INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-4, Issue-3, July-Sept-2014 ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 4th May-2014 *Revised: 25th May-2014* *Accepted: 6th June-2014*

Research article

THE EFFECT OF FENNEL (*FOENICULUM VULGARE*) ON POTENTIAL PHYTOREMEDIATION OF PELARGONIUM

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ABSTRACT: The present study was undertaken in view of providing an impeccable hypothesis that Sweet Fennel (*Foeniculum vulgare* Mill. var. dulce Mil) 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, Lead and Cadmium) by the system design. The contaminated soil was put into 50 vases in a way that fennel 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 average translocation of metals from soil to root of Fennel (*F. vulgare*) in companion by *pelargonium* was found to be in the order of Ni (2.43) > Pb (1.82) >Cd (1.33) > Cu (1.27) > Zn (1.01) and when these values were compared with control value of pelargonium samples individually it was observed to be higher in the contaminated site for Cd, Zn, Cu, Ni and Pb. In case of shoot (root to shoot) TFR was found in the order of Zn (2.03) > Ni (1.99) > Pb (1.43) > Cu (0.96) > Cd (0.88) and among the metals Ni, Zn, Co and Pb TFR was found to be higher than the control value. There was a positive correlation between the heavy metal concentrations in the leaves and root of plants and the studied soils. The Cadmium and Lead uptake rate by Fennel is significantly affected by pelargonium grown and companion in the contaminated soil ($p<0.001$).
Key words: *Foeniculum vulgare,* Phytoremediation, Nickel, Lead, Cadmium, contaminated soil.

INTRODUCTION

Aristotle was introduced four elements of life, water, soil, air and fire. Although this theory has been rejected, but this notion showed the importance of these causes in human life. 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 [1, 2, 3]. 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 contaminated 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 pigment, 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 [4, 13,14]. Therefore, the vast industrial waste materials and sewages from a lot factories and different chemical fertilizer 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 extremely expensive and difficult [5]. Plants are ideal agents for soil and water remediation because of their unique genetic, biochemical and physiological features.

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 [6, 7 ,8] . 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 six different plant-based technologies that include: Depending on contaminant , the site conditions, the level of clean-up required , and the types of plants , phytoremediation technology can be used for containment: phytoimmobilization and phytostabilization , or removal : phytoextraction and phytovolatilization [9]. The pollutants remain absorbed in or adsorbed to the roots. 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, phytoextraction, where plants absorb metals from soil an translocate them to the harvestable shoots where they accumulate. Although plants show some ability to reduce the hazards of organic pollutants [10-14], the greatest progress in phytoremediation has been made with metals [15-17]. Sweet fennel (Foeniculum vulgare Mill. var. dulce Mil) is a major aromatic plant belonging to the Apiaceae [18] and according to the other studies it could be effective in the phytoremediation **[19].** The present study was undertaken in view of providing an impeccable hypothesis that *Foeniculum vulgare* may probably be employed as a potent phytoremendiation of heavy metals from polluted soils. Aneth is the international name of fennel. It is a plant of the family *Apiaceae.* This plant has 1-2m height and is biennial. The age growth is 6 months and leaves of this plant are green and like chamomile. The fennel `s flowers are yellow and appear on the top of stem. They grow in north of *Iran, Isfahan, Golsefid,* around of *Shahrekord and Khozestan.* Its mineral and vitamin contents are Calcium, Phosphorous, Iron, Sodium, Potassium, Thiamine, Riboflavin, Niacin and vitamin C. Fennel has lots of medicinal uses and heals lots of sicknesses such as cough and rheum[20,21,22,23]. Plant part of use as crude drug is fruit[18].In the recently study about virtue of fennel on heavy metal of this plant in *India University* prove that is very useful for immune system and safe to be used in *Indian* system of medicine [24]. It belongs to *Foeniculum vulgare* and can be cultivated or grown easily in contaminated soils and a possible use for the sanitation of sludge and waste substrates. *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) [13, 14, 25, 26, 27]. The present study was undertaken in view of providing an impeccable hypothesis that *Foeniculum vulgare* 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 *Foeniculum vulgare* 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 *Foeniculum vulgare* 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 Nur county (Royan)in the north province Mazandaran -Iran, 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 $Zn(NO_3)_2$, and 5 m mol/lit $ZnCl_2$,50 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 $CuCl_2$+ $Cu(NO_3)_2$ 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. *Foeniculum vulgare* (2 month- old) 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 [28] 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 25ºC.

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 50 vases in a way that *F. vulgare* and *geranium* were grown in fifteen examined soils and vases individually, in other fifteen 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 [2, 29]. 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 , 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 [30]. 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 [31,32,33].

$U = (TSCF)\ (T)\ (C)$ (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 [34].

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.

Table 1- Physical and Chemical properties of studied soil before adding heavy metals and cultivated *pelargonium roseum* and Fennel (*F. vulgare*).

Characteristic	Quantity	Characteristic	Quantity
Soil Texture	Loam	Sand (%)	36.73
Clay (%)	25.26	Silt	
Cu (mg/kg DW)	0.7702	Zn (mg/kg DW)	1.0076
Ni (mg/kg DW)	1.6109	Cd (mg/kg DW)	0.0982
Pb (mg/kg DW)	1.0223		

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)	6.5	0.54	69.0	11.22
2 (15-35)	6.7	0.36	28.7	10.02

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 Fennel (*F. vulgare*) in companion by *pelargonium* was found to be in the order of Ni (2.43) > Pb (1.82) >Cd (1.33) > Cu (1.27) > Zn (1.01) and when these values were compared with control value of pelargonium samples individually it was observed to be higher in the contaminated site for Cd, Zn, Cu, Ni and Pb. In case of shoot (root to shoot) TFR was found in the order of Zn (2.03) > Ni (1.99) > Pb (1.43) > Cu (0.96) > Cd (0.88) 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 .

Results indicated that the rate of heavy metals uptake by *pelargonium* plant is significantly affected by the pH of soil ($p<0.01$). Translocation factors for all studied metals: Lead, Zinc, Cadmium, Copper and Nickel 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. There was a direct relationship between the age of leaves by lead, Nickel and Cadmium concentration in solution (figure 1, 2, 3and 4) and the severity of the response and the correlation coefficient reached -0.91.

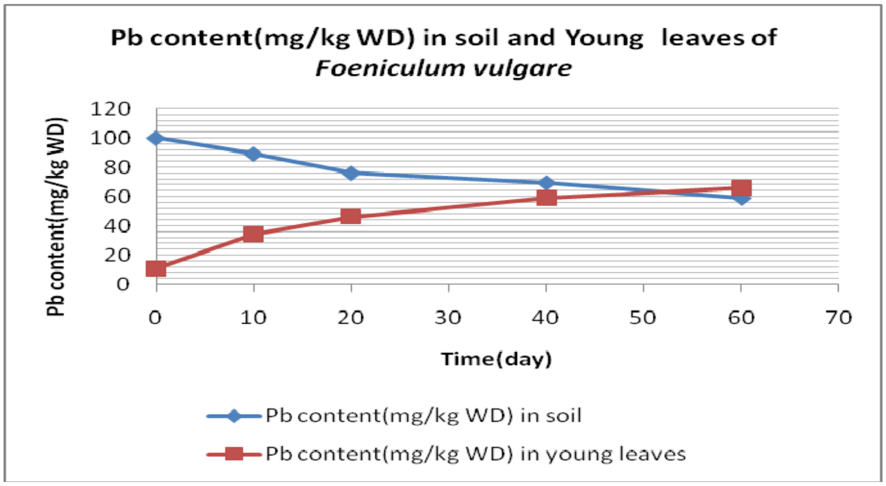

Figure 1- Lead Content (mg/kg DW) in soil and young Leaves of Fennel (*F. vulgare*) in companion by *pelargonium* during 60 days of growing in contaminated soil.

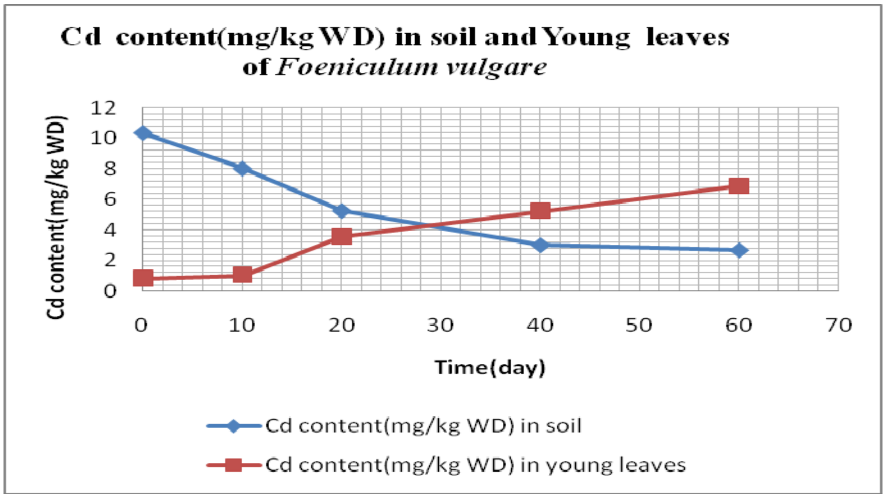

Figure 2- Cadmium Content (mg/kg DW) in soil and young Leaves of Fennel (*F. vulgare*) in companion by *pelargonium* during 60 days of growing in contaminated soil.

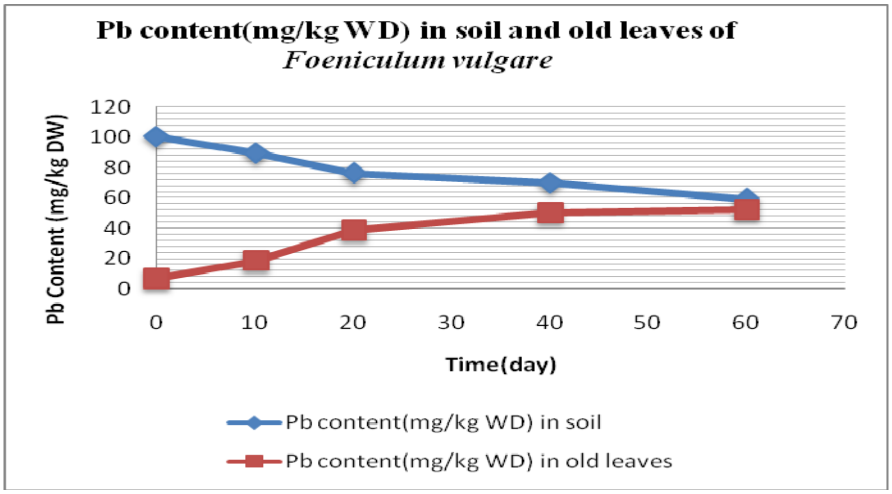

Figure 3- Lead Content (mg/kg DW) in soil and old Leaves of Fennel (*F. vulgare*) in companion by *pelargonium* during 60 days of growing in contaminated soil.

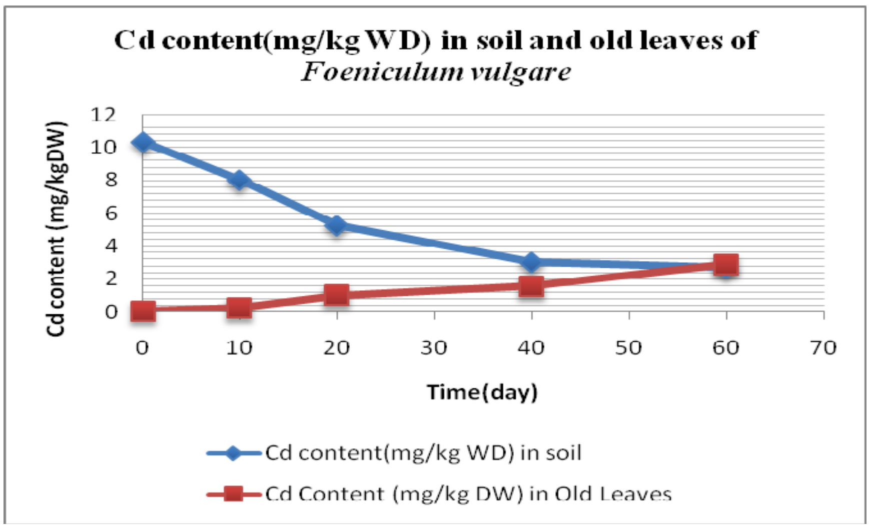

Figure 4- Cadmium Content (mg/kg DW) in soil and old Leaves of Fennel (*F. vulgare*) in companion by *pelargonium* during 60 days of growing in contaminated soil.

There was a positive correlation between the heavy metal concentrations in the leaves and root of plants and the studied soils. The Cadmium and Lead uptake rate by Fennel is significantly affected by pelargonium grown and companion in the contaminated soil ($p<0.001$) , in table 3 the potential of uptake heavy metals by fennel different parts individually grown in contaminated soil comparing with pelargonium 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 Cadmium in control soil and Fennel root grown in treated contaminated soil by *pelargonium* spices is shown in figure 5.

Table 3- Comparing %uptake heavy metals by Fennel (F. *vulgare*) (shoot/total) individually grown in contaminated soil comparing by pelargonium companion

	% Zn uptake	% Cu uptake	% Ni uptake	% Cd uptake	% Pb Uptake
Fennel	19.66	7.18	28.56	1.09	20.11
pelargonium	26.88	5.23	17.44	1.98	28.13
Rosmarinus officinalis in Companion by *pelargonium roseum*	31.22	10.36	42.77	4.05	36.09

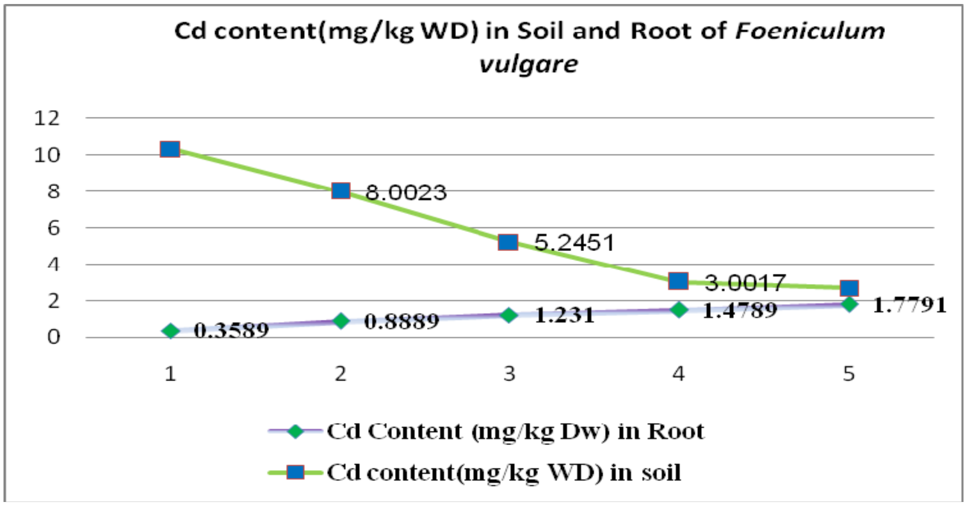

Figure 5- Cadmium Content (mg/kg DW) in contaminated soil and Root of Fennel (*F. vulgare*) in companion by Pelargonium.

CONCLUSION

The results of this research concluded that *Foeniculum vulgare* in the contaminated soil had suitable ability for phytoremediation by phytoextraction method and transmitting more Lead and Nickel in pH <7 after 35-60 days of growth of *Foeniculum vulgare* spices. 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 [35,36,37,38] , therefore our results showed that roots of Rosemary probably tolerate more metal toxicity and are active in contaminated soil. 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 translocation 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.

ACKNOWLEDGMENT

Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) is gratefully acknowledged.

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