INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 5, p. 75-79, 2014

RESEARCH PAPER OPEN ACCESS

Lead phytoremediation of *Rosmarinus officinalis* and its effect on the plant growth

Farkhonde Ardalan[1*], Mohammad Vakili[2], Soheyla Kourepaz[2]

[1]*Department of Horticulture Science, Jiroft Branch, Islamic Azad University, Jiroft, Iran*

[3]*The Faculty of Islamic Azad University, Jiroft, Iran*

Key words: Phytoremediation, hyper-accumulator, *Rosmarinus officinalis* L., lead, heavy metals.

http://dx.doi.org/10.12692/ijb/4.5.75-79 Article published on March 08, 2014

Abstract

This project was conducted to evaluate lead absorption, accumulation rate and the effect of this metal on growth of *Rosmarinus officinalis* in Padina Research and Development greenhouse, Mashhad, Iran. The pot experiment was conducted in completely randomized design with 4 replications and 5 lead concentrations (0, 200, 400, 800 and 1000 µM). Root growth, stem growth, lead accumulation rate in root, lead accumulation rate in shoot, biological and transfer factors were measured. Analysis of variance indicated that root and stem growth decreased in treated plants with lead compared to control. No symptoms of lead poisoning were observed in plants. Lead accumulation rate were increased in root and stem with 953.5mg/kg and 1507.50mg/kg, respectively. According to the results, lead transfer and biological factors were higher than 1 in all treatments and were reached to 1.81 and 23, respectively. Considering that a plant is lead hyper-accumulator, can save higher than 1000mg/kg of it and has higher than 1 of biological and transfer factors. So this plant is considered as a hyper-accumulator plant.

* **Corresponding Author:** Farkhonde Ardalan ✉ ardalan_agri_invemtor@yahoo.com

Introduction

Soil pollution by heavy materials especially lead is one of the main environmental problems in many parts of the world (Parsadoust *et al.*, 2007). Heavy metals can be entered to the biosphere through human activities such as pesticides, mining, etc (Rouzbehani and Abedi-Koupayi, 2009). Lead loss is mostly due to low mobility in environment and high sedimentation (Nourmohammadi and Rezvani, 2005). According to the Environmental Protection Agency, lead is the most important pollutant metal in the environment. 3mg/l of lead in nutrient solution is toxic to plants, reduces plant growth in 10mg/l and is fatal in 100mg/l (Henry, 2000). Lead iterance to plant more than 30mg/kg causes effects such as inhabitation of seed germination, leaves discoloration, induction of leaves necrosis, chlorosis and folding (Kopittke *et al.*, 2007; Li *et al.*, 2007).

Over the past 25 years, many chemical and physical methods have been used to clean up ecosystems that often are associated with huge costs (Rouzbehani and Abedi-Koupayi, 2009). Phytoremediation is a method of soil bio-mediation in which plants are used as a tool to purify contaminated soils (Ahmadi-Afzadi and Mousavi-Bideli, 2005; Bahreyninegad and Safari-Sobhani, 2007). Phytoremediation is 1000 times cheaper than the current technology while this technology is environmental friendly and vegetation cover leads to keeping and improving of soil properties. So far, more than 400 metal hyper-accumulator species are known that belongs to 45 plant species. According to conducted studies by Meagher, plants of *Brasicaeceae* family are zinc and lead hyper-accumulator in the land surrounding mining of zinc and lead (Meagher, 2000).

Strategies of these plants tolerance to metals high concentrations in soil can be a physiological reason for excessive metal accumulation in plants than metals collector. Also there are other reasons such as possibility of useful competition, drought resistance, metal absorption without any reason, defense against herbivores or pathogens like bacteria and fungi. Further investigation is needed to determine the exact causes of metal excessive accumulation in these plants (Mcgrath *et al.*, 2002).

Plants in terms of substances absorption control are divided to three groups: excluder, hyper-accumulator and indicator (Ahmadi-Afzadi and Mousavi-Bideli, 2005). Lead hyper-accumulator is defined according to three important qualities: 1. Metal concentration in shoot: metal rate must be >1000mg/kg of Pb, 2. Biological factor (metal concentration in plant/metal concentration in soil): must be more than 1 and sometimes also reaches 50-100 and 3. Transfer factor (metal concentration in shoot/ metal concentration in root): must be more than 1 (Wei and Zhou, 2005).

As regards, soil pollution with lead element is one of the most important environmental problems in many countries and phytoremediation of heavy metals in contaminated areas with hyper-accumulator plants is one of the effective and environmental friendly methods. On the other hand, according to medicinal importance and *Rosmarinus officinalis* used in urban green spaces, the effect of lead contamination on plant growth and metal absorption rate by the plant was studied.

Material and method

Hydroponic pot experiment was conducted in completely randomized design with 4 replications and 5 lead concentrations (0, 200, 400, 800 and 1000μM) in Padina Research and Development greenhouse, Mashhad, Iran. Its average temperature was 23 and 17°C at day and night, respectively.

Cutting cultivation

Earthen pots was selected with diameter of 12cm and height of 8cm. Cultivation bed was prepared containing sand peat and perlite by volume of 1:1. Annul cuttings of *Rosmarinus officinalis* was deployed in soil bed. Plants were irrigated with plain water for a week and were not given any specific nutrient solution in order to adapt to the greenhouse environment and decrease displacement stress effect.

Hoagland hydroponic nutrition

After a week, the plants were transferred to the new cultivation bed (sand peat and perlite by volume of 1:1) after root complete separation of soil and wash to the extent there was no soil around roots. At this time, Hoagland hydroponic nutrition was started. Shoot and root length was measured during this stage. Plants were fed with nutrient solution for 3 weeks and lead element was not added to them due to adaptation with new cultivation bed and decreases the effect of transfer stress.

Pb nitrat consumption

According to Sai Kachout and his colleagues' studies, 0, 200, 400, 800 and 1000µM of lead concentrations was applied after 3 weeks. Pb nitrate was used for required concentrations (Sai Kachout *et al.*, 2009).

Measurement of lead accumulation

35 days after treatment with lead, plants were harvested and root and stem length were measured. Plant samples were washed twice with plain water and once with distilled water. Root and shoot samples were dried separately at 100°C for 10min and were placed in an oven at 70°C until dried completely. 1gr of plant sample was become to ashes by graphics furnace and was digested by nitric acid and hydrochloric acid. Lead concentration was determined by using atomic absorption spectrophotometer.

Statistical analysis

Data were placed in Microsoft Office Excel 2010. Biological (metal concentration in plant/metal concentration in soil) and transfer (metal concentration in shoot/ metal concentration in root) factors were calculated by means of formula for data. Analysis of variance was performed by SPSS software. Comparison of means was performed by the Duncan's multiple range tests at the level of 5%.

Results

Analysis of variance indicated (Table 1) that lead significantly affected all measured traits (α≤0.05).

Table 1. Analysis of variance of the effect of lead on growth of *Melissa officinalis* and the metal accumulation rate in it.

				Mean	Squares (MS)		
SOV	Df	Stem growth	Root growth	Root lead concentration	Shoot lead concentration	Biological factor	Transfer factor
Pb	4	4.425*	4.73*	494571.62*	1582592.42*	100.09*	0.262*
Error	15	0.167	0.079	3193.81	198.61	0.198	0.034
CV(%)	-	0.77	0.77	0.62	0.71	0.26	0.19

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

The plant response to different lead concentrations

Results indicated (Table 2) that the highest stem (3.50cm) and root growth (3.25cm) belonged to control treatment. There was no significant difference between root growths in plants treated with lead toxic concentrations. Also the lowest shoot lead accumulation (24.50mg/kg), root lead accumulation (21.00mg/kg) and biological factor (1.43) was observed in control treatment. 1000µM treatment showed the highest shoot and root lead accumulation with 953.5mg/kg and 1507.50mg/kg, respectively. The lowest transfer factor was observed in 400µM treatment with 1.11. The highest biological (23.00) and transfer factor (1.81) belonged to 200 and 800µM treatment, respectively.

Root and stem growth under different lead concentrations

According to results (Fig. 1), 1000mg/kg treatment had the lowest stem (1cm) and root growth (0.63cm). The highest stem (3.5cm) and root growth (3.25cm) belonged to control treatment. Root and stem growth in control treatment was higher than treated plants with lead.

Discussion

Generally, lead accumulation in plants root and shoot was increased by lead addition to nutrient solution. These results were matched with results of other investigators (Boonyapookna, 2005; Chandra Sekhar, 2005; Prabha *et al*., 2007). Higher than 1 in biological factor indicated high capacity of plant lead accumulation. Higher than 1 in transfer factor showed plants tolerant mechanism and large amount of metal transferring from root to shoot that this is one of the important features of hyper-accumulator plants. These results were matched with Saderi and his colleagues' results on *Matricaria chemmomilla* (Saderi *et al*., 2011).

Table 2. Effect of different lead concentrations on measured traits of *Melissa officinalis*.

Pb concentrations	Stem growth (cm)	Root growth (cm)	Root lead concentration (mg/kg)	Shoot lead concentration (mg/kg)	Biological factor	Transfer factor
0µM	3.50a	3.25a	21.00e	24.50e	1.43e	1.17c
200µM	1.75b	1.00b	270.00d	393.00d	23.00a	1.46b
400µM	1.00c	0.75b	493.25c	484.75c	14.18d	1.11c
800µM	1.25ab	1.00b	808.75b	1463.75b	21.41b	1.81a
1000µM	1.00c	0.63b	953.5a	1507.50a	17.64c	1.60ab

Means in a column followed by the same letter are not significantly different at P≤0.05.

Root growth was significantly increased after coping with lead stress but was adapted with stress and more lead was not caused more stress. These results were consistent with Kopittke and his colleagues' results on *Vigna unguiculata* (Kopittke *et al*., 2007).

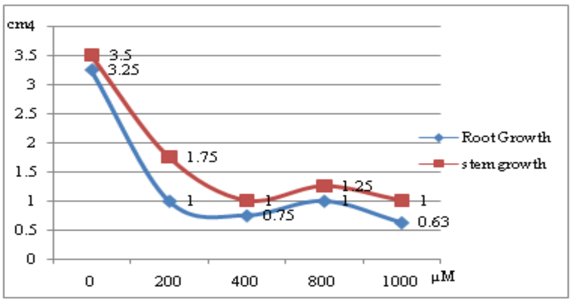

Fig. 1. The effect of lead different concentrations on root and stem growth of *Rosmarinus officinalis*.

Lead toxicity in *Elsholtiza argyi* was caused significant reduction in shoot fresh and dry weight, leaves discoloration and folding (Li *et al*., 2007). Based on the results of this study, leaves discoloration and wilting, stem hogging and root fragility were not observed in plant samples under stress of heavy metal. In fact, reduction in chlorophyll content and discoloration was not observed in resistant plants. The results were consistent with Price and Grejtovsky results (Price and Grejtovsky, 2000). According to the project, *Rosmarinus officinalis* could be considered as a lead hyper-accumulator plant.

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