An evaluation of the efficiency of cultural plants to remove heavy metals from growing medium

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ABSTRACT

Contamination of heavy metals in the environment is one of major concern because of their toxicity and threat to human life and the environment. Phytoextraction, using plants to extract heavy metals from contaminated soils is an emerging technology. In this work, in order to find a suitable plant species for use in cleaning up the soil in an industrial region, some crop species, which are cultivated by farmers on these soils, were studied. The effects of various concentrations of four heavy metals including Cd, Cr, Co and Ni were studied in two cultivars of wheat (*Triticum aestivum* L.), bean (*Phaseolus vulgaris* L.) and alfalfa (*Medicago sativa* L.) in hydroponic culture media under controlled environmental conditions. The results showed that, despite a higher tissue concentration, and because of a low biomass particularly under toxicity conditions, alfalfa was not an effective species in removing heavy metals from the medium. In contrast, regarding the biomass, metal content, as well as % recovery values, it became apparent that the bean plant was the most effective crop in removing heavy metals from medium.

Keywords: heavy metals, Cd, Cr, Co, Ni; wheat; beans; alfalfa; phytoextraction

The continuous application of large amounts of fertilizers and other soil amendments to agricultural lands has raised concern regarding the possible accumulation of elevated levels of their trace element constituents and potential harm to the environment (Colbourn and Thornton 1978, Ma and Rao 1995, Raven and Leoppert 1997). Furthermore, increasing amounts of urban and industrial wastes (Haines and Pocock 1980, Parry et al. 1981, Culbard et al. 1983, Gibson and Farmer 1983), which may contain significant quantities of heavy metals, are being disposed on the agricultural lands (Raven and Leoppert 1997). Severe heavy metal contamination in soils may cause a variety of problems, including the reduction of yield and metal toxicity of plants, animals and humans. The decontamination of these soils by engineering methods are high costing projects (Baker et al. 1991, Salt et al. 1995, Huang et al. 1997).

Over the last 15 years there has been an increasing interest in developing a plant-based technology to remediate heavy metal-contaminated soils (Chaney 1983, Chaney 1993, Cunningham and Berti 1993, Baker et al. 1994a, Raskin et al. 1994, Salt et al. 1995). Phytoextraction is the use of plants to remove heavy metals from contaminated soils (Chaney 1983, Cunningham and Berti 1993, Baker et al. 1994a, Raskin et al. 1994). Since plant cultivation and harvesting are relatively inexpensive processes as compared to traditional engineering practices that rely on intensive soil manipulation, phytoextraction may provide an attractive alternative for the clean up of heavy metal-contaminated soils (Chaney 1983, Baker et al. 1994a, Raskin et al. 1994, Salt et al. 1995). The goal of heavy metal phytoextraction is to reduce metal levels in the soil to acceptable levels within a reasonable time frame (Raskin et al. 1994, Nanda-Kumar et al. 1995, Huang et al. 1997). The process of phytoextraction generally requires the translocation of heavy metals to the easily harvestable shoots. Dried, ashed or composted plants contain highly enriched heavy metals that may be isolated as hazardous waste or recycled as metal ore (Nanda-Kumar et al. 1995).

The efficiency of phytoextraction for a given species is determined by two key factors: biomass production and the metal bioaccumulation factor (Blaylock et al. 1997). Metal hyperaccumulation is a rare phenomenon that occurs in some plants called hyperaccumulators (Brooks et al. 1977). However, hyperaccumulators are often described as slow-growing and low biomass plants (Dushenkov et al. 1995, Nanda-Kumar et al. 1995, Ebbs et al. 1997, Rouhi 1997). The lack of high-biomass and a fast-growing species that can hyperaccumulate Cd, Pb and Cr was described as a constraint in using natural hyperaccumulators for phytoremediation

(Nanda-Kumar et al. 1995, Blaylock et al. 1997, Huang et al. 1997).

The potential of some crop plants from Brassicaceae for phytoremediation has been extensively studied (Baker et al. 1994b, Brown et al. 1995b, Dushenkov et al. 1995, Huang and Cunningham 1996, Ebbs and Kochian 1997, Ebbs et al. 1997) and it was demonstrated that some efficient shoot accumulators of the genus *Brassica* contained up to 3.5% on a dry weight basis of heavy metals (Nanda-Kumar et al. 1995). However, reports on the potential of legume plants is rare. There is also no available data on the physiological responses of legumes to heavy metals, and the metal content of harvested or fed parts for example in alfalfa, which determine the potential toxicity of metals to animals is also unclear. On the other hand, it is likely that two important legume crops (bean and alfalfa) accumulate high amounts of metals in their shoots and roots, which could make them a proper species for use in cleaning up the soil. However, it is also possible that these plants tolerate high amounts of metals in soil due to exclusion.

Because of the presence of more than 7 large industrial groups, a high contamination particularly with Cd and Cr, was reported in agriculturally-used lands in West-Tabriz industrial region, Iran (EPA 1996). Some of these sites are highly contaminated and do not support any crop production, but some of them are used for cultivation of wheat, bean and alfalfa plants.

On the other hand, since the phytoextraction of contaminants depends on shoot biomass production, it is necessary to select a proper crop plant for a given soil to increase crop production and thereby to maximize the effectiveness of phytoremediation.

This work was aimed at the study of these crops in terms of growth and their accumulation of metals at elevated levels of four important heavy metals e.g. cadmium (Cd), chromium (Cr), cobalt (Co) and nickel (Ni). Cadmium, chromium and nickel are ubiquitous pollutants present in industrial, agricultural and municipal wastes (Nanda-Kumar et al. 1995). In addition to physiological responses, it was aimed to determine the potential efficiency of these crops to remediate contaminated soils in which were cultivated.

MATERIAL AND METHODS

Seeds of three crop plants including wheat (*Triticum aestivum* L. cv. Omid and cv. Alvand), bean (*Phaseolus vulgaris* L. cv. Germeze-Naz) and alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh) were provided by the Agricultural Research Center, Tabriz. These cultivars were the same which are used by farmers in agriculturally-used contaminated soils in west-Tabriz.

Plants culture and growth conditions

Seeds were surface-sterilized using sodium-hypochlorite at 5%, then were germinated in the dark on filter paper soaked with saturated $CaSO_4$ solution. Subsequently, 6-day-old seedlings with similar size were selected and transferred to the 2 l dark plastic pots. Five plants were cultured in each pot. Prior to starting the treatments, all plants were pre-cultured for another three (wheat and bean) or seven (alfalfa) days in 50% nutrient solution. The composition of nutrient solutions for wheat was as follow (Cakmak et al. 1996) (mM): $Ca(NO_3)_2$ 2.0, $MgSO_4$ 1.0, K_2SO_4 0.9, KH_2PO_4 0.25, KCl 0.1 and (μM): H_3BO_3 2.0, $MnSO_4$ 0.4, $ZnSO_4$ 1.0, $CuSO_4$ 0.4 and $(NH_4)_6Mo_7O_{24}$ 0.04 (pH = 7). The composition of the nutrient solutions for bean and alfalfa plants were as follows (Neumann et al. 1999) (mM): $Ca(NO_3)_2$ 5.0, $MgSO_4$ 1.25, K_2SO_4 1.75, KH_2PO_4 0.25, KCl 0.25 and (μM): H_3BO_3 25, $MnSO_4$ 1.5, $ZnSO_4$ 1.5, $CuSO_4$ 0.5 and $(NH_4)_6Mo_7O_{24}$ 0.025 (pH = 7). Iron was supplied as Fe-EDTA (ethylenediamine tetraacetic acid) at 100 (wheat) or 20 (bean and alfalfa) μM. After preculture, plants were transferred to treatment solutions with 100% nutrient solution plus one of each heavy metal at the concentration of 0 (control), 25, 50, 75 and 100 μM. The cloride salt of three metals including Cd, Co and Ni were used for the treatments, Cr was supplied as CrO_3 prepared at pH = 6 to release Cr^{6+} in the solution. Plants were grown under controlled environmental conditions with a temperature regime of 25/18°C day/night, 14/10 h light/dark period, and a relative humidity of 70/80% and at a photon flux density of about 300–400 $\mu mol/m^2/s$. The nutrient solutions were changed completely every 4 days.

Harvest

Plants were harvested at 25 (bean, wheat) or 29 (alfalfa) days after sowing (16 days after treatment). Each bundle was divided into shoots and roots, and then the roots were washed with distilled water for 20 min (Dushenkov et al. 1995). The shoots and roots were blotted dry on filter paper and dried at 70°C for 2 days to determine plant dry weight.

They were either 25 (bean, wheat) or 29 (alfalfa) days-old (after sowing) 6 days sowing, 3 (bean and wheat) or 7 (alfalfa) days pre-culture and 16 days of treatment.

Determination of root length and chlorophyll

After determining fresh weight, the roots of the second group were used for the measurement of root length according to Tennant (1975). Chlorophyll concentration was measured in the leaves spectrophotometrically after a 48 h extraction (at 4°C) in N,N-dimethylformamide using 4 replicates. The absorbance of chlorophyll was measured at 664, 647 and 603 nm and chlorophyll concentration was calculated using the following formula (Moran 1982):

Total chlorophyll (μg/ml) = $8.24A_{644} + 23.97A_{647} – 16.64A_{603}$

The determination of metal contents

For the determination of metal contents, oven-dried samples were ashed in a muffle furnace at 550°C for 8 h and then digested in 1:3 HNO_3. The digested samples were dried on a heating plate and subsequently ashed at 550°C for another 3 h. Samples were resuspended in 2 ml 10% HCl and made up to volume so that the end HCl concentration was 1% (Nanda-Kumar et al. 1995). The concentration of Cd, Cr, Co and Ni was determined by atomic absorption spectrophotometry (AAS).

The calculation of parameters and statistical analysis

The following Formula was used for calculation of parameters:

Bioaccumulation coefficient = μg metal/g dry weight plant/μg metal/g soil (ml of nutrient solution) (Nanda-Kumar et al. 1995).

% Recovery = metal content in shoot or root/metal content in medium (Dushenkov et al. 1995).

Statistical analysis was carried out using Sigma Stat (2.03).

RESULTS

Shoot and root dry weight of all four plants had decreased in response to all studied heavy metals in the growth medium. The most reduction in shoot and root dry weight was observed in alfalfa plants

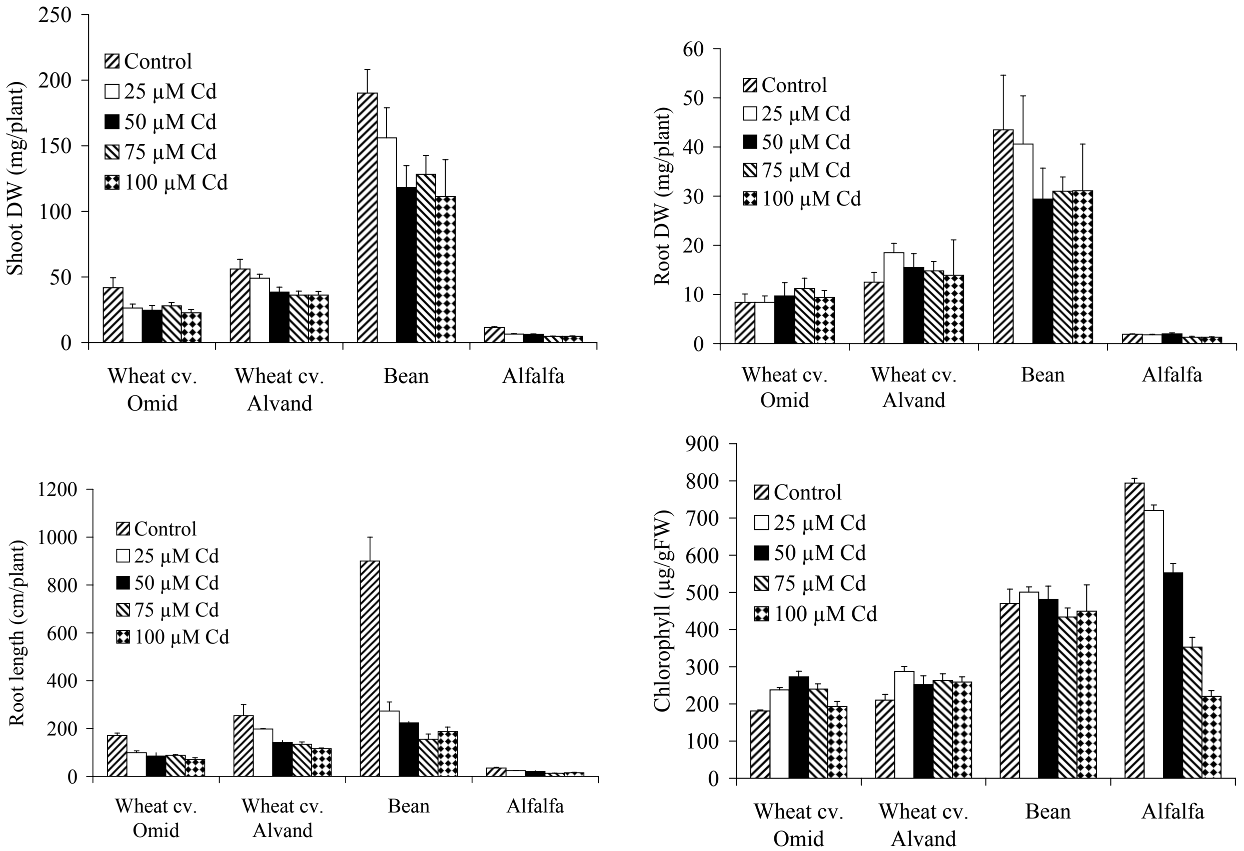


Figure 1. Shoot and root dry weight (mg/plant), root length (cm/plant) and chlorophyll concentration (μg/g fresh weight) in two cultivars of wheat (*Triticum aestivum* L. cv. Omid and cv. Alvand), bean (*Phaseolus vulgaris* L. cv. Germeze-Naz) and alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh) plants in response to elevated levels of cadmium in the growth medium (μM)

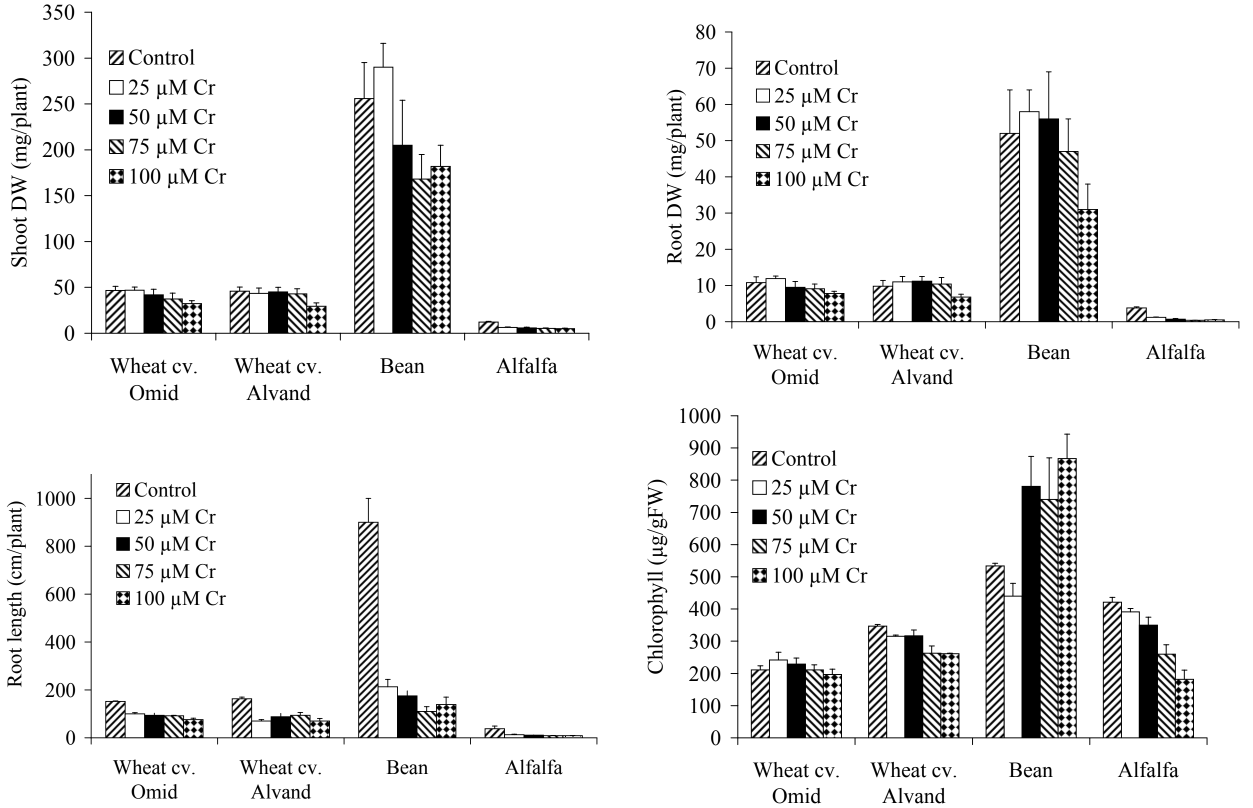


Figure 2. Shoot and root dry weight (mg/plant), root length (cm/plant) and chlorophyll concentration (µg/g fresh weight) in two cultivars of wheat (*Triticum aestivum* L. cv. Omid and cv. Alvand), bean (*Phaseolus vulgaris* L. cv. Germeze-Naz) and alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh) plants in response to elevated levels of chromium in the growth medium (µM)

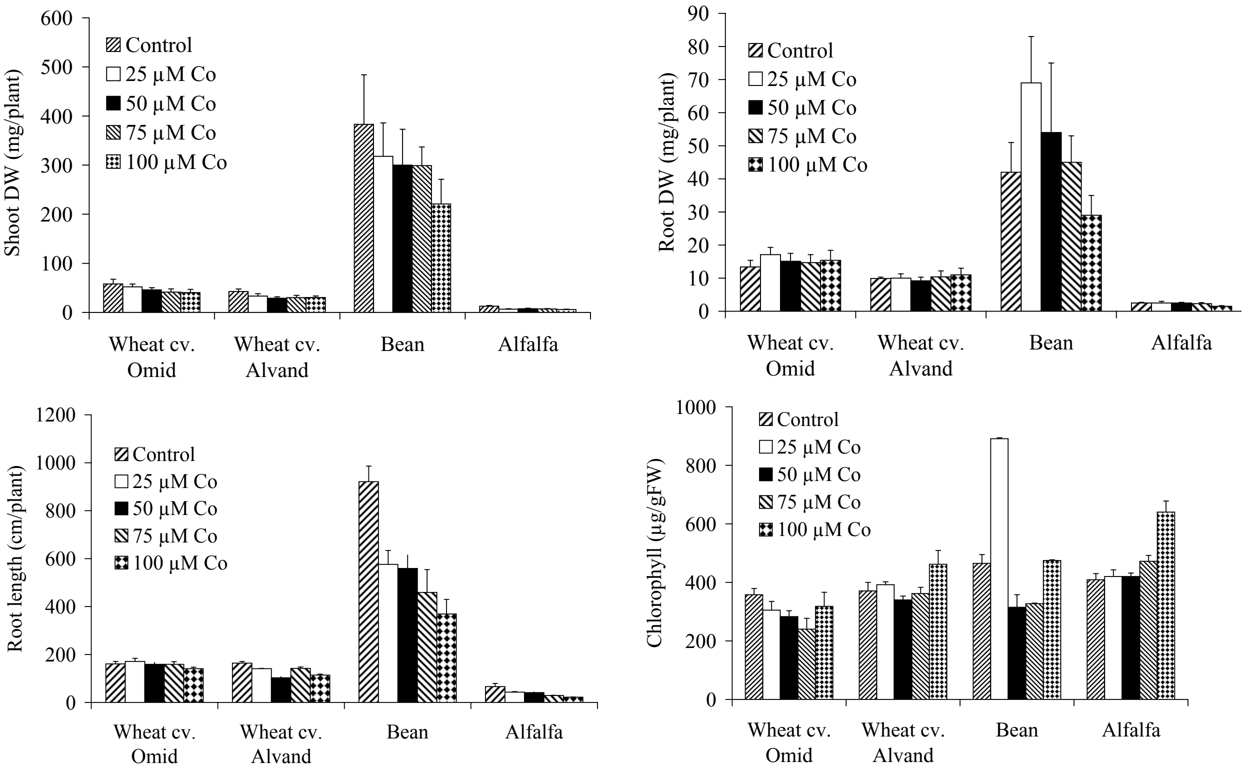


Figure 3. Shoot and root dry weight (mg/plant), root length (cm/plant) and chlorophyll concentration (µg/g fresh weight) in two cultivars of wheat (*Triticum aestivum* L. cv. Omid and cv. Alvand), bean (*Phaseolus vulgaris* L. cv. Germeze-Naz) and alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh) plants in response to elevated levels of cobalt in the growth medium (µM)

Table 1. Shoot and root concentration (μg/g dry weight) and content (μg/plant) of four heavy metals in two cultivars of wheat (*Triticum aestivum* L. cv. Omid and cv. Alvand), bean (*Phaseolus vulgaris* L. cv. Germeze-Naz) and alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh) plants grown in nutrient solution containing 75 μM of each metal ion; data are mean ± *SD*

		Concentration (μg/g dry weight)		Content (μg/plant)	
		shoot	root	shoot	root
Cadmium	wheat (cv. Omid)	391 ± 17	8 035 ± 1150	11 ± 2	90 ± 1
	wheat (cv. Alvand)	249 ± 40	7 703 ± 1100	9 ± 1	114 ± 28
	bean	305 ± 10	2 774 ± 60	39 ± 6	86 ± 6
	alfalfa	1 458 ± 49	8 692 ± 630	7 ± 2	10 ± 0.5
Chromium	wheat (cv. Omid)	213 ± 54	2 088 ± 68	8 ± 1	19 ± 2
	wheat (cv. Alvand)	74 ± 8.4	1 894 ± 31	3.2 ± 0.3	19.7 ± 1.7
	bean	117 ± 3.7	12 148 ± 37	19.7 ± 6.5	571 ± 7
	alfalfa	679 ± 0.9	6 750 ± 948	3.6 ± 0.1	2.7 ± 0.6
Cobalt	wheat (cv. Omid)	241 ± 22	1 428 ± 297	10 ± 0	21 ± 3
	wheat (cv. Alvand)	222 ± 29	1 058 ± 42	6.7 ± 0.5	11 ± 0.5
	bean	328 ± 38	4 155 ± 640	98 ± 5	187 ± 24
	alfalfa	563 ± 70	3 000 ± 280	4 ± 0.2	6.9 ± 0.2
Nickel	wheat (cv. Omid)	216 ± 23	10 654 ± 1420	5.8 ± 0.4	65 ± 3
	wheat (cv. Alvand)	193 ± 25	11 639 ± 1610	9 ± 1	142 ± 20
	bean	302 ± 39	1 119 ± 3	76 ± 10	33 ± 0.9
	alfalfa	449 ± 40	10 000 ± 190	4 ± 0.8	10 ± 1

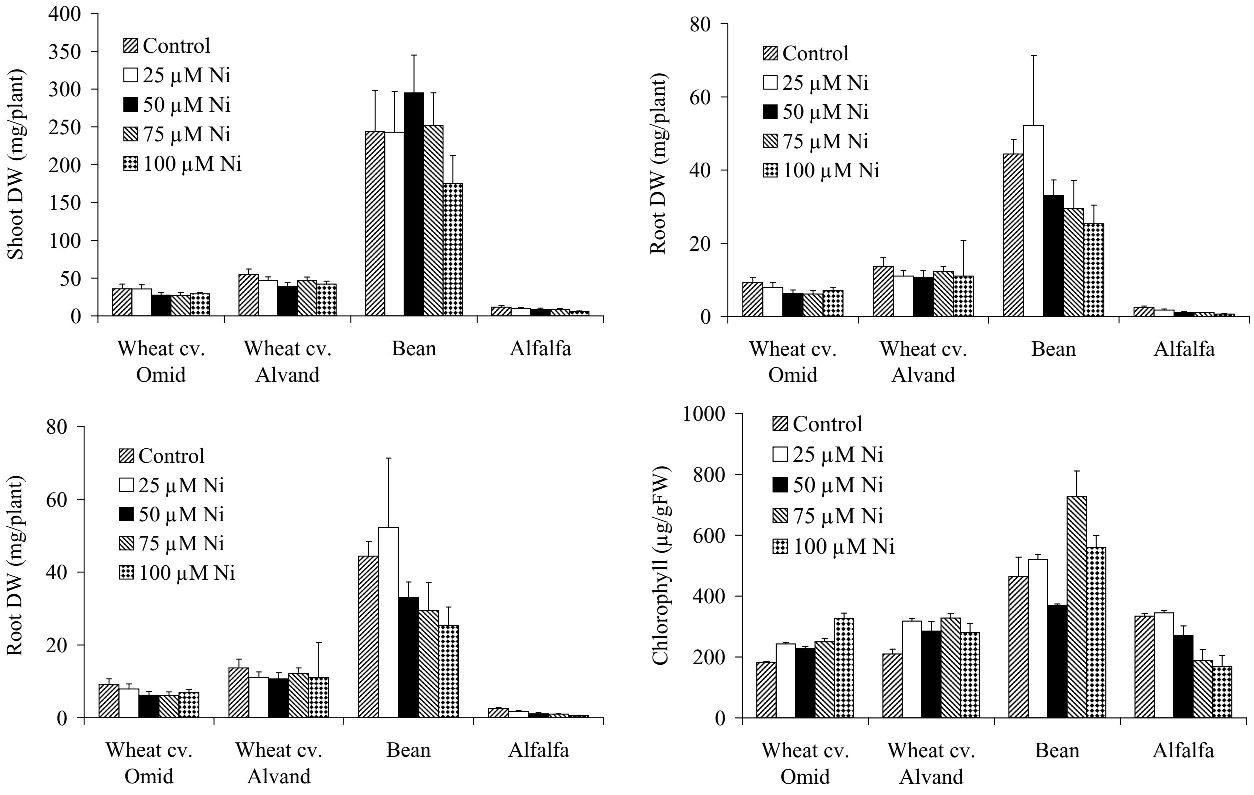
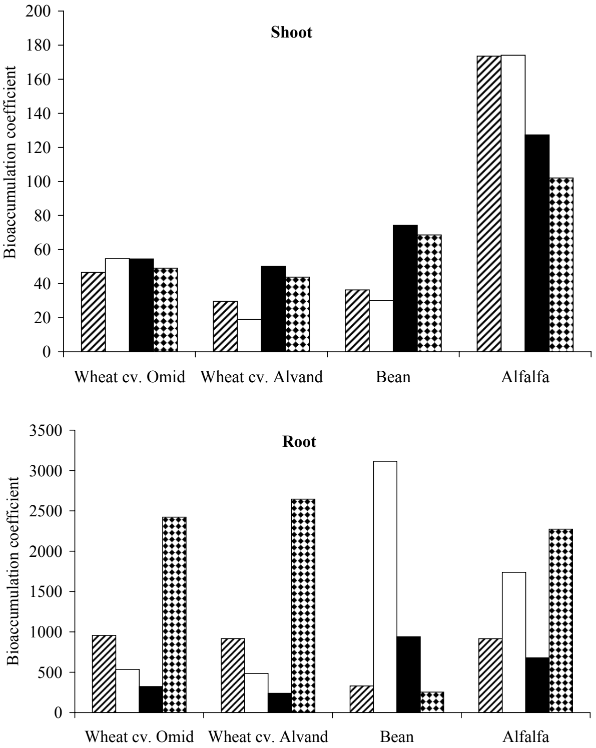


Figure 4. Shoot and root dry weight (mg/plant), root length (cm/plant) and chlorophyll concentration (μg/g fresh weight) in two cultivars of wheat (*Triticum aestivum* L. cv. Omid and cv. Alvand), bean (*Phaseolus vulgaris* L. cv. Germeze-Naz) and alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh) plants in response to elevated levels of nickel in the growth medium (μM)



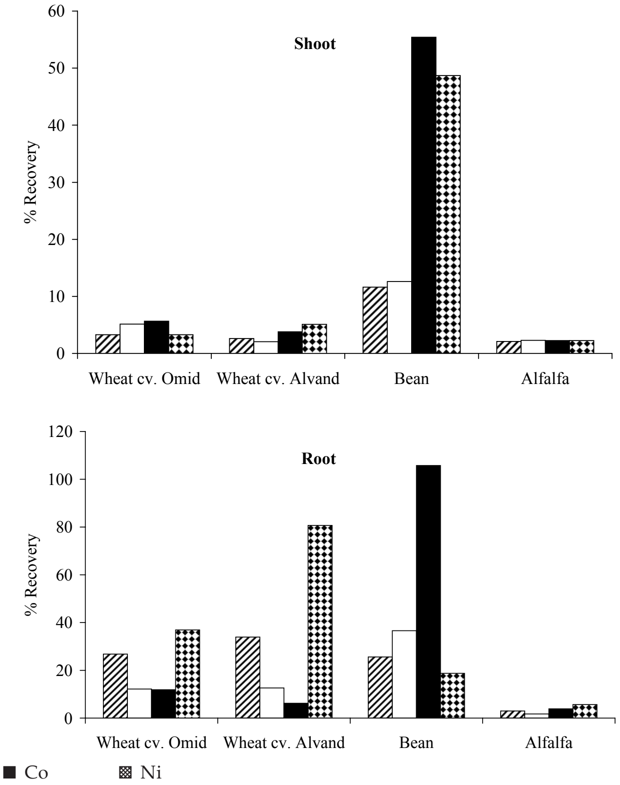


Figure 5. Biaccumulation coefficient of four heavy metals in shoots and roots of two cultivars of wheat (*Triticum aestivum* L. cv. Omid and cv. Alvand), bean (*Phaseolus vulgaris* L. cv. Germeze-Naz) and alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh) plants grown in nutrient solution containing 75 μM of each metal ion

Figure 6. % recovery of four heavy metals in shoots and roots of two cultivars of wheat (*Triticum aestivum* L. cv. Omid and cv. Alvand), bean (*Phaseolus vulgaris* L. cv. Germeze-Naz) and alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh) plants grown in nutrient solution containing 75 μM of each metal ion

in response to all of the four metals in the growth medium. This means that alfalfa plants were the most susceptible species to elevated levels of heavy metals in the growth medium (Figures 1–4).

Although a reduction in shoot and root dry weight in response to heavy metals in wheat and bean plants was similar, root length in the bean was more susceptible to elevated levels of heavy metals than that of two genotypes of wheat. The root length of the bean was affected even more than alfalfa under the toxicity of all four tested metals with the exception of cobalt (Figures 1–4).

Chlorophyll concentration was also decreased in response to heavy metals toxicity, the highest reduction was observed in alfalfa plants. However, at low and moderate concentrations of heavy metals, an increase in chlorophyll concentration was observed in all studied plants particularly in the bean under cobalt toxicity. In response to a high concentration of Ni, chlorophyll concentration in wheat and bean plants was also increased, but decreased up to 50% only in alfalfa (Figures 1–4). The enhancement of chlorophyll concentration could be attributed to the higher reduction of growth rather than larger chlorophyll synthesis.

The highest tissue concentration of heavy metals in the shoots was observed in alfalfa plants. In roots, however, the most heavy metal accumulation was recorded for Cd, Co and Ni in wheat. Chromium was mostly accumulated in the roots of alfalfa plants (Table 1).

The highest heavy metal content in the shoots was observed in bean plants. In the roots, only the chromium amount was the highest in bean, for the other three heavy metals the largest amounts were observed in wheat (Table 1).

Metal amounts in roots were higher than that in the shoot in all species and for all metal ions. Despite of the relatively long washing time (20 min), a high metal content in the roots due to localization of ions in the apoplasm could not be excluded.

The bioaccumulation coefficient, or phytoextraction rate, which was defined as the ratios between μg of metal/g dry weight of shoot or root and μg of metal/g dry weight of the soil (Nanda-Kumar et al. 1995), was also calculated for all four metals and plant species. As it was presented in Figure 5, the highest bioaccumulation coefficient for all four metals was observed in alfalfa plants. Three other plants showed mainly the same extent, particularly in bioaccumulation coefficient of shoots. In the roots, however, a high accumulation rate was also observed in two studied wheat cultivars for cadmium and in the bean for cobalt.

The potential of plants for phytoremediation is also based on % recovery or the ratios between metal amounts of shoot or root to metal amounts in the medium (Dushenkov et al. 1995). In contrast to bioaccumulation coefficient values, the highest % recovery values was shown for bean plants (Figure 6). However, the roots of both wheat cultivars showed also a high % recovery for nickel.

DISCUSSION

The ability to respond to elevated levels of heavy metal ions and to tolerate limited concentrations of metals such as cadmium appears to be ubiquitous in biological systems (Verkleij and Schat 1990, Ernst et al. 1992). In only a limited number of plant species a heritable tolerance or resistance occurs, which enables these plants to grow on metal contaminated soils (Brooks et al. 1977).

Several studies dealing with metal hyperaccumulating plants have concluded that phyto-extraction of metals was a feasible remediation technology for the decontamination of metal-polluted soils (Chaney 1983, McGrath et al. 1993, Brown et al. 1994, Brown et al. 1995a, b, Salt et al. 1995).

Nevertheles, most of the wild hyperaccumulating species, may not be suitable for many large-scale phytoremediation efforts, because these plants are small and slow-growing (Dushenkov et al. 1995, Nanda-Kumar et al. 1995). Recent studies looking at the feasibility of phytoextraction, demonstrated that both metal hyperaccumulation and good biomass yields are required to make the process efficient (Nanda-Kumar et al. 1995, Blaylock et al. 1997, Huang et al. 1997). Therefore, the most promising candidate species which were selected by researchers, were from some of the high-biomass and fast-growing crop plants e.g. from brassicaceae (Dushenkov et al. 1995, Huang and Cunningham 1996, Blaylock et al. 1997, Ebbs and Kochian 1997, Ebbs et al. 1997).

Accordingly, it could be suggested that another important criterion for a given species for using in phytoremediation, is a high tolerance to toxic effects of a given heavy metal. A high growth reduction under metal toxicity even in high-biomass but susceptible crop plants, could dramatically limit the yield of plants grown on contaminated soils.

In this work it was shown that, in the relatively short time frame of this experiment, the bean plant produced 8 times more biomass than the other three plants (Figures 1–4). Furthermore, under metal stress, bean plants showed only a low reduction in the production of their dry weight. In contrast, the growth of particularly alfalfa plants in the presence of all four studied heavy metals was very poor, so for example up to 59% of shoot growth reduction with the presence of cadmium was observed (Figure 1).

Most studies about some candidate species are mainly based on the interpretation of the analysis of metal concentrations in the shoots (Nanda-Kumar et al. 1995, Huang and Cunningham 1996, Huang et al. 1997). However, high concentration values in the shoot could be also observed as the result of the growth inhibition of plants under metal toxicity, i.e. concentration effect. Therefore, the content value of metal per plant or organ seems to be a better estimate for heavy metal extraction efficiency in a given species, and reflects the extent of metals which could be removed by an individual plant.

Accordingly, in the present work, because of a dramatic reduction in the dry weight production, alfalfa plants accumulated a high level of metals in the unit of dry weight (a high concentration and bioaccumulation coefficient values) (Table 1 and Figure 5). In this plant, the concentration value for example for Cd of the shoot was higher than that which was recorded for Cd concentration in known hyperaccumulator species *Thlaspi caerulescens* (Ebbs et al. 1997).

In contrast, metal content (extracted metal) per plant and also % recovery in alfalfa, was much lower than that of the bean (Figure 6). A lower reduction in dry weight production in bean was accompanying with a high metal content (Table 1), a high % recovery in the shoot and roots (Figure 6) therefore there was a large amount of metals removed from each pot.

The heavy metal removal by the shoots of the bean plants demonstrated in this work was approximately 195, 99, 490 and 380 μg/pot for cadmium, chromium, cobalt and nickel respectively (data not shown). This data is comparable with the values for example for Cd which were obtained in some *Brassica* species grown on soil during a four week experiment (Ebbs et al. 1997).

However, it should be noted that, the above mentioned data on efficiency of heavy metal removal from soil in the bean, reflect the extent of removal by 25-day-old plants after only 16 days of growth in the presence of metals. Had growth continued for a longer period of time, the greater biomass produced would have accumulated substantially more Cd, Cr, Co and Ni, increasing heavy metal removal. In other words, a high biomass that would be produced in a full growing season in bean plants, would remove a significantly higher percentage of metals from the soil.

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ABSTRAKT

Hodnocení schopnosti kulturních rostlin odstraňovat těžké kovy z živného roztoku

Kontaminace prostředí těžkými kovy je jedním z vážných problémů kvůli jejich toxicitě a rizikům pro člověka a životní prostředí. Fytoextrakce je nová technika, využívající rostlin k extrakci těžkých kovů z kontaminovaných půd. Cílem této práce bylo najít vhodné rostliny pro čištění kontaminovaných půd v průmyslové oblasti, které by mohly být na těchto půdách pěstovány. Vliv různých koncentrací čtyř těžkých kovů (Cd, Cr, Co a Ni) byl studován u dvou odrůd pšenice (*Triticum aestivum* L.), fazolu (*Phaseolus vulgaris* L.) a vojtěšky (*Medicago sativa* L.) v hydroponii za kontrolovaných podmínek. Výsledky ukázaly, že i přes vysoký obsah prvků v tkáních rostlin nebyla vojtěška kvůli nízkému výnosu biomasy vhodnou rostlinou k odstraňování těžkých kovů z roztoků. Oproti tomu rostliny fazolu se ukázaly jako nejvíce vhodné při odstraňování těžkých kovů z prostředí, a to jak s ohledem na výnos biomasy, tak na obsah prvků v pletivech i transportní hodnoty.

Klíčová slova: těžké kovy, Cd, Cr, Co, Ni; pšenice; fazol; vojtěška; fytoextrakce

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