Arch. Biol. Sci., Belgrade, 60 (2), 239-244, 2008 DOI:10.2298/ABS0802239G

LEAD UPTAKE, TOLERANCE, AND ACCUMULATION EXHIBITED BY THE PLANTS *URTICA DIOICA* AND *SEDUM SPECTABILE* IN CONTAMINATED SOIL WITHOUT ADDITIVES

MILENA GRUBOR

Department of Ecological Safety and Protection, Clinical Center of Serbia, 11000 Belgrade, Serbia

Abstract — Specimens of *Urtica dioica* and *Sedum spectabile* collected from plants growing at uncontaminated sites were transplanted in Pb-contaminated soil without additives (EDTA, HEDTA) to identify their natural potential for hypertolerance and hyperaccumulation of lead. The total content of Pb in the plants was determined by atomic spectroscopy. Our research showed that the concentrated toxic levels of lead (Pb) in *Sedum spectabile* and *Urtica dioica* were about 100 or more times higher than those of non-accumulator plants. It can be concluded that these plants have a high natural potential for hypertolerance and hyperaccumulation of lead, since they can hyperaccumulate it without addition of any chelating compounds (EDTA, HEDTA) to enhance lead uptake. This makes them very promising plants for use in phytoremediation of Pb-contaminated sites.

Key words: *Urtica dioica*, *Sedum spectabile*, hyperaccumulation, hypertolerance, lead, phytoremediation

UDC 582.635.5:582.711.16:504

INTRODUCTION

Generally, environmental remediation deals with the removal of pollution or contaminants from environmental media such as soil, groundwater, sediment, or surface water for the general protection of human health and the environment. The most common chemicals involved are lead and other heavy metals, pesticides, petroleum hydrocarbons, and solvents. Occurrence of this phenomenon is correlated with the degree of industrialization and intensity of chemical usage. The threat that heavy metals (such as lead) pose to human and animal health is aggravated by their long-term persistence in the environment. For instance, lead (Pb), one of the more persistent metals, has been estimated to have a soil retention time of 150 to 5000 years (Mack, 1995).

Until 1970, there was little widespread awareness of the worldwide scope of soil contamination or its health risks. In 1980 the U.S. Comprehensive Emergency Response Compensation and Liability Act (CERCLA) was passed to establish, for the first time, strict rules on legal liability for soil contamination. This act stimulated identification and cleanup of thousands of sites, and encouraged property buyers and sellers to make soil contamination a focal issue of land use. It has become standard practice in Japan and many parts of the western world.

REMEDIATION TECHNOLOGIES

Remediation technologies are many and varied but can be categorized as into *ex situ* and *in situ* methods. *Ex situ* methods involve excavation of impacted soils and subsequent treatment at the surface. *In situ* methods seek to treat the contamination without removing the soils. The more traditional remediation approach (used almost exclusively on contaminated sites from the 1970s to the 1990s) consists primarily of soil excavation and disposal on landfill dumps.

Phytoremediation, the use of certain plants to remove or stabilize harmful contaminants or to prevent soil erosion, is a relatively new technology that can be applied to large areas to remove heavy metals, in particular lead, from contaminated sites (soil or water) and to degrade or eliminate pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the media that contain them. Some extracted metals can be recycled for profit. It is clean, efficient, inexpensive, and environmentally non-disruptive, as opposed to processes that require excavation of soil. Phytoremediation processes include phytoextraction, rhyzofiltration, phytostabilization, phytovolatilization, and phytotransformation. There are different versions of phytoextraction: natural hyperaccumulation, where certain plants naturally take up the contaminants in soil unassisted, at levels that would be toxic to many other plants (> 0.1 – 1.5 ppm Pb/g dry weight); and induced or assisted hyperaccumulation, in which a conditioning fluid containing a chelator or another agent is added to soil to increase metal solubility or mobilization, so that the plants can absorb them more easily (Baker and Brooks, 1989).

LEAD UPTAKE, TOLERANCE, AND ACCUMULATION BY THE PLANTS *URTICA DIOICA* AND *SEDUM SPECTABILE*

Lead is a natural constituent of the Earth's lithosphere. It is released into the soil and water through weathering of bedrock (Mack, 1995). In contrast to toxic organics and inorganics, which in many cases can be degraded, metallic species released into the environment tend to persist indefinitely, accumulating in living tissues throughout the food chain. Metal pollutants such as lead, arsenic, cadmium, mercury, copper, chromium, and zinc cannot be broken down to non-toxic forms. Once a site is suspected of being contaminated, there is a need to assess the contamination.

The general purpose of this research was to develop better phytoremediation techniques by identifying suitable crop and plant species that show the ability to accumulate lead while producing large amounts of biomass when grown using established agricultural practices.

MATERIALS AND METHODS

Specifically, the objectives of the experiments were to: (a) identify outdoor-grown *Sedum spectabile* and *Urtica dioica*, plants that in pot studies demonstrate vigorous growth in actual lead-contaminated soils; and (b) select those plants which take up high levels of lead or which translocate lead to above-ground portions of the plant without the addition of any chelating compound (EDTA, HEDTA) to enhance lead uptake by increasing metal solubility or mobilization, so that the plants can absorb them more easily. *Urtica dioica* and *Sedum spectabile* were chosen as model plants because of their common presence throughout the tested area.

The research was done using vegetative planting materials of *Sedum spectabile* and *Urtica dioica* collected from plants growing at sites known to be not contaminated with Pb.

Some vegetating plants of *Sedum spectabile* and *Urtica dioica* growing at such sites were transplanted in the same uncontaminated soil as control plants.

The battery industry is the largest consumer of lead and the chief source of lead in the environment. Samples of Pb-contaminated soil were taken around the premises of battery manufacturers and recyclers, where the amount of lead available to plants should be different from that of normal soil, in order to evaluate the ability of *Sedum spectabile* and *Urtica dioica* to tolerate and accumulate toxic levels of lead.

Plants of *Sedum spectabile* and *Urtica dioica* were transplanted in pots containing Pb-contaminated soil three times per growing season, each experiment lasting from two to three weeks (Fig. 1). Any metal toxicity symptoms (e.g., yellowing, stunting) exhibited by the plants were visually noted during the experimental period. Two to three weeks after the initial lead treatment, all plants were harvested. The leaves, stems, and roots of *Urtica dioica* and *Sedum*

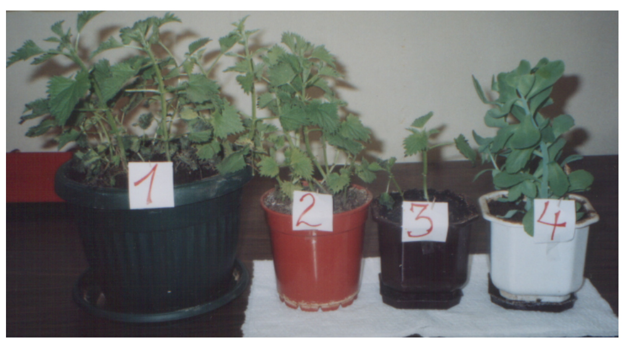

Fig. 1. Plants of *U. dioica* and *S. spectabile* growing in pots containing Pb-contaminated soil.

spectabile were digested and processed by atomic absorption spectroscopy (AAS). The lead content of each tissue was determined using described nitric acid-hydrogen peroxide procedures (USEPA, 1990) with slight modifications, and expressed as µgs of lead/g of dry weight of plant tissue.

RESULTS AND DISCUSSION

The main goal of *in situ* remediation techniques is to reduce the fraction of toxic elements that is potentially mobile or bioavailable. Soluble contaminants are subject to migration with soil water, uptake by plants or aquatic organisms, or loss due to volatilization into the atmosphere. Bioavailability refers to the fraction of a contaminant that can be taken into any biological entity, be it plant, earthworm, or human. Successful implementation of phytoextraction depends on the following: identification of suitable plants; bioavailability of the contaminant in the environmental matrix; root uptake; internal translocation of the plant; and plant tolerance. Plant productivity (i.e., the amount of dry matter that is harvestable each season) and the accumulation factor (the ratio of metal in plant tissue to that in the soil) are important design parameters (Blaylock, 2000).

In our research, plants were transplanted in Pb – contaminated soils without the use of chelators (EDTA; HEDTA, S, or citric acid) because the addition of chelating agents to such soils increase Pb solubility and mobility within plants and involves the additional risk of leaching of Pb chelates into the soil (Blaylock et al., 1997; Bowman et al., 2005; Sun et al., 2001).

Permissible Exposure Limits (PEL) of lead for plants, toxic levels, and critical concentration:

- A concentration of 1 mg/l lead harms plants.

- The critical concentration of lead for plants is 10 µg/g dry weight.

- A level of 20 µg/g dry weight of lead is toxic for plants.

- The PEL of lead for soil is 100 mg/kg.

- The normal level of lead for most plants is 0.1 do 1.5 ppm/g dry weight.

Analysis of lead concentration in the control soils with plants of *Sedum spectabile* and *Urtica dioica* showed low natural Pb concentrations, which are presented in Fig. 2. It was very important to identify natural concentrations of lead in the control soils in order to demonstrate that higher levels of lead in the experimental plants came from contaminated soil, not from soils where the plants were grown.

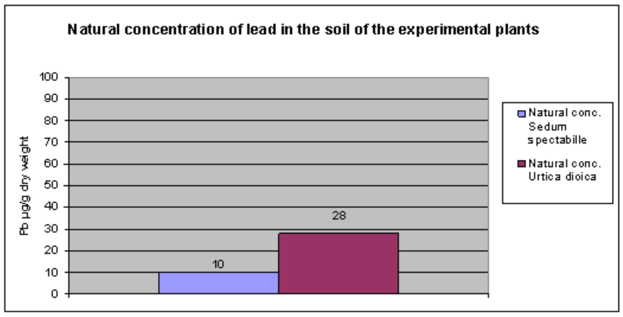

Fig. 2. Natural concentration of lead in uncontaminated soils where the control plants were grown..

Figure 3 presents the natural concentration of lead in control plants of *Urtica dioica* grown in uncontaminated soil. The content of lead in vegetative plant material was lower in control plants of *Sedum spectabile* than in control plants of *Urtica dioica*.

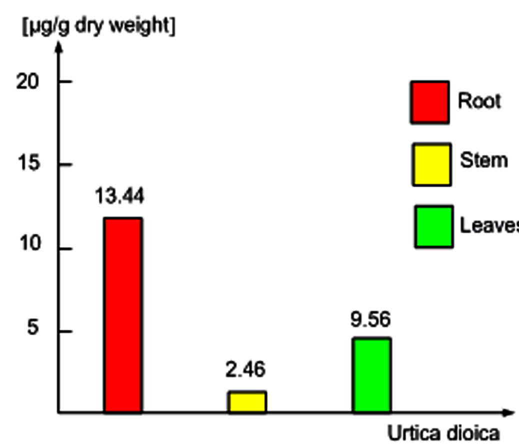

Fig. 3. Concentration of lead in vegetative plant material of *Urtica dioica* plants grown in uncontaminated soil (control plants).

Our experimental research indicated that the levels of lead (Pb) in *Sedum spectabile* and *Urtica dioica* are about 100 or more time higher than those of non-accumulator plants, these plants having been shown in the past to possess a high natural potential for hyperaccumulation and hypertolerance of lead (Pb) because they can hyperaccumulate it without the addition of any chelating compounds (EDTA, HEDTA) to enhance lead uptake (S h a w, 1990). A natural metal hyperaccumulator phenotype is much more important than a high yield potential when using plants to remove metals from contaminated soils. The results are presented in Figs. 6, 7, and 9.

Our studies showed that *Urtica dioica* was relatively sensitive to higher concentrations of lead (10500 µg/g of dry weight) (Fig. 6), but was not inhibited by lower concentrations (Fig. 7). This means that adsorption of lead by *Urtica dioica* was limited and may reach a maximum (10500 µg/g of dry weight). Because its lead tolerance was not infinite, high lead concentrations would harm the plant.

Sedum spectabile was shown to be a less effective natural hypeaccumulator (110-840 µg/g of dry weight), but a naturally hypertolerant species,

Fig. 4. Lead concentration of 10500 µg/g of dry weight harmed *U. dioica*.

Fig. 5. Lead concentration lower than 10500 µg/g of dry weight) did not harm *U. dioica*.

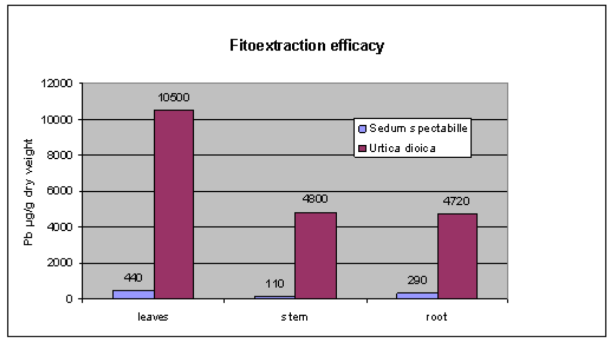

Fig. 6. Efficacy of lead phytoextraction by *Sedum spectabile* and *Urtica dioica* after three weeks (experiment conducted in June).

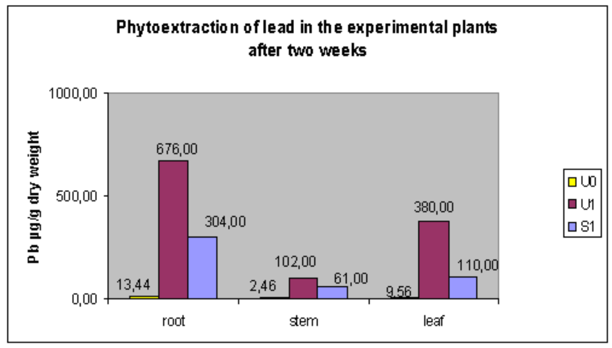

Fig. 7. Efficacy of phytoextraction by *Sedum spectabile* and *Urtica dioica* after two weeks (experiment conducted in August). U0-Natural concentration in control *U. dioica*; U1- concentration of lead in experimental *U. dioica*; S1- concentration of lead in experimental *S. spectabile*.

Fig. 8. *S. spectabile* and its infinite lead tolerance.

since its lead tolerance was infinite and high lead concentrations did not harm the plant (Fig. 8). Hypertolerance of metals is a key plant characteristic required for hyperaccumulation; vacuolar compartmentalization appears to be the source of hypertolerance in natural hyperaccumulators (Baker and Walker, 1990). The results indicated that *Sedum spectabile* developed a good natural mechanism of tolerance and reduction of lead uptake, which means that the root of *Sedum spectabile* is an excellent barrier to translocation of lead to the shoots. The discovery that *Sedum* is a naturally hypertolerant plant has great practical importance because low tolerance to higher concentrations of lead harmed or killed fast-growing plants that are able to accumulate toxic concentrations of metals such as Pb, and Cd. Its low sensitivity to Pb toxicity makes *Sedum spectabile* a very promising plant for the phytoremediation of Pb-contaminated sites.

Testing of phytoextraction of lead by *Sedum spectabile* and *Urtica dioica* after three weeks showed that the concentration of lead in the root is lower than in the shoots (Fig. 6). However, testing of it after two weeks indicated that the concentration of lead in the root is higher than in the shoots (Fig. 7). These results suggest that the rate of Pb uptake by the root decreased and the rate of translocation to the shoots increased as a function of exposure time (Jones et al., 1973).

Our results demonstrated that *Sedum spectabile* and *Urtica dioica* accumulated lead, but exhibited different tolerance of lead taken up from solid media (Pb-contaminated soil around the premises of battery manufacturers and recyclers). These results showed that *Sedum spectabile* and *Urtica dioica* are able to reduce the heavy metal content in soil to low levels, without any additives to enhance lead uptake. The results are presented in Fig. 9.

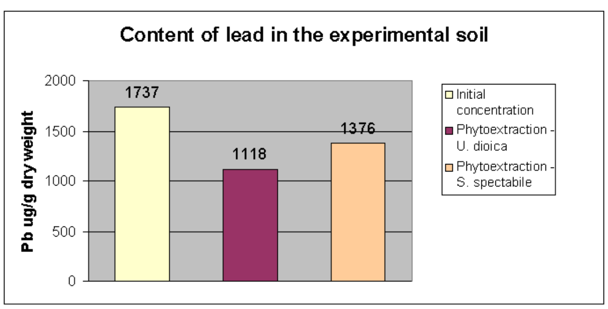

Fig. 9. Reduction of initial lead content in the experimental soil after phytoextraction by *Sedum spectabile* and *Urtica dioica* (experiment conducted in October).

Of the two species evaluated, *Urtica dioica* is the more suitable species for phytoextraction of lead from samples of Pb-contaminated soil (Pb-contaminated soil around the premises of battery manufacturers and recyclers). The different responses of various species to lead suggest that the phytotoxic mechanisms of lead involve different biochemical pathways in different plant species. The exact nature of these mechanisms was not investigated in this study.

Urtica dioica and *Sedum spectabile* have good biomass, are easy to breed using current crop production and management practices, and are not aggressive plants, characteristics that make them suitable for phytoextraction. The whole plant biomass of *Sedum spectabile* and *Urtica dioica* must be harvested before flowering to protect against dissemination of lead by pollen and because some animals obtain food (nectar, pollen, or fruit pulp) from the plants after flowering.

PHYTOREMEDIATION: FROM LABORATORY INTO THE FIELD

These results do not reflect field fluctuations in soil moisture and nutrient levels, or competition from other species growing at the lead-contaminated site. Other arrangements are therefore recommended for future tests. The actual effectiveness of *Urtica dioica* and *Sedum spectabile* for cleaning up Pb-contaminated fields can be determined more precisely by further evaluation of their growth, tolerance, and metal uptake. This can be done by quantifying lead levels in soils and plants before and after harvest. Further research is needed to understand cellular mechanisms of contaminant transport in plants and the physiology of contaminant uptake, translocation, and accumulation. Both fundamental and empirical studies will contribute to our understanding of these processes (Malone et al., 1973).

CONCLUSIONS

Urtica dioica and *Sedum spectabile*, without any additives such as chelating agents (EDTA, HEDTA), are able to accumulate, tolerate, and to some extent translocate Pb ions from contaminated soil and can be considered as natural hyperaccumulators of lead and species hypertolerant of lead toxicity. If in further studies *Urtica dioica* and *Sedum spectabile* prove capable of hyperaccumulating Pb from Pb-contaminated fields, they could become a valuable tool for the phytoremediation of Pb-contaminated sites. Methods designed to recover inorganic elements using plants and phytoextraction are in the developmental stage, and the technology of phytoremediation is, for the most part, still a concept. Research in phytoremediation could benefit mankind by helping to increase land productivity by making more land arable to feed a rapidly increasing population in the world (Moffat, 1995).

REFERENCES

Baker, A. J. M., and *R. R. Brooks* (1989). Terrestrial higher plants which hyperaccumulate metallic elements: a review of their distribution, ecology, and phytochemistry. *Biorecovery* **1**:81126.

Baker, A. J. M., and *P. L. Walker* (1990). Ecophysiology of metal uptake by tolerant plants, In: *Heavy Metal Tolerance in Plants: Evolutionary Aspects* (Ed. A. J. Shaw), 155-177. CRC Press, Boca Raton.

Blaylock, M. J. (2000). Field demonstrations of phytoremediation of lead-contaminated soils, In: *Phytoremediation of Contaminated Soil and Wate*r (Eds. N. Terry and G. Banuelos), 1-12. Lewis Publishers, Boca Raton.

Blaylock, M. J., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B. D., Salt, D.E., and *I. Raskin* (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technol.* **31**, 860-865.

Bouwman, L. A., Bloem, J., Romkens, P. F. A. M., and *J. Japanega* (2005) Edge amendment of slightly heavy metal-loaded soil affects heavy metal solubility, crop growth, and microbes and various nematodes, but not bacteria and herbivorous nematodes. *Soil Biol. Biochem*. **37**, 271-278.

Jones, L. H. P., Clement, C. R., and *M. J. Hopper* (1973). Lead uptake from solution by perennial ryegrass and its transport from roots to shoots. *Plant Soil* **38**, 403414.

Mack, R. B. (1995). Lead in history. *Clin.Toxicol. Bull.* **3**, 7-44.

Malone, C. D., Koeppe, D. E. and *R. J. Miller* (1974). Localization of lead accumulated by corn plants. *Plant Physiol.* **53**, 388394.

Moffat, A. S. (1995). Plants proving their worth in toxic metal cleanup. *Science* **269**, 302303.

Shaw, A. J. (1990), In: *Heavy Metal Tolerance in Plants: Evolutionary Aspects* (Eds. A. J. Shaw), 268. CRC Press, Boca Raton.

Sun, B., Zhao, F. J., Lombi, E., and *S. P. McGrath* (2001). Leaching of heavy metals from contaminated soils using EDTA. *Environ. Pollution* **113**, 111-120.

U.S. Comprehensive Emergency Response Compensation and Liability Act (CERCLA). Public Law, codified as 42 U.S.C. §§ 9601 to 9675, enacted by the United States Congress on December 11, 1980.

АПСОРПЦИЈА, ТОЛЕРАНЦИЈА И АКУМУЛАЦИЈА ОЛОВА БИЉКАМА *SEDUM SPECTABILE* И *URTICA DIOICA* БЕЗ ДОДАТАКА У КОНТАМИНИРАНО ЗЕМЉИШТЕ

МИЛЕНА ГРУБОР

Одељење за еколошку безбедност и заштиту, Клинички центар Србије, 11000 Београд, Србија

По први пут, изабране биљке *Urtica dioica* и *Sedum spectabile* одгајане на Pb-незагађеном земљишту су тестиране у лабораторијским условима, засађене у Pb-контаминираном земљишту са тачно одређених Pb-загађених локалитета, са циљем да се одреде њихови природни потенцијали на хиперакумулацију и хипертолеранцију, што подразумева недодавање хелата (EDTA, HEDTA) у загађено земљиште. Укупан садржај Pb у биљкама је одређиван атомском спектроскопијом. Наша истраживања су показала: да је токсична концентрација олова у *Urtica dioica* и *Sedum spectabile* стотину и више пута већа него код не-акумулаторних биљака, па су ове биљке показале природна хипер-акумулаторна и хипер-толерантна својства, јер су акумулирале велике количине Pb без икаквих додатака - хелационих једињења (EDTA, HEDTA), за повећање преузимања олова из земљишта. Ова открића потврђују да се такве биљке могу успешно употребити у фиторемедијацији Pb-загађеног земљишта.