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Research article

COMPANION *PELARGONIUM ROSEUM* AND *ROSMARINUS OFFICINALIS* IN CLEANING UP CONTAMINATED SOIL BY PHYTOEXTRACTION TECHNIQUE THE ROLE OF COMPANION PLANTS IN BOOSTING PHYTOREMEDIATION POTENTIAL

[1]Parisa Ziarati, [2]Sara Iranzad-Asl, [3]Jinous Asgarpanah

[1]Department of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran
[2]Pharmacy School, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran
[3]Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran
Corresponding Author: Parisa Ziarati e-mail: ziarati.p@iaups.ac.ir
Islamic Azad University, Pharmaceutical Sciences Branch (IAUPS), Faculty of Pharmacy, Toxicogenomics lab. No 99, Yakhchal, Gholhak, Dr. Shariati, Tehran-Iran.
Tel: +98-21-22633980; Fax: +98-21-22600099

ABSTRACT: The present study was undertaken in view of providing an impeccable hypothesis that Rosmarinus officinalis L. and pelargonium roseum in companion of each other may probably be employed as potent phytoremediators with respect to easily grown in contaminated soil and considering the possibility of boosting hyper accumulation of heavy metals (Nickel, Copper, Zinc, Chrome, Cobalt, Lead and Cadmium) by the system design. The contaminated soil was put into 45 vases in a way that rosemary and geranium were grown in ten examined soils and vases individually, in other ten vases both of plants together in order to find the effect of companion plant in possible potential phytoremediation and no plants were grown in five others as they have been considered as control group in soils. The translocation factor (TF) / mobilization ratio of metals from soil to root (TFS) and root to shoot (RFR) have been estimated. The average translocation of metals from soil to root of Rosemary in companion by pelargonium was found to be in the order of Ni (1.96) > Pb (1.70) >Cd (1.05) > Zn > Cr (0.82) > Cu (0.66) and when these values were compared with control value of rosemary samples individually it was observed to be higher in the contaminated site for Cd, Zn, Cu, Ni, Cr and Pb. The Cadmium and Lead uptake rate by rosemary is significantly affected by geranium grown and companion in the contaminated soil ($p<0.005$).
Key Words: Phytoextraction, *pelargonium roseum, Rosmarinus officinalis*, heavy metals

INTRODUCTION

During industrialization, emissions of metals have been tremendously raised and significantly exceed those from natural sources for practically all metals. Due to this mobilization of metals into the biosphere, their circulation through soil, water, and air has greatly increased [1, 2, 3, 4, 5]. 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 [6,7,8,9]. 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). Phytoremediation is the use of plants to make soil contaminants nontoxic, and is often also referred to as bioremediation, botanical bioremediation and, Green Remediation. Phytoremediation technique is a widely accepted, aesthetically pleasant, solar-energy driven, passive technique that can be used to clean up sites with shallow, low to moderate levels of contamination [10,11,12,13,14,15]. The revegetation of trace element polluted areas is a difficult task because of the presence of many-limiting factors [16].

The idea of using rare plants that hyper accumulate metals to selectively remove and recycle excessive soil metals was introduced in 1983, gained public exposure in 1990, and has increasingly been examined as a potential practical and more cost-effective technology than the soil replacement, solidification and washing strategies presently used [17,18 ,19] . Elevated concentrations of both essential and non-essential heavy metals lead to symptoms of toxicity with growth and development processes affected. The factor that determines the appropriateness of a particular contaminant for phytoremediation is solubility of contaminant [18]. Heavy metal phytotoxicity may result from alterations of numerous physiological processes caused at cellular/molecular level by inactivating enzymes, blocking functional groups of metabolically important molecules, displacing or substituting for essential elements, and disrupting membrane integrity [19, 20, 21, 22]. A rather common consequence of heavy metal poisoning is the enhanced production of ROS due to interference with electron transport activities. This increase in ROS exposes cells to oxidative stress leading to lipid peroxidation, biological macromolecule deterioration, membrane dismantling, ion leakage, and DNA-strand cleavage. About 360 species worldwide are known to act as Ni hyperaccumulators [23]. The plant families most strongly represented are the *Brassicaceae, Euphorbiaceae, Asteraceae, Flacourtiaceae, Buxaceae, and Rubiaceae*. About 90 other species are from more than 30 families, distributed throughout the plant kingdom. Normal concentrations of Co and Cu in plants are in the ranges 0.03–2 and 5–25 mg kg^{-1}, respectively. However, even on cobalt-enriched soils, such as those derived from ultramafic rocks, plant Cu rarely exceeds 20 mg kg-1. Phytostabilization appears to have strong promise for two toxic elements, chromium and lead. The reduction of Cr6+, which poses an environmental risk, to Cr3+, which is highly insoluble and not demonstrated to pose an environmental risk [24] James in the research has shown that if chromate is reduced to chromic by chemical or biological methods, the inertness and insolubility of chromic oxides in soil will limit the formation of chromate and limit environmental risk. Phytoremediation offers the ability to reduce chromate below the tilled soil layer, which is not provided by identified soil amendments to reduce chromate.

The Co and Cu accumulators have been found in more than dozen families. Extensive screening of many sites of mining and smelting activity throughout .The 30 hyper accumulators of cobalt and 32 of copper, with 12 species being common to the two lists are identified [25,26,27,28,29,30].
Rosemary (*Rosmarinus officinalisLinné*) , from the botanical family *Lamiaceae*, is a scented, evergreen shrub with a very pungent odor that is native to the Mediterranean region and Portugal; the odor is sometimes defined as camphor-like and is a common household plant, grown in many parts of the world. It is used for flavoring food and as a beverage, as well as in cosmetics [31, 32]. In folk medicine it is used as an antispasmodic in renal colic and dysmenorrheal, in relieving respiratory disorders and in stimulating the growth of hair. Rosemary extract relaxes the smooth muscles of the trachea and intestine and has choleretic, hepatoprotective and antitumerogenic activity [33, 34, 35]. Rosemary is used as a decorative plant in gardens and has many culinary and medical uses. The plant is said to improve the memory. The leaves are used to flavor various foods, such as roast meats. In this research we have studied in other plant as companion for *Rosemary* is *Geranium* as in the other previous studies we found it very potent plant for phytoremediation of Lead and Cadmium by high translocation factors.
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) [8, 9, 36, 37, 38]. The present study was undertaken in view of providing an impeccable hypothesis that *Rosmarinus officinalis* L. and *pelargonium roseum* in companion of each other may probably be employed as potent phytoremediators with respect to easily grown in contaminated soil and considering the possibility of boosting hyper accumulation of heavy metals by these system design. There are three main purposes of research:

1-Determining *Rosmarinus officinalis* L. and its companion *pelargonium roseum* for Cleaning Up Contaminated Soil and their potential ability of to phytoextract different metals (Nickel, Copper, Zinc, Chrome, Cobalt, Lead and Cadmium).
2- Compare of the phytoextraction rates for both plants based on different growth stages of the plant.
3- Determine metal transfer factors from soil (TFS) of *Rosmarinus officinalis* L. and its companion *pelargonium roseum* individually and in companion of each other due to find the possible role of companion plants in boosting phytoremediation potential.

MATERIAL AND METHODS

As a model with realistic parameters was needed to move forward, 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 waste 30 m mol/lit of $Pb(NO_3)_2$, 15 m mol/lit $Cd(NO_3)_2$, 10 m mol/lit $ZnSO_4$ and 5 m mol/lit $Zn(NO_3)_2$, 10 m mol/lit $Co(NO_3)_2 \cdot 6H_2O$, and 5 m mol/lit $CoCl_2$, 30 m mol/lit Cu Cl_2 10 m mol/lit $Ni(NO_3)_2$, and 5 m mol/lit $Ni_3(PO_4)_2$ and $NiCl_2$, 30 m mol/lit $CaHPO_4$, 5 m mol/lit $CrCl_3$ + Cr $(OH)_3$ and K_2SO_4 and 500 gram dried black and green tea leaves residues (ratio 3:1) were added.

The purpose of the model designed with respect to estimate optimal conditions for phytostabilization and its viability for a given set of contaminant and environmental conditions. 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, Co, Cu, Cr, Ni, Zn and Cu, using at least two standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines. 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. Rosemary (six month old plants) was grown in a local nursery until transplant into the research study.

Soil pH: Approximately 10 g±0.1 of prepared soil sample was weighed and about 25 mL of deionized water was added, and left to stand for one hour to equilibrate [39] Soil pH in water was recorded using a potable combo probe (Hanna Instruments,) calibrated using buffer solutions of pH 4.0 and 7.0 and pH 7.0 and 10 at 25 ºC.

Electrical conductivity

Soil suspension prepared with soil and deionized water in 1:5 ratios (10 grams of soil and 50 mL of water) was allowed to stand for one hour. Soil electrical conductivity was analyzed using a potable combo probe (Hanna Instruments).

The soil was put into 45 vases in a way that rosemary and *geranium* were grown in ten examined soils and vases individually, in other ten vases both of plants together in order to find the effect of companion plant in possible potential phytoremediation and no plants were grown in five others as they have been considered as control group in soils like the other method studies [7, 40]. 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. Samples were watered each day by deionized water. The studied samples have been managed by the same light situation and some circumstances in order to be compared with each other due to determine the effect of companion plant ability in phytoextraction of Lead, Zinc, Cadmium, Cobalt, Copper, and Nickel from soil.

Physical and chemical properties and concentrations of heavy metals in soils before and after adding heavy metals 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 [42]. Different parts of Plant samples (shoots, roots and leaves) were separated and washed and digested by wet method according the standard protocol for measuring Cadmium and Lead. Mean values were calculated, and one way ANOVA using the Minitab 15.0 statistical software was used for the analysis of data in all studies except for ageing of leaves experiments. Bioaccumulation factors (BAF-s) were calculated for heavy metal contents of plant parts (mg/kg) / heavy metal content of soil (mg/kg), for each metal. The uptake rate is given by the following equation [43, 44, 45].

$U = (TSCF) (T) (C)$ (1)

Where U = uptake rate of contaminant, mg/day

TSCF = transpiration stream concentration factor, dimensionless

T = transpiration rate of vegetation, L/day

C = aqueous phase concentration in soil water or groundwater, mg/L.

The ratios were higher than one it was considered as suitability of plant at that condition for use in phytoremediation. The enrichment factor (EF) has been calculated to derive the degree of soil contamination and heavy metal accumulation in soil and in plants growing on contaminated site with respect to soil and plants growing on uncontaminated soil [46].

RESULTS

Chemical extraction of the soil profile before adding specified amounts of heavy metals is shown in the table 1 and electrical conductivity and nitrate content in different layers is indicated in table 2. Data is averages of the profiles. Translocation factor (TF): The translocation factor (TF) / mobilization ratio of metals from soil to root (TFS) and root to shoot (RFR) have been estimated. The average translocation of metals from soil to root of Rosemary in companion by pelargonium was found to be in the order of Ni (1.96) > Pb (1.70) >Cd (1.05) > Zn > Cr (0.82) > Cu (0.66) and when these values were compared with control value of rosemary samples individually it was observed to be higher in the contaminated site for Cd, Zn, Cu, Ni, Cr and Pb. In case of shoot (root to shoot).

TFR was found in the order of Zn (1.81) > Ni (1.43) > Pb (1.22) > Co (0.89) > Cu (0.78) > Cd (0.72) > Cr (0.70) and among the metals Ni, Zn, Co and Pb TFR was found to be higher than the control value. Comparatively the translocation values from soil to root and root to shoot demonstrated lower values than the enrichment factors and did not follow the similar pattern indicating that distribution of metals in contaminated soil is quite high and their translocation from soil to root and root to shoot in a manner that is not clear is constrained .

Table 1- Physical and Chemical properties of studied soil before adding heavy metals and cultivated *pelargonium roseum* and *Rosmarinus officinalis.*

Characteristic	Quantity	Characteristic	Quantity
Soil Texture	Loam	Sand (%)	37.99
Clay (%)	23.96	Silt	38.05
Co (mg/kg DW)	0.6529	Zn (mg/kg DW)	1.0341
Cr (mg/kg DW)	0.5520	Cu (mg/kg DW)	4.2205
Ni (mg/kg DW)	1.5556	Cd (mg/kg DW)	0.1025
Pb (mg/kg DW)	1.0112		

Table 2- The characteristics of soil samples comparing with their depth and pH

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	0.51	69.1	10.33
2 (15-35)	6.7	0.33	29.9	9.11

There was a direct relationship between the age of leaves by lead, Nickel and Cadmium concentration in solution (figure 1, 2 and 3) and the severity of the response and the correlation coefficient reached -0.94.

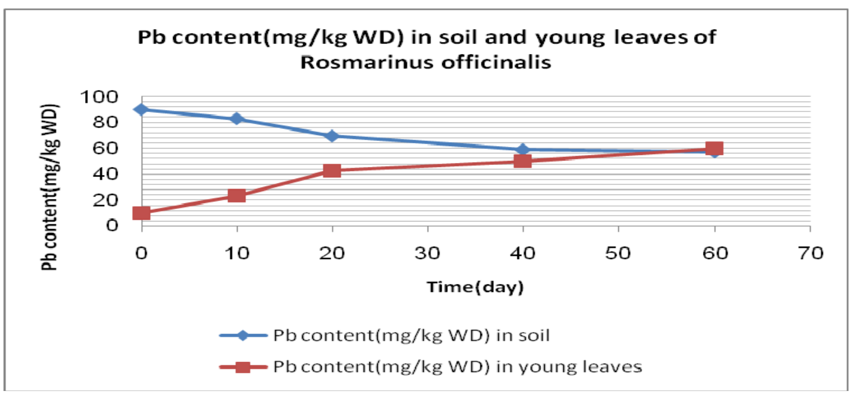

Figure 1-Lead Content (mg/kg DW) in soil and old Leaves of *Rosemary* in companion by *geranium* during 60 days of growing in contaminated soil.

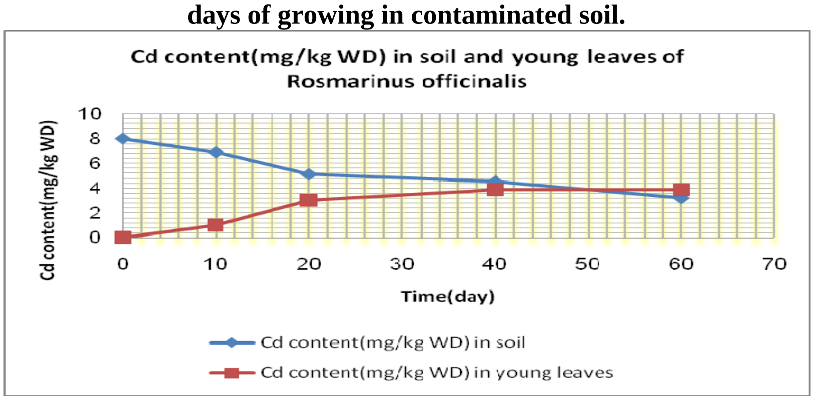

Figure 2-Cadmium Content (mg/kg DW) in soil and young Leaves of *Rosemary* in companion by *geranium during* 60 days of growing in contaminated soil.

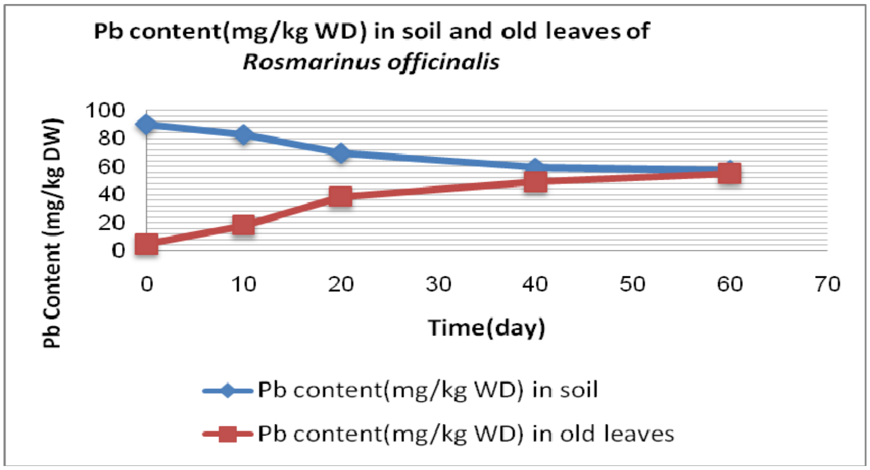

Figure 3- Lead Content (mg/kg DW) in soil and old Leaves of *Rosemary* in companion by *geranium* during 60 days of growing in contaminated soil.

The Cadmium and Lead uptake rate by rosemary is significantly affected by geranium grown and companion in the contaminated soil ($p<0.005$) , in table 3 the potential of uptake heavy metals by rosemary different parts individually grown in contaminated soil comparing with geranium companion is shown.
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. The fractionation of pb,Co,Zn,Cu, Cr, Ni, and Cd in control soil, *rosmary* grown and in soil treated by *pelargonium* spices in companion by rosemary is shown in table 3.

Table 3- Comparing %uptake heavy metals by Rosemary (shoot/total) individually grown in contaminated soil comparing by Geranium companion

	% Zn uptake	% Cu uptake	% Ni uptake	% Cd uptake	% Pb Uptake	% Co uptake	% Cr uptake
Rosemary	23.11	7.18	14.78	1.34	22.23	1.91	1.28
Geranium	27.14	5.23	25.2	1.98	28.13	2.02	1.63
Rosmarinus officinalis in Companion by *pelargonium roseum*	29.85	7.99	44.04	3.01	39.77	2.25	3.19

CONCLUSION

Mullins and Li et al. demonstrated that among the different tissues of a plant, roots are the preeminent and crucial organs due to transfer a large amount of soil metal from soil to plant shoots [43, 47]. Phytoextraction, or phytoaccumulation, is referred to as the uptake and translocation of metal contaminants in the soil via the roots into the aboveground portions of the plants [48, 49, 50, 51], therefore our results showed that roots of Rosemary probably tolerate more metal toxicity and are active in contaminated soil. The larger root length, root transfer and root volume of Rosemary can result in higher concentrations of metals in leaves and stems. Uncovering the underlying molecular mechanisms in plants, especially those capable of hyper accumulation, will provide further insights for proceeding phytoremediation technique in the future. Mechanisms for toxicity should be examined in a risk-based approach in order to determine impacts of metal speciation for other companion plants. Harvesting and investigating the certain plants to perform as companion accumulator , up taken and translocating metals from roots to shoots for removing toxicity of heavy metals and other toxic materials such as petroleum or nitrate from the soil and preventing vegetables, crops and other products to absorb them seems indispensable . Regarding the results of the present study, it is recommended to study more on the species belong to other companion plant families that have potential ability to hyperaccumulate heavy metals more especially the inedible plants.

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