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The Phytoremidiation Technique for Cleaning Up Contaminated Soil by Geranium (*pelargonium roseum*)

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ABSTRACT: Phytoremediation is the biotechnological application of plants to detoxify pollutants, and is an ideal and modern technique for environmental clean-up. Regarding the vast industrial waste materials and sewages from a lot factories and different chemical fertilizers and pesticides have caused contamination of soils in capital city Tehran , potential ability of Geranium (*pelargonium roseum*) to phytoextract lead, cadmium, Cobalt, chrome and nickel was investigated. Results indicated that the younger plants have more potential to uptake and concentrate the studied heavy metals than the older ones and the uptake rate is significantly affected by the plant accumulation and the soil pH. Our studies demonstrated the ability of several high biomass *pelargonium roseum* cultivars to hyper-accumulate lead, cadmium, nickel and chrome on contaminated soils in Tehran and other polluted cities and preventing vegetables, crops and other products to absorb toxic heavy metals. Besides helping farmers who grow crops on acid soils, phytoremediation research findings can be used by other organization in government and academia and by environmental consultants, and industry groups complying with cleanup of contaminated sites. Other fast growing plants with high biomass and good metal uptake ability are needed in Iran in order to clean contaminated soils especially near the mines.

Keywords**:** *pelargonium roseum,* hytoremidiation, Cadmium, Chrome, Lead, Nickel, soil

INTRODUCTION

According to a report released by a U.S. environmental action group, the world's most polluted places threaten the health of more than 10 million people in many countries (ENS, 2006). Most of the soil contaminants can be removed by many other physical methods but the heavy metal pollution of vast cultivated land areas are a serious threat to the agricultural biology. During the industrial developments, the level of toxic heavy metals has been increased in soil and has caused environmental contamination especially in capital city Tehran. According to a report from Department of the Environment Tehran, I.R .Iran in July 2005, some significant sources of Lead and cadmium are: Nickel-Cadmium Batteries, Cadmium pigments, Cadmium stabilizers, Cadmium Coating , fossil fuels, cement, phosphorous fertilizers, Lead batteries, Glasses & ceramic industries, paint manufacturers, Cadmium electronic compounds, Metal plating , Factories with the process of extraction, production and concentration of Lead ore, Industrial wastewater , solid waste and Municipal waste waters (Department of The Environment Tehran, I.R. Iran, 2005). Therefore, the vast industrial waste materials and sewages from a lot factories and different chemical fertilizers and pesticides in Tehran have caused contamination of soils. Heavy metal bioaccumulation in food chain could be highly dangerous to human health and on the other hand preventing heavy metal pollution is critical because cleaning contaminated soils is extremely expensive and difficult (United States Department of Agriculture / Natural Resources Conversation Service 2000).

Plants are ideal agents for soil and water remediation because of their unique genetic, biochemical and physiological features. Phytoremediation is the most emerging field of environmental biotechnology. The plant roots have natural ability to absorb the heavy metals of the soil, behaving as natural phytoremediates.

Phytoremediation consists of four different plant-based technologies that include: rhizofiltration, which involves the use of plants to clean various aquatic environments; phytostabilization, where plants are used to stabilize rather than clean contaminated soil; phytovolatilization, which involves the use of plants to extract certain metals from soil and then release them into the atmosphere through volatilization; and phytoextraction, where plants absorb metals from soil and translocate them to the harvestable shoots where they accumulate. Although plants show some ability to reduce the hazards of organic pollutants (Cunningham et al., 1995; Gordon et al., 1997; Carman et al., 1998) , the greatest progress in phytoremediation has been made with metals (Salt et al., 1995; Watanabe 1997; Blaylock and Huang 2000). Geranium(*pelargonium roseum* belongs to Geraniaceae family and There are around 800 species in the family, distributed in from 7 to 10 genera, according to the database of the Royal Botanic Gardens, Kew. Numerically, the most important genera are *Geranium* (430 species), *Pelargonium* (280 species) and *Erodium* (80 species) (Aldasoro et al., 2002; Watson and Dallwitz 2012; Stevens 2012).
They can be cultivated or grown easily in contaminated soils and a possible use for the sanitation of sludge and waste substrates.

The aims of this research were to determine the relationship between the soil pH and the phytoextraction rate based on different growth stages of the plant and the potential ability of to phytoextract different metals (Zinc, Copper, Lead, Cadmium and Nickel).

MATERIALS AND METHODS

Soil sampling

A composite soil sample was collected from depth of 0-35 cm from a yard in the center of Tehran in order to simulate the conditions of soils in the contaminated lands with industrial sewages**.** 30, 15, 10, 5, 10, 5, 5, 30 and 30 mM/L of $Pb(NO_3)_2$, Cd $(NO_3)_2$, $CrSO_4$, $Cr(NO_3)_3$, $Ni(NO_3)_2$, $Ni_3(PO_4)_2$, $zncl_2$, $CaHPO_4$ and K_2SO_4 respectively and 200 g of remaining and separating after infusion green and black tea leaves (ratio 3:1) were added. Composite soil samples (depth of 0-35 cm) were collected of Heavy metal contaminated soil by two different conditions: Geranium grown and not grown in it by different pHs after every 15 days . Metal contents were detected by Atomic Absorption Spectrophotometer by wet digestion method in Research Laboratory in Pharmaceutical Sciences Branch University.

At the beginning of study, soil profile characteristics were observed and recorded by a packet penetrometer (Cl-700A, soil Test Inc., USA). Soil samples were mixed, homogenized and separated into three parts, 1/3 of each samples was air-dried and pass through a 2 mm sieve in order to determine p and k content, pH and electrical conductivity and particle-size distribution. The other 2/3 was passed through a 2 mm sieve without drying and 1/3 of it used to determine heavy metals concentration by Atomic Absorption Spectroscopy (AAS) after digestion with aqua-regia. The samples were analyzed by an Atomic Absorption Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame for heavy metals – Pb, Cd, Cr, Ni and Zn, using at least three standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines (AOAC 1998).

Sampling method

Different parts of *pelargonium roseum* Plant samples (shoots ,roots and leaves) were separated and washed and digested by wet method according the standard protocol for measuring Cadmium, chrome, zinc, nickel and Lead. Mean values were calculated, and analysis of variance (ANOVA) and Student's t-test were performed. Bioaccumulation factors (BAF-s) were calculated for heavy metal content of plant parts (mg/kg) / heavy metal content of soil (mg/kg), for each metal.

The last port used to determine nitrate and ammonium 2M KCl extraction followed by determination using flow injection method. All the soil data are expressed on a dry basis. The soil by different pH put into eight vases and samples were grown in six examined soils and no plants were grown in two others as they have been considered as control group in soils, as the same procedure in the other reports of scientists who have investigated the effects of soil acidification on Zn and Cd phytoextraction (Moteshare Zadeh et al., 2008). As soil acidification might cause some negative side effects such as increasing solubility of some toxic metals and leaching them into the groundwater and creating another environmental risk. Therefore, at the beginning of study, we tried to control pH at the range of 5.9 up to 6.9 in samples of soils.

All samples were watered each day by tap water (Tehran tap water). The studied samples were managed by the same light situation and some circumstances in order to be compared with each other due to determine the ability of Geranium (*pelargonium roseum*) in phytoextract Lead, Zinc, Cadmium, chrome and Nickel from soil.

Physical and chemical properties and concentrations of heavy metals in soils before and after adding Cadmium, Nickel, Lead, Chrome , and zinc, and also, after the growth period measured. In order to assess amount of heavy metals transfer from soil to plant (shoot and root), translocation factor was determined by dividing metal concentration at shoot by its concentration at root Marchiol (Marchiol et al., 2004). The ratios were higher than one it was considered as suitability of plant at that condition for use in phytoremediation.

RESULTS AND DISCUSSION

Results showed significant differences in lead and cadmium up taking by different parts of plant. The best results for uptake of Nickel, cadmium and chrome was in the soil with pH=6.3 among different samples while for lead up taking was in 6.5 and for zinc up taking was in pH=6.1. This range of pH had no affecting in zinc up taking . Chemical extraction of the soil profile before adding specified amounts of heavy metals is shown in the table 1. Data is averages of the profiles.

Table 1. chemical characteristics of the soil profile at the studied vases (before adding chemical substances and pre growing of Geranium (*pelargonium roseum*)

Layer (depth cm)	pH (H_2O)	Electrical conductivity dS/cm 1:1	NO_3-N mg/kg DW	NH_4-N mg/kg DW
1 (0-15)	6.4	0.42	60.3	9.79
2 (15-35)	6.5	0.23	20.5	8.49

Plant availability of certain heavy metals depends on soil properties such as soil pH and contain exchange capacity and on the distribution of metals among several soil fractions (Plant soil Environ). The fractionation of Pb, Zn, Ni, Zn, and Cd in control soil and in soil treated by Geranium (*pelargonium roseum*) is shown in table 2.

Table 2. The characteristics of soil samples and their potential of uptake heavy metals comparing with their pH

Soil samples	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
First Crop harvest	*pelargonium roseum*	*pelargonium roseum*	*pelargonium roseum*	*pelargonium roseum*	*pelargonium roseum*	*pelargonium roseum*	--------	--------
Growth period	30 days	30 days	35 days	45 days	45 days	55 days	-----	------
Soil type (0-15cm) pH 1:1	5.9	6.1	6.3	6.5	6.7	6.9	6.7	6.9
EC 1:1 dS/m	0.29	0.46	0.45	0.52	0.38	0.44	0.46	0.47
NO_3 –N mg/kg DW	21.9	25.1	22.4	28.0	39.5	33.3	56.3	57.8
NH_4-N mg/kg DW	2.66	2.72	2.48	3.16	3.11	2.72	4.83	5.25
% Pb uptake	11.5	29.2	29.5	32.1	28.6	27.9	-----	-----
% Cd uptake	12.9	13.2	14.3	14.1	14.1	13.5	-----	-----
% Zn uptake	9.42	10.24	9.82	8.43	8.50	8.11	----	-----
% Ni uptake	6.36	8.11	8.53	6.18	5.98	5.99	-----	-----
% Cr uptake	10.95	11.55	11.66	10.76	10.62	10.20	-----	------

Results indicated that the rate of heavy metals uptake by this plant is significantly affected by pH ($p<0.02$).

Younger plants had more potential to uptake and concentrate heavy metals than older ones. Translocation factor in all conditions were higher than one which indicates that metal concentrations in shoots were higher than roots and the plant is suitable for phytoremediation .

The maximum Cadmium , lead, Chrome and Nickel uptake rate was in pH= 6.3and by 35 day *pelargonium roseum* while for zinc the best situation is pH= 6.1 and after 30 days of growing.

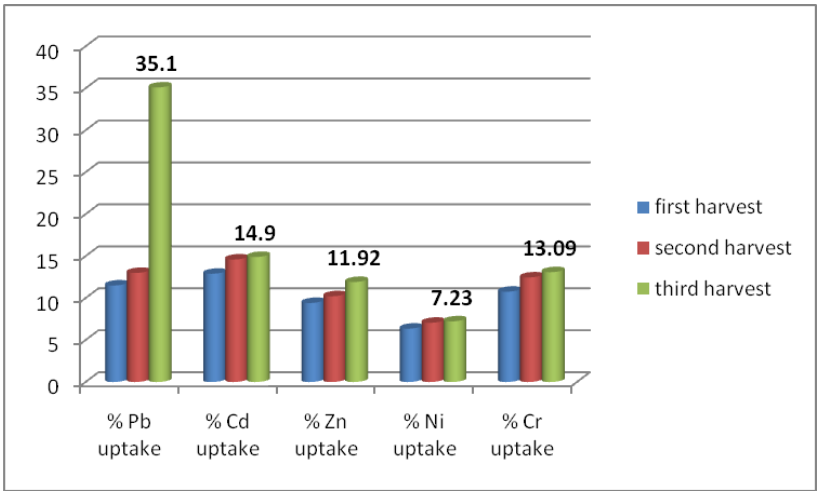

Figure 1. *pelargonium roseum* potential uptake ability of heavy metals in soil (Tea leaves added) with pH 6.5 comparing by number of harvesting

As expected the additional number of Geranium plants cultivated in sample soils increased the potion of Pb, Cd, Zn, Cr and Ni. The heavy metals uptake rate by this plant is significantly affected by number of plant cultivated as for lead uptake ($p<0.03$) while for Cd and nickel and chrome r the *p*-value was less than 0.01.

It is obvious that many countries throughout the world such as Iran and other developing countries that are grappling with the issue of contaminated environments need a low cost and effective strategy. By determining the characteristics that influence environmental contamination by heavy metals especially Lead and Cadmium, scientists will be able to map high and low risks areas of contamination. Harvesting the certain plants to accumulate, up taken and translocating metals from roots to shoots can remove toxic and heavy metals from the soil and preventing vegetables, crops and other products to absorb them. This technology is not only low cost but also applied strategy to agriculture and to environmental problems.

The results of this research concluded that in the soil which consisted of dried black and green tea leaves had suitable ability for phytoremediation by phytoextraction method and transmitting more Lead and Cadmium in pH 6.3 – 6.5 after 35-45 days of growth.

Regarding the results of the present study, it is recommended to study more on the species belong to other plant families that have potential ability to hyperaccumulate heavy metals especially the inedible plants.

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