Considering the levels of concentrated heavy metals in plant tissues which exceeded 1 mg g^{-1} (Pb) and 0.1 mg g^{-1} (Cd) as standards. Therefore, Brooklime could be introduced as a new and potent hyperaccumulator of Pb and Cd and has the potential to be used for remediation of contaminated water resources.

Key words: *Veronica Beccabunga* L. ssp. *abscondita* M.A. Fischer, brooklime, aquatic plants, heavy metals, hyperaccumulator, phytoremediation

INTRODUCTION

Since the beginning of the industrial revolution, contamination of the biosphere by organic and inorganic contaminants has dramatically been accelerated (1). This contamination has created a serious problem for both human health and environment. Heavy metals, which are released into environment at increasing rates naturally or as a result of various human activities such smelting, mining, agriculture and waste disposal technologies, constitute the main group of inorganic pollutants and a considerable large area of land is contaminated by them (2-3). Since these chemicals are not degradable, therefore, they can remain in the environment for an almost indefinitely time, although their bioavailability changes considerably depending on interactions with the various soil constituents (4).

Environmental remediation is the removal process of contaminants from the impacted sites. Based on ion exchange, electrodialysis, reverse-osmosis and chemical precipitation, several methods of removing heavy metals from water have been developed and used with some success. These technologies have different efficiencies for different metals and may be very

***Correspondence to**: *Naser Hosseini, Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak 38156-8-8349, Iran, Tel.: +98 861222726; n-hosseini@araku.ac.ir*

costly if large volumes, low metal concentrations and high clean up standards are involved (5, 6, 7-8).

Over the last decades, phytoremediation has gained increasing attention due to its cost effective, simplicity, sustainability, large-scale applicability, aesthetically more attractive and environmentally friendly nature for *in situ* removal of pollutants (4-9). This strategy uses plants and their associated rhizospheric microorganisms to remove, degrade, transform, immobilize or stabilize contaminants located in the atmosphere and soil and water resources (9-10). It has been shown that some plant species have the ability to uptake and accumulate heavy metals from their environment which have even no known biological function (11). Of the known aquatic species which have the ability to remove heavy metals from water are *Eichhornia crassipes* (12-13), *Pistia stratiotes*, *Spirodela polyrrhiza* and *E. crassipes* (6), *Hydrocotyle umbellata* (14), *Lemna minor* (15, 16-17), *Potamogeton pectinatus* and *P. malaianus* (18), and *Myriophyllum spicatum* (19).

Hyperaccumulators are plants which have the potential to uptake and retain large quantities of metals into their organs (20) quite suitable for phytoremediation purposes. A hyperaccumulator should be able to concentrate at least 10 mg g^{-1} of Zn or Mn, 1 mg g^{-1} of Ni, Co, Cr, Cu or Pb and 0.1 mg g^{-1} of As or Cd in its tissues. Different species of the genera *Brassica*, *Alyssum*, *Arabidopis* and *Petrisis* are among the most widely studied plants of the more than 400 hyperaccumulating vascular plants (11). However, the efficiency of metal removal by aquatic plants is low because of their small size and slow-growing roots. Therefore, exploration of new plant species capable of tolerate and accumulate high amounts of heavy metals with the fast growth and robust growth habit has become an important aspect of phytoremediation technology.

As a part of our screening program for aquatic plants of the flora of Iran with the high capacity of heavy metals accumulation, we have found *Veronica Beccabunga* L. ssp. *abscondita* M.A. Fischer (21) as a hyperaccumulator of lead (Pb) and cadmium (Cd) in a preliminary investigation. The plant is a member of figwort family (Scrophulariaceae) and grows widely on the margins of brooks and ditches in some parts of the country including west and center. Therefore, the overall objective of the present study was to evaluate the potential of this species to uptake and accumulate major pollutant metals Pb and Cd from tested solutions under greenhouse condition.

MATERIALS AND METHODS

1. Chemicals

All the chemicals used in the present study including cadmium nitrate ($Cd(NO_3)_2.4H_2O$) and lead nitrate ($Pb (No_3)2$) were purchased from Merck (Darmstadt, Germany).

2. Plant Materials and Metal Exposure

Seedlings of *V. Beccabunga* L. ssp. *abscondita* M.A. Fischer plant were collected from its natural habitat in Abbasabad, Shazand, Markazi province, Iran, in 2009. After collection, they were gently washed with distilled water and then planted in plastic basins containing Hoagland's nutrient solution (22) under greenhouse conditions (14 h photoperiod; 15 °C day/11 °C night).

After two weeks, adopted and uniform plants were placed in pots containing 3% Hoagland's nutrient mediums supplemented with twelve concentrations of Pb (0, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 200 and 300 mg L^{-1}) and eleven concentrations of Cd (0, 0.025, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100 mg L^{-1}). Plants that were cultured in nutrient mediums without heavy metals were treated as control. The pH of all solutions was adjusted to 6.5. Cultures were maintained under above mentioned conditions for further two weeks.

3. Measurement of metals in plant tissues

At the end of experiment, in each treatment three replicates of 40 g fresh weights were harvested and subjected to analysis of metals. For this purpose, plant samples were rinsed under distillated deionized water to remove residue of nutrient solutions. The extraction of heavy metals was performed according to the method of with a minor modification (23). Briefly, plant tissues were dried at 70 °C for 48 h and coarsely ground and sieved. Afterwards, 1 g of the triturated samples was burned and then heated to 600 °C for 6 h. Plant ash were wet washed in

Teflon beaker using a 2:1 HNO_3/HCl acid mixture and heated to 110 °C for 6 h. After cooling, the extracts were filtered and then diluted with distilled deionized water to a total volume of 100 ml. Finally, heavy metal content of plant extracts was determined by using a flame atomic absorption spectrophotometer (Model Perkin-Elmer, 2380).

4. Experimental design and data analysis

The analysis of variance (One-way ANOVA) of obtained data was performed using a SPSS 11.0 (SPSS Inc.; Chicago, IL) statistical computer package. Also, Duncan's multiple range test was used to compare mean values at the 5% probability level.

RESULTS AND DISCUSSION

Brooklime plants exposed to higher concentrations of cadmium (more than 50 mg L^{-1}) showed various degrees of toxicity symptoms. As can be seen in **Fig. 1**, plants placed in solutions supplemented by 100 mg L^{-1} of this chemical died at the first day of exposure while those grown in 50 mg L^{-1} for 7 days showed toxicity symptoms including chlorosis and early senescence mostly on the mature leaves. However, Pb did not induced considerable symptoms of phytotoxicity and plants survived in any concentration of this metal.

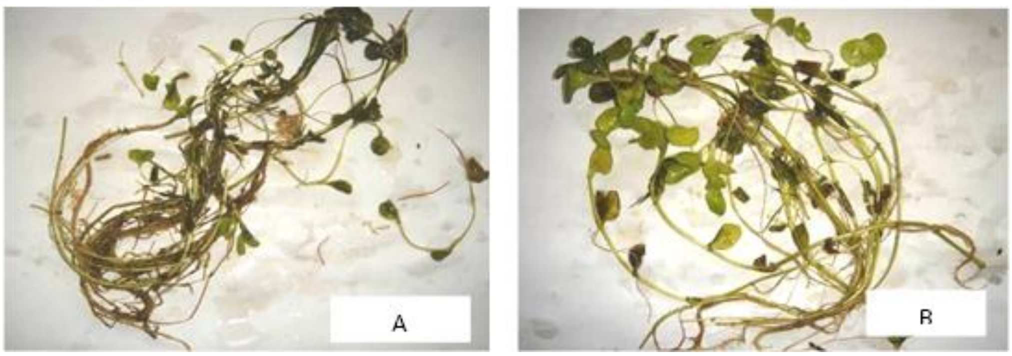

Fig. 1. Phytotoxicity symptoms in *V. Beccabunga* ssp. *abscondita* plants following exposure to 100 (A) and 50 (B) mg l^{-1} Cd.

The uptake and accumulation of Cd and Pb in *V. Beccabunga* ssp. *abscondita* plants were in general increased with the increase in metal concentration of nutrient solutions (**Fig. 2**). The highest amounts of Pb (34.0 mgg^{-1}) and Cd (123.1 mgg^{-1}) were detected in the tissues of plants grown in concentrations of 50 and 300 mg L^{-1} of these metals, respectively **(Tables 1 and 2).** This nearly linear pattern of metal uptake suggests that both active and passive transport mechanisms are involved.

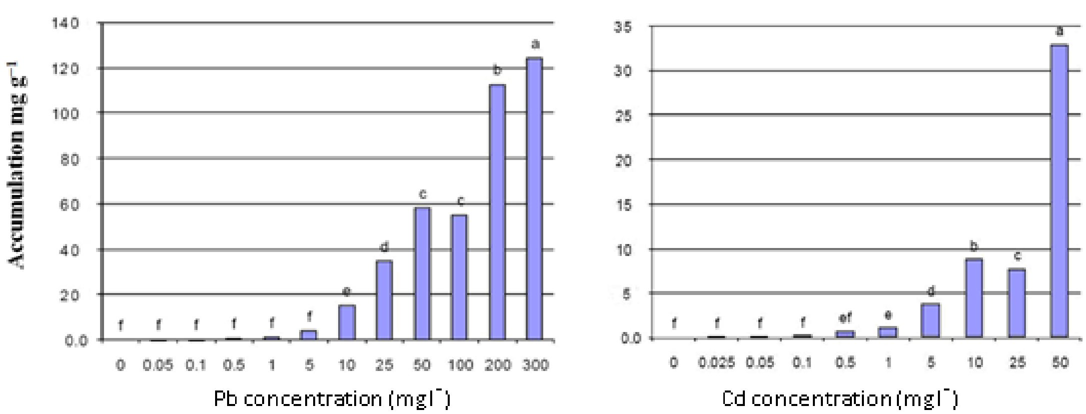

Fig. 2. Pb and Cd content of *V. Beccabunga* ssp. *abscondita* plants cultivated in various concentrations of these elements.

In recent years, restoration of soils and water resources contaminated by different classes of pollutants has become a major global issue. On the base of constitutive and adaptive mechanisms of plants for tolerance or accumulation of high concentrations of contaminants in their rhizospheres, they offer a promising and inexpensive method of decontamination (3). This important completes in several ways including phytoextraction, phytodegradation, rhizofiltration, phytostabilisation and phytovolatilisation of contaminants (24).

According to the literature, plants that are able to concentrate at least 1 mg g^{-1} Pb and 0.1 mg g^{-1} Cd in their tissues could be considered as hyperaccumulators of these chemicals (11). The amounts of accumulated Pb and Cd in brooklime plants grown in the solutions containing high concentrations of these metals exceeded 37.5 mg g^{-1} and 6.25 mg g^{-1}, respectively, which are considerably greater than standard levels. Considering results of present study and heavy metals accumulating capacity of other aquatic hyperaccumulators such as *Potamogeton natans* (24), *Typha latifolia* (25) and *Spirulina platensis* (26), *V. Beccabunga* ssp. *abscondita* has the potential to be considered as a potent hyperaccumulator of lead and cadmium for remediation of impacted water resources.

The bioconcentration factor (BCF) was calculated as the ratio of the trace element concentration in the plant tissues at harvest to the concentration of the element in the external environment (27). It has been shown in this investigation that the plant has high bioconcentration factors for tested elements even at low concentrations of these metals. For instance, at the lowest concentrations of Pb (0.05 mgl^{-1}) and Cd (0.025 mgl^{-1}) BCF was calculated to be as high as 1600 and 2000, respectively (**Tables 1 and 2**).

Table 1. *Bioadsorption and BCF of Pb in V. Beccabunga ssp. abscondita plants after two weeks.*

BCF	Pb accumulation mg g?1 DW	Pb concentration mgl -1
0	0.0±0 f	Cont.
1600	0.08±0.003 f	0.05
2800	0.28±0.005 f	0.1
1280	0.64±0.019 f	0.5
1140	1.14±0.013 f	1
644	3.22±0.436 f	5
1506	15.06±1.111 e	10
1385.6	34.64±0.589 d	25
1180.8	59.04±1.989 c	50
655.8	65.58±3.007 c	100
547.65	109.53±3.848 b	200
410.36	123.11±3.006 a	300

Means followed by the same letters within columns are not significantly different at the 5% probability level.

Table 2. *Bioadsorption and BCF of Cd in V. Beccabunga ssp. abscondita plants after two weeks.*

BCF	Cd accumulation mg g?1 DW	Cd concentration mgl?1
0	0±0 f	Cont.
2000	0.05±0.01 f	0.025
1400	0.07±0.01 f	0.05
1500	0.15±0.10 f	0.1
1740	0.87±0.27 ef	0.5
1130	1.13±0.24 e	1
734	3.67±0.54 d	5
805	8.05±2.09 b	10
430	10.75±0.88 c	25
680.2	34.01±6.45 a	50

Means followed by the same letters within columns are not significantly different at the 5% probability level.

CONCLUSION

Although metals appear as natural constituents of Earth's crust and many are essential for normal growth and development of living organisms, all of them are considered to be contaminant when occur in unwanted sites or in forms or concentrations that cause detrimental effects for human health or environment. At present, they are the major class of contaminant substances and a considerable large area of land is contaminated by them (2-3). Results of the present study confirm that *V. Beccabunga* ssp. *abscondita* is a new hyperaccumulator of Pb and Cd with an extraordinary accumulation capacity. Since the plant grows rapidly and densely and easily adapts to *ex situ* cultivation conditions, therefore, it has the great potential to reclaim contaminated water resources with Pb and Cd.

ACKNOWLEDGMENT

Authors are grateful to Arak University research department for financial supports of this project.

REFRENCES

1. Nriogo, J.O., Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. *Nature*, 279: 409-411. 1979.
2. Begonia, M.T., Begonia, G.B., Ighoavodha, M., Gilliard, D., Lead accumulation by tall fescue (*Festuca arundinacea* Schreb) grown on a lead-contaminated soil. International *Journal of Environmental Research*, 2: 228-233. 2005.
3. Yang, X., Feng, Y., He, Z., Stoffella, P.J., Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. *Journal of Trace Elements in Medicine and Biology*, 18: 339-353. 2005.
4. Doumett, S., Lamperi, L., Checchini, L., Azzarello, E., Mugnai, S., Mancuso, S., Petruzzelli, G., Del Bubba, M., Heavy metal distribution between contaminated soil and *Paulownia tomentosa* in a pilot-scale assisted phytoremediation study: Influence of different complexing agents. *Chemosphere*, 2: 1481-1490, 2008.
5. Martin, I. 1nd Bardos, R.P., A review of full scale treatment technologies for the remediation of contaminated soil (Final Report for royal commission on environmental pollution). EPP Publications.172 p, 1996.
6. Mishra, V.K., Tripathi, B.D., Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. *Bioresource Technology*, 99: 7091-7097. 2008.
7. Raskin, I., Kumar, P.B.A.N., Dushenkov, S., Salt, D., Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology*, 5: 285-290. 1994.
8. Salehi, P., Asghari, B., Mohammadi, F., Removal of heavy metals from aqueous solutions by *Cercis siliquastrum* L. *Journal of Iranian Chemistry Society*, 5: 80-86. 2008.
9. Nunez-Lopez, R.A, Meas, Y., Gama, S.C., Borges, R.O, Olguın, E.J., Leaching of lead by ammonium salts and EDTA from *Salvinia minima* biomass *produced during aquatic phytoremediation. Journal of Hazardous Mateerial*, 154: 623-632. 2008.
10. Ouyang, Y., Phytoremediation: modeling plant uptake and contaminant transport in the soil-plant-atmosphere continuum. Journal of Hydrology, 266: 66-82. 2002.
11. Baker, A.J.M. and Brooks, R.R., Terrestrial higher plants which hyperaccumulate metallic elements: A review of their distribution ecology and phytochemistry. *Biorecovery*, 1: 81-126, 1989.
12. Muramoto, S., Oki, Y., Removal of some heavy metals from polluted water by water hyacinth (*Eichhornia crrassips). Bulletin of Environmental Contamination and Toxicology*, 30: 170-177. 1983.
13. Zhu, Y.L., Zayed, A.M., Qulan, J.H., De-Souza, M., Terry, N., Phytoaccumulation of trace elements by wetland plants: water hyacinth. *Journal of Environmental Quality*, 28: 339-344. 1999.
14. Dierberg, F.E., Debusk, T.A., Goulet, N.A., Removal of copper and lead using a thin-film technique In: Reddy KR, Smith WH. (eds), Aquatic plants for water treatment and resource recovery. Magnolia Publishing, INC, pp 497-504, 1987.
15. Jain, S.K., Vasudevan, P., Jha, N.K., Removal of some heavy metals from polluted water by aquatic plants: studies on duckweed and water velvet. *Biological Wastes*, 28: 115-126. 1989.

16. Mo, S.C, Choi, D.S, Robinson, J.W., Uptake of mercury from aqueous solution by duckweed: the effect of pH copper and humic acid. *Journal of Environmental Health*, 24: 135-146. 1989.
17. Mohan, B.S. and Hoseti, B.B., Phytotoxicity of lead and cadmium to *lemna minor* grow in sewage stabilization ponds. *Environmental Pollution*, 98: 233-238. 1997.
18. Peng, K., Luo, C., Lou, L., Li, X., Shen, Z., Bioaccumulation of heavy metals by the aquatic plants *Potamogeton pectinatus* L and *Potamogeton malaianus* Miq and their potential use for contamination indicators and in wastewater treatment. *Science of Total Environment*, 392: 22-29. 2008.
19. Keskinkan, O., Goksu, M.Z.L, Yuceer, A., Basibuyuk, M., Forster, C.F., Heavy metal adsorption characteristics of a submerged aquatic plant (*Myriophyllum spicatum*). *Process Biochemistry*, 39: 179-183. 2003.
20. Roosens, N., Verbruggen, N., Meerts, P., Ximenez-Embun, P., Smith, J., Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. *Plant Cell and Environment*, 26: 1657-1672. 2003.
21. Fischer, M.A., *Veronica* L. In: Rechinger KH (eds), *Flora Iranica*, Graz, Akad Druck U Verlagsansalt, 147: 146-149. 1981.
22. Hoagland, D.R. and Arnon, D.I, The water culture method for growing plants without soil. *California Agricultural Experiment Station***,** 34: 71-39. 1938.
23. Topper, K. and Kotuby-Amacher, J., Evaluation of a closed vessel acid digestion method for plants analyses using inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis*, 21: 1437-1455. 1990.
24. Pulford, I.D. and Watson, C., Phytoremediation of heavy metal-contaminated land by trees-a review. *Environmental International*, 29: 529-540. 2003.
25. Fritioff, A. and Greger, M., Uptake and distribution of Zn Cu Cd and Pb in an aquatic plant *Potamogeton natans*. *Chemosphere*, 63: 220-227. 2005.
26. Alonso-Castro, A.J., Carranza, A., lvarez, C., Alfarodela-Torre, M.C., Chavez-Guerrero, L., Garcia-Dela, C.R.F., Removal and accumulation of cadmium and lead by *Typha latifolia* exposed to single and mixed metal solutions. *Archives of Environmental Contamination and Toxicology*, 57: 688-696, 1989.
27. Solisio, C., Lodi, A., Soletto, D., Converti, A., Cadmium biosorption on *Spirulina platensis* biomass. *Bioresource Technology*, 99: 5933-5937. 2008.