Tájökológiai Lapok 89 (1): 111–125. (2011)

SEED GERMINATION, GROWTH AND METAL UPTAKE OF *MEDICAGO SATIVA* L. GROWN IN HEAVY METAL CONTAMINATED CLAY LOAM BROWN FOREST SOIL

LAILA M.H. ABUSRIWIL[1], HOSAM E.A.F. BAYOUMI HAMUDA[2], ALAELDDIN A. ELFOUGHI[1]

[1]Szent István University, Environmental Sciences Ph.D. School,
H-2103 Gödöllő, Páter K. Str. 1.
[2]Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Protection Engineering,
Environmental Protection Engineering Institute
H-1034 Budapest Doberdó Str. 6. E-mail: hosameaf@gmail.com

Keywords: Alfalfa, Heavy metal uptake, Plant growth and biomass, Clay loam brown forest soil

Summary: The use of plants for heavy metal-polluted soil rehabilitation is an emerging ecologically sound and safe technique. An *in vitro* laboratory experiment was conducted for 15 days in Petri dishes containing 3 layers of Whatman filter papers wetted by 50 ml of heavy metals Cd, Cu, Ni, Pb and Zn, separately at 0, 10, 20, 40, 80 to 160 µM to determine their impacts on germination rate, root and shoot growth of surface sterilized alfalfa seeds. An *in vivo* experiment was also conducted in a greenhouse setting, and the alfalfa seeds were grown for 8 weeks in plastic pots containing 2 kg of heavy metal (Cd, Cu, Ni, Pb and Zn) contaminated clay loam brown forest soil at different concentration levels (0, 10, 20, 40, 80 to 160 mg/kg). The results of *in vitro* demonstrated that the rates of seed germination, root and shoot growth were affected by Cd, Cu, Ni and Pb metals at higher concentrations of 80 and 160 µM. The inhibitory decreasing order of metal toxicity on seed germination was Cd > Cu > Ni > Pb > Zn. However, seed germination increased at all Zn dosages. Meanwhile, the lower concentrations of investigated metals (10 and 20 µM) stimulated the root and shoots length and at 10 and 20 mg/kg concentration levels increase plant biomass. It was found that alfalfa was able to grow efficiently at all Zn concentrations except at 160 mg/kg. The results showed that alfalfa was able to uptake the heavy metals at the applied concentrations ranging from 10 to 160 mg/kg by various degrees. Finally, the alfalfa plants, to some extent, demonstrated their potential in cleaning the soil environment from heavy metals.

Introduction

Soil contamination with heavy metals, either by natural causes or pollution, often has pronounced effects on the vegetation, resulting in the appearance of metallophytes, and heavy metal tolerant plants. Nicholson et al. (2003) concluded that the major sources of soil heavy metals include atmospheric deposition, sewage sludge, livestock manures, inorganic fertilizers and lime, agrochemicals, irrigation water, industrial by-product 'wastes' and composts. Joshi and Luthra (2000) found that the main sources of soil heavy metal pollution are geogenic, mining and smelting, disposal of municipal industrial wastes, use of fertilizers, pesticides and fumes from auto exhaust. Rapid growth of urbanization as well as transportation and industries are leading to serious environmental hazards (Urban 2007). Alvarez-Ayuso (2008) mentioned that heavy metal contamination of soils derived from agricultural or industrial activities is one of the major environmental problems in many parts of the world. Among chemical elements and compounds which are regarded as environmental pollutants, toxic metals such as Pb and Cd are the most widely spread and found around the urban agriculture areas (Mico et al. 2006, Fitamo et al. 2007). Guiweia et al. (2010) found that the concentration of Cd in the plant shoots, and the activities of catalase and ascorbate peroxidase decreased in plants from polymer-amended soil compared to an unamended control. The determination of risks dealing with the release or binding of toxic compounds in soils is a complex problem because a range of chemical, physical or biological soil properties can directly or indirectly affect these processes (Giller et al. 1998). The movements and levels of accumulation of heavy metals in a plant depend on soil type, plant and environmental factors (Alloway 1995). Deposition of metals to soil may be deleterious to crop growth and soil productivity and may also produce crops containing unacceptably high metal levels that may impact negatively on animal and human health (Nouri, 1980). The mobility of Cd and Pb metals in terms of bioavailability to plants may depend not only on the total concentration in solution but also on the speciation of the metals (Bingham et al. 1984). The speciation, adsorption and distribution of Cd in soils are governed by pH, soluble organic matter content, hydrous metal oxide content, clay content and type, presence of organic and inorganic ligands, and competition from other metal ions (Holm et al. 1995; Bingham et al. 1984). Soil pH affects the speciation and adsorption of heavy metals in soil, determining the mobility, bioavailability and toxicity of the metal.

Heavy metal uptake by plants occurs *via* the soil solution. Free metal ion activities are usually better indices of metal bioavailability and toxicity than are total soluble metal concentrations. As the mass of the water transpired by the plant increases, the Cu concentration in both the root and shoot increase (Cheng and Allen 2001). Vasiliadou and Dordas (2009) found that the Cd level affected the number of leaves and dry matter accumulation, and there were differences among the varying cultivars that were used. Baker and Brooks (1998) found that some native plant species were able to accumulate unusually high concentrations of potentially phytotoxic elements such as Cd, Cu, Pb, Ni and Zn from metalliferous soils. Ma et al. (2009) established that compared with the single factor pollution index (SFPI) of heavy metals calculated for the control site, the average SFPI from the sampling sites decreased in the order of Cr > Cd > Pb > Zn > Ni > Cu. There were notable negative correlations between the integral pollution index of soil heavy metals at all sampling sites and the distances from the railroad. Heavy metals interferes with several metabolic processes, causing toxicity to the plants as exhibited by reduced seed germination, root and shoots growth and phytomass, chlorosis, photosynthetic impairing, stunting and finally plant death (Roy et al. 2005). Plant roots participate primarily in the heavy metal cation uptake (Lasat 2002). Although heavy metal release in Germany has been decreasing in recent years (Ilyin et al. 2008), the environment still faces the problem of accumulated heavy metal deposits in soils. While some heavy metals, like Cu and Zn, are essential for plants and animals and others like Cd and Pb are toxic even at low concentrations and have no biological functions. The techniques that involve the use of living organisms include: bioremediation, phytoextraction, phytovolatilization, phytostabilization, rhizofiltration and phytoremediation (Yang et al. 2005) and are more inexpensive than chemical, mechanical or other techniques. Most metal uptake occurs in the root system, usually *via* absorption, where many mechanisms are available to prevent toxic effects due to the high concentration of metals in the soil and water.

Plants have shown the capacity to withstand relatively high concentration of contaminants without toxic effects. A wide range of plants can be useful in cleaning up the environment from waste hazards. The plant material may be used for non-food purposes; alternatively, it can be ashed followed by recycling the metals or disposing them in a landfill (Angel and Linacre 2005). Chhotu and Fulekar (2008) mentioned that the seed germination, root and shoot growth were affected by Cd, Cu, Ni, Pb and Zn metals at higher concentration of 40 and 50 ppm. However, the lower concentration of heavy metals ranging from 5 to 10 ppm doses were observed to be stimulating the root and shoot length and increase biomass of the alfalfa plant. The objective of this study was to evaluate, *in vitro,* the affect of various concentrations of different heavy metals on alfalfa seed germination rate, and their affects after application to clay loam brown forest soil on root/shoot growth biomass and the rate of metal uptake.

Materials and method

Soil sampling, and soil properties

Soil samples were collected from a depth of about 0-20 cm along uncultivated clay loam brown forest soil collected from the Gödöllő region, in Hungary. Stones and plant residues and other soil impurities were carefully removed from the soil prior to the drying process under laboratory conditions. The soil samples were screened through a 2 mm stainless steel sieve and stored in a plastic bag at room temperature until use. Concentrations of Pb, Zn, Cu, Ni and Cd were measured by atomic absorption spectrophotometer. The soil moisture content was calculated by the weight difference before and after drying at 105°C to a constant weight. The pH was measured after 30 minutes of vigorous mixed samples at 1:2.5 (Solid: distilled water ratio). The physico-chemical parameters were measured by standard methods (Table 1).

Table 1. Physico-chemical characteristics of experimental soil
1. táblázat A vizsgált talaj fizikai és kémiai jellemzői

Parameters	Clay loam brown forest soil, Gödöllő
$pH_{(H2O)}$	5.33
Humus content %	1.240
Total N content $mg \cdot kg^{-1}$	8.411
NO_3-N, $mg \cdot kg^{-1}$	133.080
NH_4-N, $mg \cdot kg^{-1}$	410.690
Ca, $mg \cdot kg^{-1}$	856.000
Mg, $mg \cdot kg^{-1}$	203.000
Na, $mg \cdot kg^{-1}$	21.000
P_2O_5, $mg \cdot kg^{-1}$	121.310
K_2O, $mg \cdot kg^{-1}$	107.000
Zn, $mg \cdot kg^{-1}$	38.100
Cu, $mg \cdot kg^{-1}$	22.900
Mn, $mg \cdot kg^{-1}$	136.000
Fe, $mg \cdot kg^{-1}$	1187.000
Cd, $mg \cdot kg^{-1}$	0.180
Pb, $mg \cdot kg^{-1}$	15.100
As, $mg \cdot kg^{-1}$	7.400

Heavy metal salts

Heavy metal solutions used in *in vitro* and *in vivo* experiments were prepared from the following heavy metal salts: Cd as $Cd(NO_3)_2.4H_2O$; Cu as $CuSO_4.5H_2O$; Ni as $Ni(NO_3)_2$; Pb as $Pb(NO_3)_2$, and Zn as $Zn(NO_3)_2.6H_2O$.

Laboratory experiment

The laboratory experiment was carried out to evaluate the impacts of the various concentration levels of heavy metals Cd, Ni, Cu, Pb and Zn on alfalfa (*Medicago sativa* L.) seed germination, root and shoot length. Following Vincent (1970) instructions, alfalfa (*Medicago sativa* L.) seeds were sterilized with 70% ethanol for 30 seconds followed by sterilization with 0.1% mercuric chloride for 5 min. The seeds were thoroughly washed with sterilized distilled water several times to avoid fungal contamination. To measure the effect of heavy metals on seed germination; 10 sterilized seeds were placed in large Petri dishes of 24 cm in diameter with 3 Whatman filter papers and were gotten wet with 50 ml of the following treatment solutions: 0, 10, 20, 40, 80 and 160 µM of each of Cd, Ni, Cu, Pb and Zn. The control Petri dishes were treated by distilled water. Petri dishes were sealed with Parafilm to prevent evaporation. Seeds were incubated at alternating temperatures of 25°C (16 hours) and 18°C (8 hours). The experiment was carried out by three replicates and 10 seeds were used for each treatment. Germination was counted every 3 days for 21 days. Seeds were considered to have germinated when the radical was at least 5 mm long. The germinating seedlings were harvested after 21 days and germination rate (%), shoot and root length were recorded in comparison with heavy metal free control.

Greenhouse experiment

In vivo, ten seedlings were grown in 2 kg capacity plastic pots for studying root and shoot growth biomass and metal uptake. Soil moisture content was adjusted to about 45% of water-holding capacity with distilled water. Soil samples were treated by different concentrations: 0, 10, 20, 40, 80 and 160 mg metal/kg soil of each of Cd, Ni, Cu, Pb and Zn. The control Petri dishes were treated by distilled water.

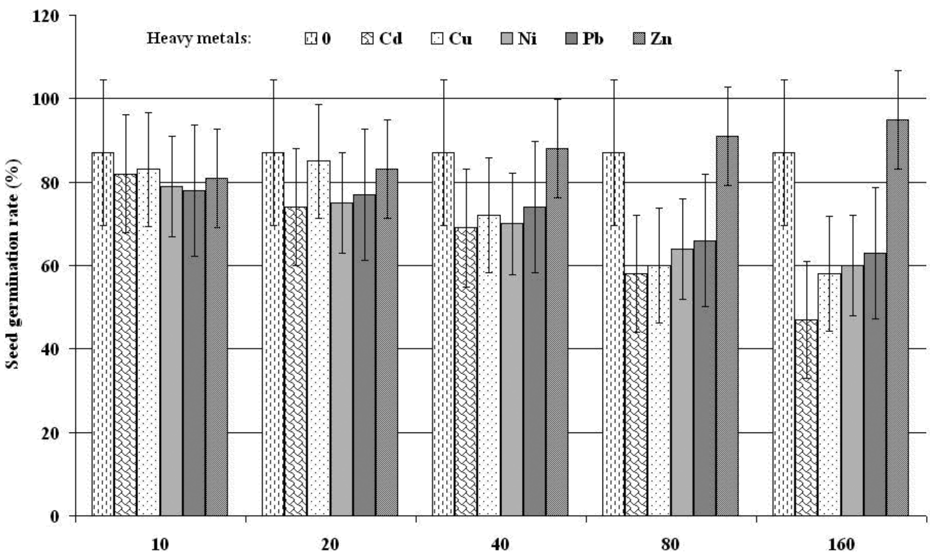
To prevent loss of nutrients and trace elements out of the pots, plastic trays were placed under each pot and the leachate collected was put back in the respective pot. Each treatment of the plants consisted of three replicates for statistical purpose. The seedlings were set under photoperiod of 14/10 hrs light/dark cycle and temperatures of 26±2°C during the day and 20±2°C during the night. The average relative humidity was recorded to be 72%. For the metal uptake study, plants were harvested after 8 weeks. The plants were then separated in to roots and shoots. The plant samples were washed with distilled water and dried in an oven at 75°C for 2 days and the dry weight of biomass was determined, after which these samples were stored in paper bags. The samples were considered for analysis of metal content digested with concentrated nitric acid and 30% hydrogen peroxide and then the heavy metal content was determined by an atomic absorption spectrophotometer. The experimentation layout was done in a complete randomized block design. The means of three replicates per treatment for each strain were analyzed using ANOVA to determine statistical differences among treatment and LSD at $P = 95\%$ was calculated as well as S.D.

Results

Accumulation of heavy metal in soil after its discharge from different pollution sources increases the metal concentration in soil environments up to dangerous levels in living systems including human beings. The present study has been carried out in laboratory and greenhouse settings in pot experiments to evaluate the effects of heavy metals on seed germination and plant growth and biomass as well as root and shoot metal uptake.

Effect of heavy metals on seed germination

The result of the *in vitro* study demonstrated a concentration dependent inhibition of the seed germination with regards to metal and alfalfa tolerance (Figure 1).



Heavy metal concentrations (μM)

Figure 1. In vitro effects of heavy metals on alfalfa seed germination after 21 days

1. ábra A nehézfémek hatása a lucerna magvak csírázására, 21 nap elteltével, in vitro körülmények között

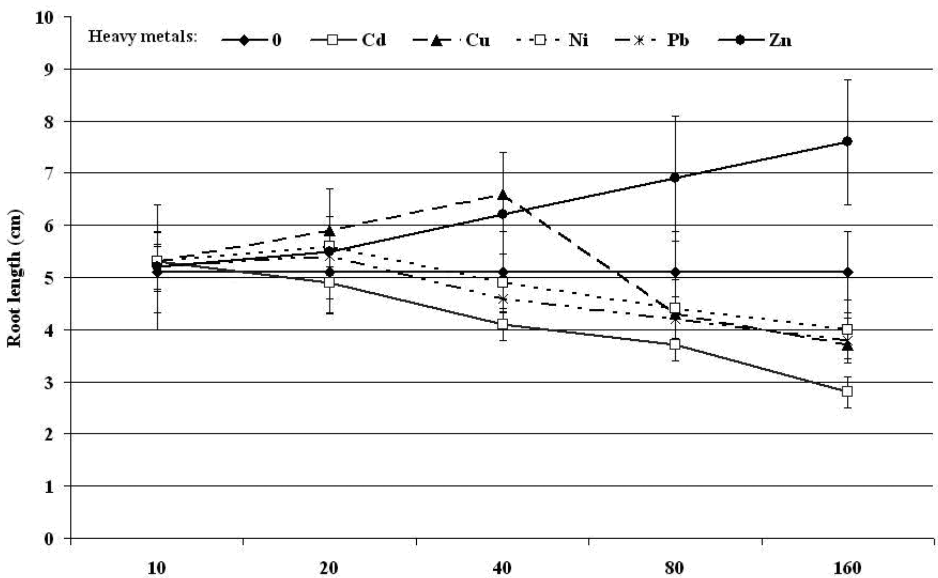
The result of this investigation indicated that Cd, Ni and Pb at 10 μM concentration levels had very low toxic effects on seed germination while Cu at the same dose increased seed germination. At the 20 and 40 μM concentrations of Cd, Ni and Pb reduced seed germination, while the seeds were germinated more at 10 and 20 μM levels of Cu. The seed germination was inhibited at 80 and 160 μM levels as compared to the control for all the four metals Cd, Ni, Pb and Cu.

Delayed germination was also observed in all cases at higher (80 and 160 μM) concentrations. However, in the same study Zn was the only metal which did not reduce the seed germination. The decreasing order of toxicity for metals on seed germination was Cd > Cu > Ni > Pb >Zn. However, seed germination increased at all Zn concentration levels.

Effect of heavy metals on root length

The increases in the heavy metal concentration caused a root length decrease with stunted growth of roots (Figure 2). The dose of 10 µM of all investigated heavy metals promoted the root length of the plants as compared to the root growth of the control plants. The heavy metals Cu, Ni, Zn and Pb at 20 µM concentrations further increased the root growth over the control root size. However at the same dose Cd reduced the root length on comparison with the control root elongation.

The metals Cd, Ni, Cu and Pb demonstrated a concentration dependant inhibition of root growth at 40, 80 and 160 µM levels. All Zn concentrations increased the root length in comparison to the control root length of the alfalfa plants. Root toxicity symptoms included: browning, reduced number of root hairs and growth.



Heavy metal concentrations (µM)

Figure 2. In vitro effects of heavy metals on alfalfa root length after 21 days of germination

2. ábra A nehézfémek hatása a lucerna gyökérfejlődésére, 21 nap csírázást követően, *in vitro* körülmények között

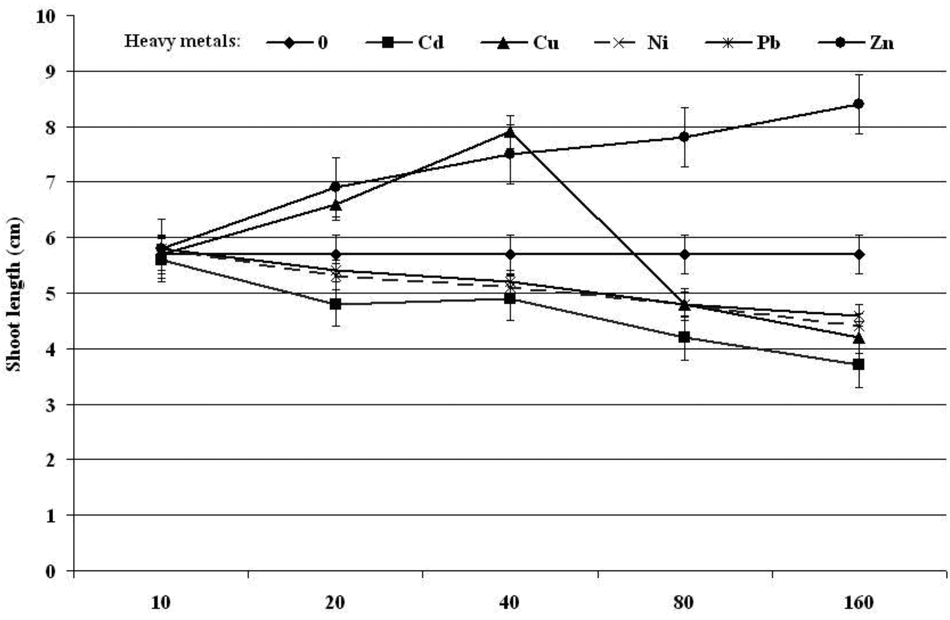
In comparison to the control, plant roots were healthy and normal. The colours of the roots receiving higher heavy metals treatment (80 and 160 µM), except with Zn, changed gradually over time from a creamy white colour to dark brown; an indication of intense suberification. Plants treated at lower concentrations were not significantly affected by the metals. Lateral roots were observed in almost all treated samples of Zn, Cd, Cu, Pb and Ni demonstrated concentration dependant inhibition of root growth at higher concentrations.

Effect of heavy metals on shoot elongation

The impacts of heavy metals on the shoot elongation are different from their effects on root growth and length (Figure 3). The shoot length was found slightly reduced than in the control alfalfa plants at the 10 µM Cd level.

On the other hand, the 10 µM dose of Cu, Pb, Ni and Zn increased the shoot lengths as compared to the control treatment. These results indicate that low concentrations of Cd, Cu, Ni and Pb have micronutrient-like effects on the alfalfa plants and all the plants appeared to be healthy.

The heavy metals Cd, Ni and Pb at 20 and 40 µM doses reduced the shoot growth; however, Cu at the same dose increased the shoot length. When the concentration of these metals was increased to 80 and 160 µM concentrations, the shoot length of the alfalfa plants found a concentration dependant inhibition of shoot growth as compared to the control plants. All plants grown in the soil contaminated with Zn showed increase in the shoot elongation than the plants grown in soil without Zn contamination.



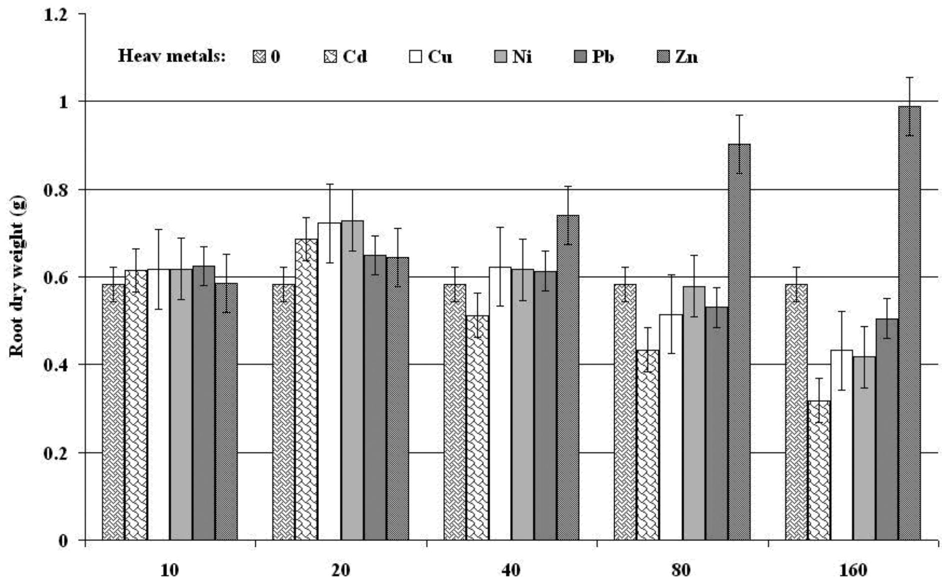
Heavy metal concentrations (µM)

Figure 3. In vitro effects of heavy metals on alfalfa shoot length after 21 days of germination

3. ábra A nehézfémek hatása a lucerna hajtásnövekedésére, 21 nap csírázást követően, *in vitro* körülmények között

Effect of heavy metals on plant biomass

The results of *in vivo* experiment indicated that the mean plant biomass (root and shoot dry weights) of alfalfa showed an increasing tendency as the concentrations increased from 10 to 20 for Cd, Cu and Ni. It was found that 40 mg/kg is better for plants grown in soil contaminated by Cu and Ni than in Cd-contaminated soil (Figs. 4a and 4b).

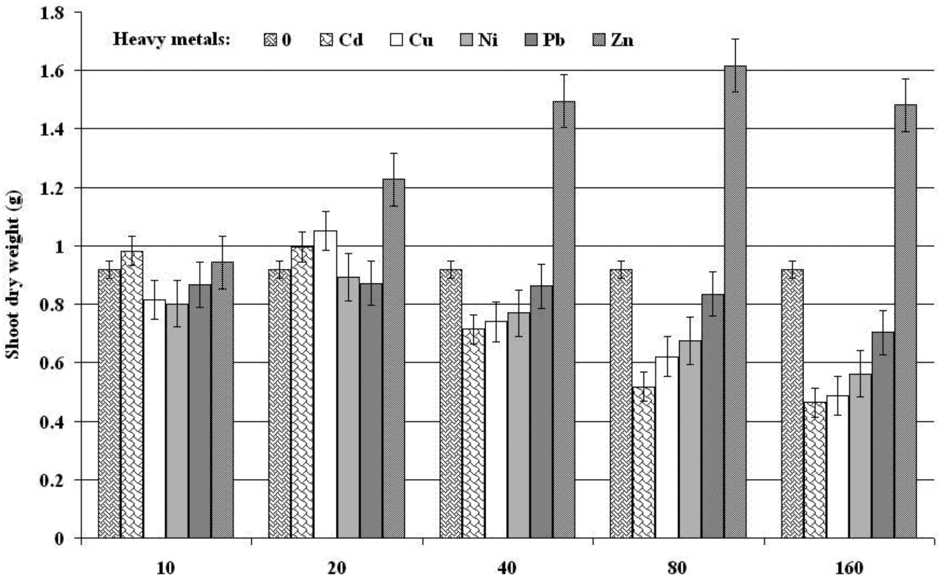


Heavy metal concentrations (mg/kg)

Figure 4a. In vivo effects of heavy metals on alfalfa root dry weight after 8 weeks plantation in clay loam brown forest soil

4.a ábra A nehézfémek hatása a lucerna gyökerének száraztömegére, 8 hetes fejlődést követően agyagbemosódásos barna erdőtalajon, *in vivo* körülmények között

The dry weights of root and shoot decreased gradually as the concentration of Cd, Cu and Ni in the soil ecosystem increased to 80 and 160 mg/kg.



Heavy metal concentrations (mg/kg)

Figure 4b. In vivo effects of heavy metals on alfalfa shoot dry weight after 8 weeks plantation in clay loam brown forest soil

4.b ábra A nehézfémek hatása a lucerna hajtásának száraztömegére, 8 hetes fejlődést követően agyagbemosódásos barna erdőtalajon, in vivo körülmények között

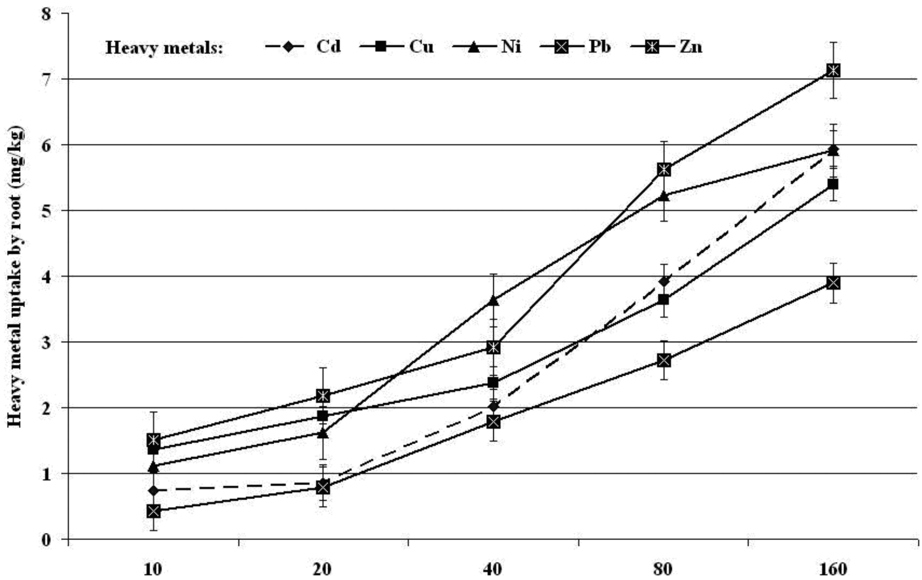
The plant dry weights of root and shoot yield affected by the higher concentration levels of Cd caused reduction in the plant biomass. Lead showed low effect on dry weights of roots and shoots of the plant. There was a positive effect seen in all Zn concentrations and an increase in biomass yield as compared to the control ones.

Heavy metal uptake by plant roots and shoots systems

The heavy metals concentration in the plant is affected by many factors such as the metal content supplied in the soil ecosystem and the plant tissue as well as by the interaction between these factors.

The mean uptake of metals Cd, Ni, Pb, Cu and Zn by roots (Figure 5a) and shoots (Figure 5b) of alfalfa plants increased as the concentrations of these metals in the soil ecosystem increased.

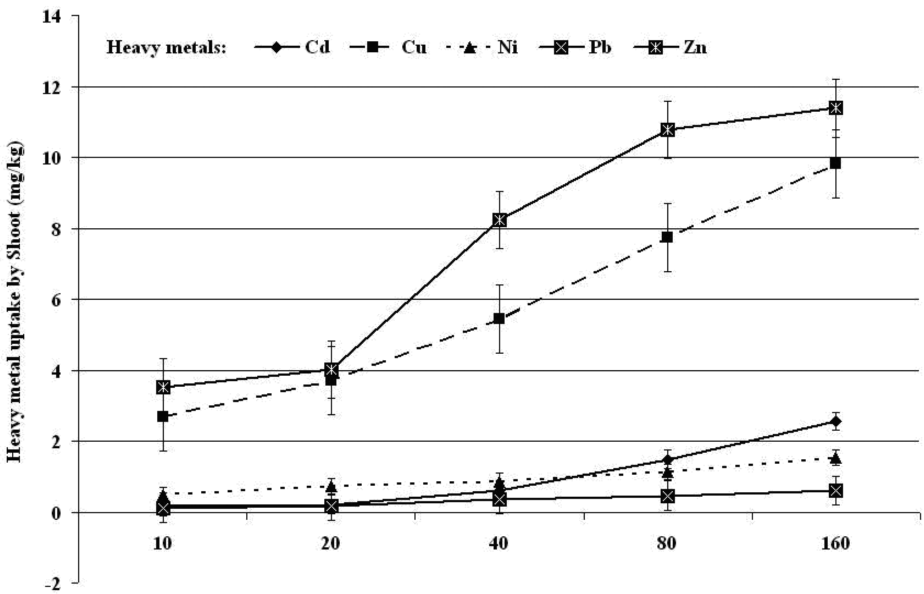
The results illustrated that the absorption of heavy metal by root system was directly proportional, increased by the increasing of the concentration of heavy metals. Zinc showed the highest metal uptake by root system, while Pb was the lowest metal absorbed by root system. It was found that the shoot system (Figure 5b) accumulated Zn and Cu more Cd, Ni and Pb.



Heavy metal concentrations (mg/kg)

Figure 5a. Uptakes of heavy metals by alfalfa roots system after 8 weeks plantation in clay loam brown forest soil

5.a ábra A lucerna gyökerének nehézfém felvétele 8 hetes fejlődést követően agyagbemosódásos barna erdőtalajon



Heavy metal concentrations (mg/kg)

Figure 5b. Uptakes of heavy metals by alfalfa shoot system after 8 weeks plantation in clay loam brown forest soil

5.b ábra A lucerna szárának nehézfém felvétele 8 hetes fejlődést követően agyagbemosódásos barna erdőtalajon

In the plants, shoots and roots, were observed to have a characteristic uptake capacity for different metals. The decreasing order of uptake of heavy metals by the alfalfa plants tissues was in the following order: Zn > Cu > Cd > Ni > Pb.

Discussion

The results demonstrated that the concentration was dependent on the inhibition of the seed germination. To some extent, our results are in agreement with PERALTA *et al.*, (2004) who investigated alfalfa plants grown in soil at different growth stages using separate batches of Cr^{6+} at 100 mg/l, and Cd^{2+}, Cu^{2+}, Ni^{2+}, or Zn^{2+} at 500 mg/l. In his case, four days after germination, all metals except Zn^{2+} had lethal effects on the seedlings. When applied 16 days after germination, Cr^{2+} and Ni^{2+} still had lethal effects on the seedlings and Cd^{2+} and Cu^{2+} destroyed more than 50% of the plant population. While approximately 90% of the plants exposed to Cd^{2+}, Cu^{2+} and Zn^{2+} were able to grow without apparent negative effects 20 days after germination, Cr^{2+} and Ni^{2+} still showed lethal effects. These results demonstrated that the tolerance of alfalfa plants to Cd, Cu and Zn was positively correlated with the age of the plants. Thus, alfalfa seedlings tolerated Zn^{2+} at 500 mg/l at the growth stage of 4 days after germination. Alfalfa plants could be considered potentially feasible to be transplanted in uncontaminated soils where the concentrations of Cd, Cu or Zn are high enough to interfere with alfalfa seed germination.

An increase in the heavy metal concentration in the soil ecosystem caused root length decrease with stunted growth of roots. One of the explanations for roots to be more responsive to toxic metals in environment might be that roots are a specialized absorptive organ, which means that they are affected earlier and subjected to accumulation of more heavy metals than any of the other plant organs This could be the main reason why root length is usually used as a measure for determining heavy metal tolerance of plants (XIONG 1998). According to CHAIGNON and HINSINGER (2003), higher concentrations of Cu can inhibit root growth before shoot growth and can accumulate in the roots without any significant increase in its content of the aerial parts. Heavy metals are found to be more toxic for root growth because they accumulate on roots and retard cell division and cell elongation. A similar conclusion was drawn by this examination in regards to results in Figure 2. The results indicate that low concentrations of Cd, Cu, Ni and Pb have micronutrient-like impacts on the alfalfa plants. These results agreed with the observation of CHHOTU and FULEKAR (2008). ORMROD et al. (1986) found that Ni caused stunted and deformed growth of shoots with symptoms of chlorosis. GYAWALI and LEKHAK (2006) noted a 11%, 22% and 41% reduction in plant height, respectively, over the control. Generally, it was seen that degrees of inhibition of shoot and root growth started from 10 µM concentration. In this respect PERALTA-VIDEA et al. (2004) reported that that Cd affected young plants more than old plants of *P. coccineus*, and Cd applied to the younger plants caused a stronger reduction in growth parameters such as leaf area and fresh weight accumulation and reduced shoot growth by reducing the chlorophyll content and the activity of photosystem I.

The biomass yield affected by the higher concentrations of metals, caused reduction in the plant biomass. Higher heavy metal concentrations can affect physiology and reduce plant growth and dry biomass yield (GRIFFERTY and BARRINGTON 2000). Authors showed that the increased Zn concentration from 25 to 50 mg/kg had a significantly positive effect on dry biomass yield. The plant biomass may be incinerated either to reduce volume, recover energy, dispose of off-using appropriate techniques or recycled to recover valuable metals (ANGEL and LINACRE 2005). That alfalfa produced greater biomass, which result in a higher concentration uptake of metals, was reported by PIVETZ (2001). The phytotoxicity of Cd on growth and dry matter production of a number of cultivated plants have been determined by GONDEK and FILIPEK-MAZUR (2003). Our results were in agreement with those mentioned above according to our results presented in Figs 4a and 4b.

The mean uptake of Cd, Ni, Pb, Cu and Zn by alfalfa plant systems increased as the concentrations of these metals in the soil ecosystem increased. Alfalfa shoot biomass has demonstrated the ability to bind an appreciable amount of Cu, Ni, Cd, Cr, Pb and Zn from aqueous solutions (TIEMANN et al. 1998). Increase in Pb uptake by alfalfa using EDTA and a plant growth promoter, was reported by LOPEZ et al. (2005). The large surface area of roots and their intensive penetration of soil may reduce leaching, runoff and erosion *via* stabilization of soil, and offer advantages for metal uptake. Most crop species tend to accumulate Cd at the highest concentrations in the root tissue, followed by leaves, then by seeds or storage organs . Several studies have demonstrated that the metal concentration in the plant tissue is a function of the heavy metals content in the growing environment (CUI et al. 2004). CHENG and ALLEN (2001) found that Cu uptake was linearly related to free Cu^{2+} ion activity and was independent of total Cu concentration in solution. In Regards to the effect of Cu on alfalfa plant growth and biomass, this investigation showed similar results to WU and HENDERSHOT (2010) who mentioned that the accumulation and toxicity of Cu to pea (*Pisum sativum* L.) roots were investigated. The root uptake of Cu and Ca varied with Ca and H activities. Calcium, H, and Cu competed for root binding with high pH and low Ca favoring more Cu uptake. Root elongation was highly sensitive to root Ca content and correlated better with root-bound Ca and Cu content than with merely dissolved Cu concentrations. A multi-element uptake model was developed to describe Cu and Ca accumulation by treating the pea roots as a collection of three biotic ligands with known site densities and proton-binding constants.

The essential elements (Cu and Zn) are required in low concentrations and hence are known as trace elements or micronutrients, whereas nonessential elements (Cd and Pb) are phytotoxic (GERARD et al. 2000). Zn is relatively mobile in soils and is the most abundant metal in the roots and shoots of contaminated plants as it is in soils. This metal is necessary as a minor nutrient and it is known that plants have special Zn transporters to absorb this metal (ZHU et al. 1999). The bioavailable of Pb is usually very low due to its strong association with organic matter, Fe-Mn oxides, clays and precipitation as carbonates, hydroxides and phosphates (SHEN et al. 2002). Cadmium also is considered to be mobile in soils but is present in much smaller concentrations than Zn (ZHU et al., 1999). Moreover, many studies have demonstrated that Cd taken up by plants accumulates at higher concentrations in the roots than in the leaves (BOOMINATHAN and DORAN, 2003). In addition, exudation of organic compounds by plant roots, such as organic acids, influence ion solubility and uptake (KLASSEN et al. 2000) through their effects on microbial activity, rhizosphere physical properties and root-growth dynamics (Yang et al. 2005). The higher concentrations heavy metals uptake by alfalfa was reported by REHAB et al. (2002). It can be concluded that the low-doses of tested heavy metals applied, stimulated the root and shoot elongation of alfalfa plants. At higher concentrations (over 80 µM or 80 mg/

kg), Cd, Cu, Ni and Pb reduced the germination rates and phytobiomass of alfalfa plants. This study shows that heavy metals were efficiently taken up by alfalfa plants at all concentrations and the uptake was increased with the increasing concentrations in soil. Finally, the alfalfa plants, to some extent, demonstrated their potential in cleaning the soil environment from heavy metals.

References

Alloway B.J. 1995: Soil processes and the behaviour of heavy metals. pp.11–37. In. B.J. Alloway (ed.) Heavy metals in soils. Blackie Academic and Professional, London.

Alvarez-Ayuso E. 2008: Cadmium in soil-plant systems: an overview. Intern. J. Environ. Pollution, 33: 275–291.

Angel J.S., Linacre N.A. 2005: Metal phytoextraction-A survey of potential risks. Intern. J. Phytoremediation, 7: 241–254.

Baker A.J.M., Brooks R.R. 1998: Terrestrial higher plants which accumulate metals elements: A review of their distribution, ecology and phytochemistry. Biorecovery, 1: 81–126.

Bingham F.T., Sposito G., Strong J.E. 1984: The effect of chloride on the availability of cadmium. J. Environ. Qual., 13: 71–74.

Boominathan R., Doran P.M. 2003: Cadmium tolerance antioxidative defenses hyperaccumulator, *Thlaspi caerulescens*. Biotechnol. Bioengineering, 83: 158–167.

Chaignon V., Hinsinger P. 2003: A biotest for evaluating copper bioavailability to plants in a contaminated soil. J. Environ. Qual., 32: 824–833.

Cheng T., Allen H.E. 2001: Prediction of uptake of copper from solution by lettuce (*lactuca sativa* romance). Environ. Toxicol. Chem., 20: 2544–2551.

Chhotu J.D., Fulekar M.H. 2008: Phytotoxicity and remediation of heavy metals by alfalfa (*Medicago sativa*) in soil-vermicompost media. Adv. Nat. Appl. Sci., 2: 141–151.

Cui Y., Wang Q., Christie P. 2004: Effect of elemental sulphur on uptake of cadmium, zinc and sulphur by oilseed rape growing in soil contaminated with zinc and cadmium. Commun. Soil Sci. Plant Anal., 35: 2905–2916.

Fitamo D., Itana F., Olsson M. 2007: Total contents and sequential extraction of heavy metals in soils irrigated with wastewater, Akaki, Ethiopia. Environ. Mgmt., 39: 178–193.

Gerard E., Echevarria G., Sterckeman T., More J.L. 2000: Cadmium availability to three plant species varying in cadmium accumulation pattern. J. Environ. Qual., 29: 1117–1123.

Giller K.E., Witter E., McGrath S.P. 1998: Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils. Soil Biol. Biochem., 30: 1389–1414.

Gondek K., Filipek-Mazur B. 2003: Biomass yields of shoots and roots of plants cultivated in soil amended by vermicomposts based on tannery sludge and content of heavy metals in plant issues. Soil Environ., 49: 402–409.

Grifferty A., Barrington S. 2000: Zinc uptake by young wheat plants under two trand piration regimes. J. Environ. Qual., 29: 443–446.

Guiweia Q., de Varennesa A, Martinsa L.L., Mouratoa M.P., Cardosoa A.I., Motab A.M., Pintoc A.P., Gonçalvesb M.L. 2010: Improvement in soil and sorghum health following the application of polyacrylate polymers to a Cd-contaminated soil. J. Hazardous Materials, 173: 570–575.

Gyawali R., Lekhak H.D. 2006: Chromium tolerance of rice (*Oryza sativa* L.) cultivars from Kathmandu valley. Nepal. Scientific World, 4: 4–10.

Holm P.E., Christensen H.T., Tjell J.C., McGrath S.P. 1995: Heavy metals in the environment. Speciation of cadmium and zinc with application to soil solutions. J. Environ. Qual., 24: 183–190.

Ilyin I., Rosovskaya O., Tranikov O., Aas W., Hettelingh J.P., ReindsG.J. 2008: Heavy metals: trand boundary pollution of the environment. EMEP Status Report 2/2008 (JointMSC-E and CCC and CCE Report). Convention on Long-range Trand boundary Air Pollution.

Joshi U.N., Luthra Y.P. 2000: Diversification of agriculture for human nutrition. Current Sci., 78: 1–4.

Klassen S.P., McLean J.E., Grossel P.R., Sims R.C. 2000: Fate and behavior of lead in soils planted with metal resistant species (River birch and small wing sedge). J. Environ. Qual., 29: 1826–1834.

Lasat M.M. (2002): Phytoextraction of toxic metals: A review of biological mechanisms, J. Environ. Qual., 31: 109–120.

Lopez M.L., Peralta-Videa J.R., Benitez T., Gardea-Torresdey J.L. 2005: Enhancement of lead uptake by alfalfa (*Medicago sativa*) using EDTA and a plant growth promoter. Chemosphere, 61: 595–598.

Ma J.H., Chu C.J., Li J., Song B. 2009: Heavy metal pollution in soils on railroad side of Zhengzhou-Putian section of Longxi-Haizhou Railroad, China. Pedosphere, 19: 121–128.

Mico C., Rectala L., Peris M., Sanches J. 2006: Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. Chemosphere, 65: 863–872.

Nicholson F.A., Smith S.R., Alloway B.J., Carlton-Smith C., Chambers B.J. 2003: An inventory of heavy metals inputs to agricultural soils in England and Wales. The Science of the Total Environment, 311: 205–219.

Nouri J., Peterson P.J., Alloway J.B. 2001: Effect of variation in harvest time on the uptake of metals by barley plants from soil amended with sludge and compost. J. Biol. Sci., 1: 1056–1059.

Ormrod D.P., Hale J.C., Allen O.B. 1986: Joint action of particulate fall-out Nickel and rooting medium nickel on soybean plants. Environ. Pollution, 41: 277–291.

Peralta-Videa J.R., de la RosaG., Gonzalez J.H., Gardea-Torresdey J.L. 2004: Effects of the growth stage on the heavy metal tolerance of alfalfa plants. Adv. Environ. Res., 8: 679–685.

Pivetz B.E. 2001: Phytoremediation of contaminated soil and ground water at hazardous waste sites. United States Environmental Protection Agency, 1–35.

Rehab F.B., Prevost D., Tyagi R.D. 2002: Growth of alfalfa in sludge-amended soils and inoculated with rhizobia produced in sludge. J. Environ. Qual., 31: 1339–1348.

Roy S., Labelle S., Mehta P., Mihoc A., Fortin N., Masson C., Leblanc R., Chateauneuf G., Sura C., Gallipeau C., Olsen C., Delisle S., Labrecque M., Greer C.W. 2005: Phytoremediation of heavy metal and PAH-contaminated brownfield sites. Plant and soil, 272: 277–290.

Shen Z.G., Li X.D., Wang C.C., Chen H.M., Chua H. 2002: Lead phytoextraction from contaminated soil with high-biomass plant species. J. Environ. Qual., 31: 1893–1900.

Tiemann K.J., Gardea-Torresdey J.L., Gamez G., Dokken K. 1998: Interference studies for multimetal binding by *Medicago sativa* (alfalfa). Depart. Chem. Environ. Sci. Eng., University of Texas. Proceedings of the 1998 Conf. on Hazardous Waste Res., pp. 63.Urban H. 2007: Impact of urban agriculture. Highlights of urban harvest research and development, 2003–2006. pp. 66.

Vasiliadou S., Dordas C. 2009: Increased concentration of soil cadmium affects on plant growth, dry matter accumulation, Cd, and Zn uptake of different tobacco cultivars (*Nicotiana tabacum* L.). Intern. J. Phytoremediation, 11: 115–130.

Vincent J.M. 1970: Manual of techniques for the study of root nodule bacteria. IBP Handbook. Blackwell Scientific Publications. Oxford.

Wu Y., Hendershot W. 2010: Effect of Calcium and pH on Copper Binding and Rhizotoxicity to Pea (*Pisum sativum* L.) Root: Empirical Relationships and Modeling. Archives of Environmental Contamination and Toxicology, 59: 109–119.

Xiong Z.T. 1998: Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumulator *Brassica pekinensis* Rupr. Bull. Environ. Contam. Toxicol., 6: 258–291.Yang X.E., Peng H.Y., Jiang L.Y. 2005: Phytoremediation of copper from contaminated soil by *Elsholtzia splendens* as affected by EDTA, citric acid and compost. Intern. J. Phytoremediation, 7: 69–83.Zhu D., Schwab A.P., Banks M.K. 1999: Heavy metal leaching from mine tailings as affected by plants. J. Environ. Qual., 28: 1727–1732.

A *MEDICAGO SATIVA* L. CSÍRÁZÁSA, FEJLŐDÉSE ÉS FÉMFELVÉTELE NEHÉZFÉMMEL SZENNYEZETT AGYAGBEMOSÓDÁSOS BARNA ERDŐTALAJON

[1]ABUSRIWIL M.H. LAILA, [2]BAYOUMI HAMUDA E.A.F. HOSAM, [1]ELFOUGHI A. ALAELDDIN

[1]Szent István Egyetem, Környezettudományi Doktoi Iskola
2103 Gödöllő, Páter K. u. 1.
[2] Óbudai Egyetem, Rejtő Sándor Könnyűipari és Környezetmérnöki Kar, Környezetmérnöki Intézet
1034 Budapest, Doberdó u. 6. E-mail: hosameaf@yahoo.com

Kulcsszavak: lucerna, nehézfém felvétel, növény növekedés és biomassza, agyagbemosódásos barna erdőtalaj

Összefoglalás: A növényeknek a nehézfémekkel szennyezett talajok javítása érdekében történő felhasználása biztonságos ökológiai módszer. Laboratóriumi kísérletben, Petri csészében 3 réteg Whatman típusú szűrőpapírt áztattunk 50 ml, 0, 10, 20, 40, 80 és 160 µM koncentrációjú Cd, Cu, Ni, Pb és Zn oldattal, 21 napon keresztül. A kísérlet célja az volt, hogy meghatározzuk a nehézfémeknek a fertőtlenített lucerna magvak csírázására, gyökér és hajtás növekedésére történő hatását. Az *in vivo* kísérleteket üvegházban végeztük, ahol lucerna magvakat csíráztattunk 2 kg nehézfémekkel (Cd, Cu, Ni, Pb and Zn) szennyezett agyagbemosódásos barna a erdőtalajt tartalmazó műanyag ládákban, 8 héten keresztül. A talaj a nehézfémeket 0, 10, 20, 40, 80 to 160 mg/kg koncentrációban tartalmazta. Az *in vitro* kísérletek azt mutatják, hogy a Cd, Cu, Ni és Pb 80 és 160 µM-nál magasabb koncentrációja gátolta a csírázást, a gyökér és hajtás növekedést. A csírázást gátló fémek toxicitása a következőképpen alakult: Cd > Cu > Ni > Pb > Zn. Habár, a csírázás mértéke fokozódott a Zn összes vizsgált koncentrációjánál. A vizsgált fémek 10 és 20 µM koncentrációnál stimulálták a gyökér és hajtás növekedést, míg 10 és 20 mg/kg koncentrációnál növelték a növény biomassza tömegét. Megállapítottuk, hogy a lucerna – a 160 mg/kg koncentrációt kivéve – hatékony fejlődésre képes a Zn összes vizsgált koncentrációjánál. Az eredmények azt mutatják, hogy a lucerna alkalmas a talajhoz 10-160 mg/kg koncentrációban adott nehézfémek különböző mértékű felvételére. Végül megállapítottuk, hogy a növény bizonyos mértékben felhasználható a nehézfémekkel szennyezett talajok tisztításához.