Hyperaccumulation of heavy metals by some crop species and the feasibility for using in phytoremediation

Roghieh Hajiboland
Plant Science Department, University of Tabriz, Tabriz 51666, Iran Phone: +98-411-3356031 Email: ehsan@tabrizu.ac.ir

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. Phytoremediation, using plants to remediate contaminated soils is an emerging technology. In this work, in order to find suitable plant species for using in cleaning up the soils, some crop species were studied in the presence of various concentrations of six important heavy metals including Cd, Cr, Co, Ni, Cu and Mn. The growth and accumulation of metals were studied in five crop species including two cultivars of wheat, bean and alfalfa for Cd, Cr, Co and Ni and rice, maize and sunflower for Cu and Mn. Experiments were carried out using hydroponic culture media under controlled environmental conditions. Because of different growth responses to high metal concentrations depending on species, the accumulation of metals was not reliable for evaluating of the potential of a given species for using in phytoremediation. Therefore, in addition of bioaccumulation coefficient, %recovery of each heavy metal in shoot and root of studied plants were also calculated. Taking into account the biomass, metal content as well as %recovery values together, it could be concluded that bean plants is the most effective crop in removing Cd, Cr, Co and Ni from medium. This crop species recovered up to 50% of Ni supplied by medium in shoots and roots and up to 25% of Cd in roots and 15% in shoots. For Cu, a great bioaccumulation coefficient and also a high %recovery was observed in rice. This plant, demonstrated a high Cu tolerance was associated with a high metal accumulation in both shoot and roots, giving rise a %recovery as high as 174% in shoot and roots. For Mn, in contrast, a high bioaccumulation and %recovery especially in shoot was detected in sunflower. Interestingly, in maize, though a low uptake and accumulation, a high susceptibility to heavy metals was observed. In conclusion, assuming the biomass production under toxicity conditions which is attainable for bean, rice and sunflower, and also the corresponding bioaccumulation factors, it is possible to achieve a halving of soil metals in fewer than five crops.

Key words: Alfafa, Bean, Heavy metals, Maize, Phytoremediation, Rice, Sunflower, wheat

Introduction
Contamination of heavy metals in the environment is one of major concern because of their toxicity and threat to human life and the environment. The continuous application of large amounts of fertilizers and other soil amendments to agricultural lands has raised concern regarding the possible accumulation of toxic levels of their trace element constituents and potential harm to the environment (Ma and Rao, 1997, Raven and Leoppert, 1997). Furthermore, increasing amounts of urban and industrial wastes 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 reduction of yield and metal toxicity of plants, animals and humans. Decontamination of these soils by engineering methods are high costing projects (Baker, et al., 1991, Salt, et al., 1995, Huang, et al., 1997).
Phytoextraction is the use of plants to remove heavy metals from contaminated soils (Raskin et al., 1994). The goal of heavy metal phytoextraction is to reduce metal levels in soil to
acceptable levels within a reasonable time frame (Nanda-Kumar et al., 1995). The potential of some crop plants from Brassicaceae for phytoremediation is extensively studied (Dushenkov et al., 1995, Huang and Cunningham, 1996, Ebbs and Kochian, 1997) and it was demonstrated that some efficient shoot accumulator of genus *Brassica* contained up to 3.5% on a DW basis of heavy metals (Nanda-Kumar, et al., 1995). However, reports on the potential of some other important crops with high biomass is rare. This work was aimed to study of some crop species in terms of growth and accumulation of metals in the presence of toxic levels of some important heavy metals are ubiquitous pollutants present in industrial, agricultural and municipal wastes (Nanda-Kumar et al., 1995). In addition of physiological responses, it was aimed to determine the potential efficiency of these crops to remediate contaminated soils.

**Materials and Methods**

Seeds of six crop plants including wheat (*Triticum aestivum* L. cv. Omid and cv. Alvand), bean (*Phaseolus vulgaris* L. cv. Germeze-Naz), alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh), rice (*Oryza sativa* L. cv. T. Hashemi), maize (*Zea mays* L. cv. Sc 704) and sunflower (*Helianthus annuus* L. cv. Mehr) were used in this study. Seeds were surface-sterilized using sodium-hypochlorite at 5%, then were germinated in the dark on filter paper soaked with saturated CaSO₄ solution or on sand. Subsequently, 6-day-old seedlings with similar size were selected and transferred to the 2 L dark plastic pots. Prior to starting the treatments, all plants were pre-cultured for 3-7 days depending on species in 50% nutrient solution. 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. Plants were grown under controlled environmental conditions with a temperature regime of 25°C/18°C day/night, 14/10 h light/dark period, a relative humidity of 70/80% and at a photon flux density of about 300-400 µmol m⁻² s⁻¹. Nutrient solutions were changed completely each 4 days. This study was carried out as two separate experiments. In the first experiment, two cultivars of wheat, alfalfa and bean were used to study the toxicity effect of Cr, Cd, Co and Ni. In the second experiment, rice, maize and sunflower were used to study the effect of excess Cu and Mn. Plants were harvested 16 days after treatment. Each bundle was divided into shoot and roots, then roots were washed with distilled water for 20 min (Dushenkov et al., 1995). Shoots and roots were blotted dry on filter papers and dried at 70°C for 2 days to determine plant dry weight. For 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 HNO₃. 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). Concentration of Cd, Cr, Co, Ni, Cu and Mn was determined by atomic absorption spectrophotometry (AAS). The following formula were used for calculation of parameter: Bioaccumulation coefficient = µg metal g⁻¹ DW plant / µg metal g⁻¹ soil (ml⁻¹ 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 were carried out using Sigma Stat (2.03).

**Results**

Shoot and root dry weight of all studied plants was decreased in response to all tested heavy metals in growth medium. The most reduction in shoot and root dry weight was observed in alfalfa plants in response to four metals in the first experiment (Fig.1) and in maize and sunflower in response to Cu in the second experiment (Fig. 2).

In the first experiment, The highest tissue concentration of heavy metals in shoots was observed in alfalfa plants. In roots, however, the most heavy metals accumulation was
recorded for Cd, Co and Ni in wheat. Chromium was mostly accumulated in roots of alfalfa plants. In the second experiment, the highest Cu and Mn accumulation was observed in rice and sunflower respectively (data not shown).

In the first experiment, as it was presented in Fig. 3, 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 roots, however, a high accumulation rate was also observed in two studied wheat cultivars for cadmium and in bean for cobalt. In contrast to bioaccumulation coefficient values, the highest % recovery values was shown for bean plants (Fig. 3). However, roots of both wheat cultivars showed also a high %recovery for nickel.

In the second experiment, the highest bioaccumulation coefficient was observed in rice and sunflower for Cu and Mn respectively (Fig. 3). In this experiment, relative great values was obtained for %recovery (Fig. 3). The highest values were in rice and sunflower for Cu and Mn respectively.

Discussion

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

Nevertheless, most of 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 some of 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).

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

Rice was also the most tolerant species to Cu toxicity, and concomitantly, showed a high bioaccumulation coefficient and % recovery of this metal in comparison with other two species (Fig. 8 and 10). In the case of Mn, the lowest reduction and highest bioaccumulation as well as % recovery was observed in sunflower (Fig. 8 and 10).

However, it should be noted that, above mentioned data on efficiency of heavy metal removal from soil in bean, rice and sunflower, 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 heavy metals, increasing heavy metal removal. In other word, a high biomass that would be produced in a full growing season in candidate species, would remove a significantly higher percentage of metals from the soil.
References