Cd accumulation abilities of annual alfalfa (*Medicago scutellata* L.) by humic acid and growth promoting bacteria association

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Abstract

This experiment was carried out to study the effect of humic acid and growth promoting bacteria on uptake of cadmium heavy metal by annual alfalfa (*Medicago scutellata* L.). The experiment was arranged in pot experiment conditions in Isfahan, Iran in 2011. A factorial design was used based on completely randomized block design with four replications. Experimental treatments were humic acid and growth promoting bacteria. Biofertilizers used in the experiment were bacterial species *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Pseudomonas putida* in a mixture form and *Glomus intraradices* was used as mycorrhiza fungi. Cadmium heavy metal was applied at 0, 40, and 80 mg kg^{-1} soil cadmium chloride ($CdCl_2$) levels. Root and shoot cadmium concentrations and translocation factor were measured in the plants under study. The results suggested that there were significant differences between treatment levels and their interactions for all measured characteristics. A significant trend in cadmium accumulation in root and shoot organs were observed with increasing in the cadmium concentration.

Keywords: Cadmium, translocation factor rate, metal extraction rate, *Medicago scutellata* L.

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Introduction

Heavy metal contaminated soils due to human activity act on human health as the contaminant elements can enter into the food chain (Naidu et al., 1996). Cadmium (Cd) is one of the nonessential and highly phytotoxic trace element contaminants (Das et al., 1997). In fact, the main threats to human health from heavy metals are associated with exposure to cadmium. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries, though emissions have declined in most developed countries over the last 100 years. Cadmium compounds are currently mainly used in re-chargeable nickel–cadmium batteries. Cadmium emissions have increased dramatically during the 20th century, one reason being that cadmium-

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containing products are rarely re-cycled, but often dumped together with household waste.

Phytoremediation is the technique of reducing, removing, degrading, or immobilizing environmental toxins by plants in contaminated soil and water (Wendy et al., 2006). There is a bulk of studies on application of various plants for phytoremediation of different heavy metals including *Brassica junicea* for selenate and molibdate (Schiavon et al.2012), alfalfa for Cd, Ni, Zn, and Cu (Peralta-Videa and Gardea-Torresdey 2002), and rapeseed for Cd(Wang et al. 2002).

Alfalfa *Medicago scutellata* is a plant species of the genus *Medicago*, one of the most important plants in food chain that is found throughout the Mediterranean basin. It forms a symbiotic relationship with the bacterium *Sinorhizobium meliloti*, which is capable of nitrogen fixation. This plant produces high quality forage which can fix air nitrogen in the soil, and thus increase the soil fertility (Baladi, 2010).

Some soil microorganisms improve plant growth (Vessey 2003). Growth promoting bacteria increase plant growth directly with stimulating plant growth or indirectly by increasing the bioavailability of nutrients and biological control of pests and plant disease (Glick, 1995, Kloepper, 1993) and are currently used as one of the most important types of biofertilizers (Zahir et al, 2004).

Humic acids improve nutrient uptake, and biological and physical properties of soils (Khaled and Fawy, 2011). Humic acid improves growth (Arancon et al., 2003, Atiyeh et al., 2002), increasing fresh and dry weights of plants (Chen et al., 2004).

The current experiment was an attempt to determine the effect of soil contamination with cadmium on the content of Cd in alfalfa. The experiment also involved the application of organic matter to the soil to reduce the uptake of cadmium by the experimental plants.

Materials and Methods

The Experiment was carried out to study the effect of humic acid and growth promoting bacteria on uptake of the cadmium heavy metal by annual alfalfa (*Medicagoscutellata* L.) in polyethylene pots which were filled with 10 kg of loamy sand (soil test results are shown in Table 1) and located in the greenhouse conditions in Isfahan in 2011. Experiment was performed as a factorial arrangement based on completely randomized block design with four replications. Experimental treatments were humic acid (+H), growth promoting bacteria (biofertilizers used in this experiment were bacterial species *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Pseudomonas putida* in a mixture form and the applied mycorrhiza fungi was *Glomus intraradices* (+G). Cadmium heavy metal was applied at three levels of 0, 40, and 80 mg kg^{-1} in the form of cadmium chloride ($CdCl_2$).

The characteristics measured in this study were root cadmium rate, shoot cadmium rate, metal extraction rate, and translocation factor. Samples of the shoots and roots of the harvested plants were taken, disintegrated, dried, and ground. Cadmium content of the plant samples were determined with the atomic absorption spectrometry. ANOVA was carried out using SPSS. Mean comparison was performed using Duncan's Multiple Range Test ($p \leq 0.05$).

Results

Root Cd accumulation abilities

With increasing Cd concentration an ascending trend was observed in Cd accumulation in roots. Figure (III) shows the interaction effect between levels of cadmium heavy metal and separate and combined treatments of humic acid and growth promoting bacteria on amount of absorbed cadmium by

Table 1
Various chemical and physical parameters in soil

Cd Mg/Kg	K (ava)	P (ava)	N (%)	pH	EC Ds/m	Soil texture			Depth
						Sand	Silt	Clay	
0.05	285	31	0.04	7.7	3.76	44%	22%	34%	0-30

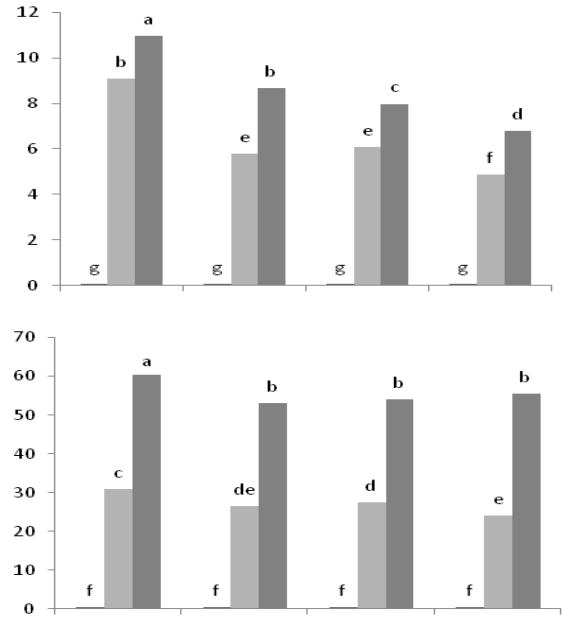

Fig.I. The interaction between different levels of cadmium (0, 40 and 80 mg/ka soil) , application of the growth promoting bacteria (G1 and G2) and humic acid (H1 and H2) on cadmium accumulation in roots (A) and shoot (B) (mg/ka)

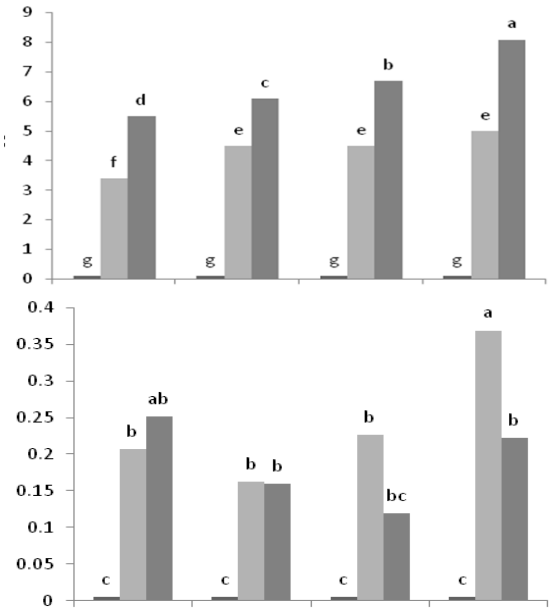

Fig. II. The interaction between different levels of cadmium (0, 40, and 80 mg/ka soil), application of the growth promoting bacteria (G1 and G2), and Humic acid (H1 and H2) on translocation factor (C) and soil cadmium extraction rate(D) (mg/ka)

roots of *Medicago scutellata*. According to this figure, it was observed that the treatment G1H1 (combined application of growth promoting bacteria + humic acid) at the highest Cd concentration (80 mg/kg) had the highest amount of absorbed cadmium by the root tissues in the studied species.

Shoot Cd accumulation abilities

Fig. (II) shows the rate of Cd accumulation in the shoots of the studied species. With increasing Cd concentration an ascending trend was observed in Cd accumulation in shoot. According to this figure, treatment G1H1 (combined application of growth promoting bacteria + humic acid) had the highest accumulation rate in shoot at the highest Cd concentration (80 mg/kg of soil).

Translocation factor

Significant difference ($p<0.01$) was observed between combined treatments for translocation factor in this study (Fig. II, C). An ascending trend was observed in translocation factor with increase in the cadmium concentration. The highest translocation factor was observed at the maximum level of Cd concentration (80 mg/kg).

Table 2
Translocation factor, metal extraction, and ANOVA for cadmium concentration in root and shoot

SOV	DF	Mean square			
		Cadmium concentration		Translocation factor	Metal extraction rate
		Root	Shoot		
Growth Promoting Bacteria (GPB)	1	25.711	31.525	7.728	0.007
Humic acid (H)	1	21.054	66.035	4.189	0.002
GPB.H	1	3.167	32.835	0.006	0.031
Cadmium heavy metal (C)	2	323.490	1247.618	181.234	3.301
GBP.C	2	6.655	8.501	2.676	0.037
H.B	2	5.505	17.435	1.088	0.024
GBP.H.C	2	1.016**	23.910**	0.594**	0.083*

Metal extraction rate

Results showed a significant difference ($p<0.01$) between treatments and also between

their interactions for metal extraction rate from soli (Fig. II, D). An ascending trend was observed in metal extraction rate from soli with increasing in the cadmium concentration, as the highest metal extraction rate was observed at the 40 mg/kg of Cd concentration in soli in the G2H2 treatment. However, in G1H1 treatment, the highest metal extraction rate was observed in the 80 mg/kg Cd concentration in soil.

According to the results of the experiment, it was observed that the annual alfalfa (*Medicago scutellata*) had high potential to absorb cadmium from contaminated soils by their roots and shoots, but accumulation rate of Cd uptake in shoot was much higher than root and this indicates that the studied species in this experiment has a high transmission factor for the transfer of heavy metals from the root to shoot which can improve the phytoremediation of the contaminated land.

Discussion

The results in general demonstrated that among different treatments, G1H1 showed the highest resistance to stress and in view of extraction of heavy metals this treatment at the highest concentration of the study (80 mg/kg of soil) had the highest cadmium uptake by shoot and root tissues. Therefore, because of reinforcing effects of growth promoting bacteria (PGPR) and humic acid in uptake and Cd accumulation potential from contaminated land these materials can be used to increase the efficiency of phytoremediation as well as to extract high levels of heavy metals at these regions.

These effects may be because of the combined influence of the experimented substances on the development of root system. The plant has been able to increase its resistance against stress induced by heavy metals through a symbiotic relationship with PGPR bacteria and humic acid. Researches indicated that Cd accumulation in plant tissues is directly related to concentration of this element in the soil and by increasing the concentration it in the soil so does the cadmium concentration in plant tissues (Davis, 1984).

PGPB increased growth of plant in the presence of heavy metals such as cadmium and zinc (Burd et al., 2000). Kuffner et al. (2008) reported that the improving uptake of heavy metals by beneficial bacteria in soil is a result of siderophores production in contaminated soils by Zn, Cd, and Pb. Abou-shanab et al. (2007) investigating the potential of native plants for remediation of metal contaminated soils also reported that by increasing the metal concentration in soil, rate of plant uptake increased.

The treatment of G1H1 with 80 mg kg^{-1} cadmium showed the highest translocation factor in this experiment. PGPB moderated some negative effects of cadmium on plants by providing nutrients for plants exposed to cadmium toxicity (Vassilev et al., 2002).

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