

# Bioavailability and mobility of Pb after soil treatment with different remediation methods

N. Finžgar, B. Kos, D. Leštan

*Biotechnical Faculty, University of Ljubljana, Slovenia*

## ABSTRACT

The risk of Pb contaminated soil after treatment with different remediation techniques was assessed. Soil vitrification (microwaves) reduced Pb orally available from the stomach and intestinal phase measured by a physiologically based extraction test. Solidification of Pb (Slovakite) did not reduce the concentration of orally available Pb and increased the Pb uptake by *Thlaspi goesingens* for 2-times compared to plants grown on the original soil. Soil extraction with 40 mmol/kg EDTA removed 57.6% of Pb. However, the concentration of orally available Pb and Pb uptake by *T. goesingens* increased. The concentration of mobile soil Pb also increased for 14-times measured by a toxicity characteristic leaching procedure. Soil leaching with 10 mmol/kg biodegradable chelator [S,S] ethylenediamine disuccinate and using a horizontal permeable reactive barrier for Pb accumulation from the washing solution removed 17.8% of Pb, but increased Pb uptake by *T. goesingens* by 3-times and slightly increased the concentration of Pb in the intestinal phase. Leaching did not significantly effects Pb availability from the stomach phase or Pb mobility.

**Keywords:** lead; soil remediation; risk assessment; heap leaching

Management of agricultural ecosystems, as well as remediation of former industrial sites which have been exposed to diffuse pollution with heavy metals, are of major concern world-wide. There are two main types of remediation for heavy-metal contaminated soils: immobilization technologies that leave heavy metals in the soil but minimize their availability and migration, and technologies that remove heavy metals from the soil (Peters 1999).

Heavy metal immobilization can be achieved by solidification/stabilization by liming, and using different soil amendments, such as zeolites, cement or red mud. For Pb contaminated soils, different phosphates (i.e. apatite, rock phosphate), which form insoluble salts with Pb (e.g. pyromorphite), have often been successfully applied (Laperche et al. 1997). Another immobilization method is vitrification by heating the contaminated soil to as much as 2000°C. Vitrification usually involves imposing an electrical current between electrodes inserted into the contaminated soil. Due to its low electric conductivity, the soil begins to heat to produce a melt that hardens into a blocks of

glasslike material (Buel and Farnsworth 1991). Abramovitch et al. (2003) proposed an alternative vitrification method, which uses microwave energy to heat the soil.

Various soil washing technologies are most commonly used for removing heavy metals from the soil. Soil washing involves the separation of heavy metals from the soil solid phase by solubilizing them in a washing solution. Acid and chelator soil washing are the two most prevalent removal methods (Peters 1999, Aydinalp and Katkat 2004). Soil washing currently involves soil flushing – an *in situ* process in which the washing solution is forced through the in-place soil matrix, *ex situ* extraction of heavy metals from the soil slurry in reactors, and soil heap leaching. Another heavy metal removal technology is electroremediation, which mostly involves electrokinetic movement of charged particles suspended in the soil solution, initiated by an electric gradient (Reed et al. 1996). The metals can be removed by precipitation at the electrodes. A publicly appealing (green) remediation technology is phytoextraction (Vysloužilová et al. 2003). However, phytoextraction can be

---

Supported by the Slovenian Ministry for Education, Science and Sport, Grant J4-6134-0481-04/4.03.

effectively applied only for soils contaminated with specific heavy metals and metalloids, e.g. Ni, Zn, Cd, As, for which hyperaccumulating plants with high biomass are known (McGrath and Zhao 2003).

The goal of soil remediation is to reduce the risk of heavy metals entering into organisms either by soil ingestion, breathing contaminated soil dust particles, through food produced on contaminated soil, or by drinking water polluted with leachable metals. There are a number of tests that have been used to evaluate the bioavailability and migration of heavy metals, particularly of Pb, which is the metal of interest in our study, before and after remediation. Bioavailability and mobility tests have been used primarily in studies of Pb solidification in soils. This is understandable, since immobilization technologies leave heavy metals in the soil, presumably in an inactive form, less available for plants and other organisms. However, heavy metals can still be harmful through soil ingestion or inhalation of soil dust.

Soil washing methods aim to remove as high as possible share of heavy metals from the soil, or to reduce the total concentration of heavy metals in the soil below limits set by legislation (in Europe: Council Directive 86/278/EEC 1986). However, even in small-scale laboratory studies, where just a few grams of soil were treated, sometimes only a minor part of the heavy metals was removed, especially from soils with a high clay and silt content (Peters and Shem 1992). Nevertheless, a survey of the literature revealed no studies on phyto- or bioavailability of Pb after treatment of contaminated soil with different soil washing techniques.

The food chain transfer of Pb is one of the major pathways for human exposure to Pb contamination in soil. The accumulation of toxic metals in indicator plants can be used to assess the extent of metal contamination and the exposure risk to indigenous biota. The Pb hyper-accumulating plant *Thlaspi goesingense* has recently been indicated by Puschenreiter et al. (2001). We used *T. goesingense* in this study as a possible indicator of phytoavailable Pb in soil.

In order to predict the oral bioavailability of Pb from soil when animal study results are not available Ruby et al. (1993) developed a physiologically based extraction test (PBET). PBET is an *in vitro* test and incorporates gastrointestinal tract parameters representative of a human (stomach and small intestinal pH and chemistry, soil-to-solution ratio, mixing). Using animal models has validated results from the PBET model. It has been reported

(Ruby et al. 1996) that results of PBET linearly correlated with results from a Sprague-Dawley rat model ( $r^2 = 0.93$  between *in vitro* and *in vivo* results).

Transport of heavy metals in soils is a potential threat for groundwater contamination and thus for human health. When the heavy metal concentration in soil is elevated, the toxicity characteristic leaching procedure (TCLP) can be used to assess heavy metal mobility (US EPA 1995). The TCLP regulatory limit for Pb is currently set at 5 mg/l.

The aim of the study was to use a battery of tests: phytoextraction into *T. goesingense*, PBET, and TCLP, to assess the risk of Pb remaining in the soil after soil treatment with laboratory simulations of different soil remediation techniques. Two Pb immobilization techniques were tested: soil Pb solidification with Slovakite, a new commercially available mixture of different heavy metal sorbents; and soil vitrification using microwaves as an energy source for soil heating. Two soil washing techniques were also tested: extraction of Pb from the soil slurry phase in a reactor with benchmark heavy metal chelator ethylenediamine tetraacetate (EDTA); and a novel method proposed in this study: soil heap leaching using biodegradable chelator [S,S] isomere of ethylenedioamine disuccinate ([S,S]-EDDS) and a horizontal permeable reactive barrier constructed at the heap bottom, with reuse of the soil washing solution in a closed remediation cycle.

## MATERIAL AND METHODS

**Soil properties.** Soil samples were collected from the 0–30 cm surface layer at an industrial site of a former Pb smelter in the Mežica Valley in Slovenia. Sequential extractions (Leštan et al. 2003) were used to determine fractionation of heavy metals into six soil fractions. Selected soil characteristics and heavy metal contents are summarized in Table 1.

**Sorption of Pb from water solution by Slovakite and apatite.** Five hundred ml of 100 and 20mM solutions of  $PbNO_3$  in deionized water were circulated with a peristaltic pump (7 ml/min) through approx. 14 cm high permeable beds consisting of 40 g vermiculite, 40 g vermiculite and 50 g Slovakite, and 40 g vermiculite and 50 g apatite, placed in a 6 cm diameter polypropylene cylinder with perforated bottom. The ratio of the initial molar concentration of Pb in the water solution and apatite in the permeable bed was 1:1 and 1:5, respectively. Pb in

Table 1. Physical and chemical characteristics and fractionation of Pb in the soil used in this study

Soil properties	
pH (CaCl <sub>2</sub> )	7.1
Organic matter (%)	10.1
P (mg/kg)	248.5
CO <sub>3</sub> <sup>-</sup> (g/kg)	153.6
CEC (mmol C <sup>+</sup> /100 g)	21.0
Sand (%)	53.0
Silt (%)	41.5
Clay (%)	5.5
Texture	sandy loam
Total Pb (mg/kg)	1170
Fractionation	Pb (%)
In soil solution	0.13 ± 0.02
Exchangeable	0.40 ± 0.12
Bound to carbonate	35.36 ± 0.54
Bound to Fe and Mn oxides	0.47 ± 0.04
Bound to organic matter	31.48 ± 0.41
Residual fraction	10.19 ± 0.12
Recovery	78.3

solution exiting the bed was measured after one and after five passes of Pb solution through the permeable bed with sorbents. Experiments were performed in triplicate.

**Solidification of soil Pb with Slovakite.** After being air-dried, the soil was passed through a 5-mm mesh sieve. 1.5 kg of soil was mixed with 10.6 g/kg Slovakite, to immobilize soil Pb. Slovakite amended soil was watered to 80% field water capacity, stored in a bucket and weighed, covered with wet paper tissue to reduce evaporation, and incubated in the dark at 14°C for 1 month. To keep the soil moisture constant, we weighed the bucket with soil each week and compensated for evaporated moisture by watering the soil with spraying.

**Soil vitrification.** Air-dried soil was sieved through a 5-mm mesh sieve. 50 g of dry soil was placed in a ceramic jar and microwaved in a laboratory microwave oven (CEM Matthews NC, model MDS-2000, 650 W at 2455 MHz) for 5 min. The soil vitrified into a solid pellet with a glasslike core.

**Extraction of Pb from the soil slurry phase.** Bench-scale soil washing tests, imitating the technology of *ex situ* soil extraction in a reactor, were conducted on two scales: small scale with 15 g of soil, and larger scale with 1.5 kg of soil. In the

small scale experiments, 50 ml plastic tubes were filled with soil slurry consisting of 15 g of dry Pb contaminated soil and 15 ml of 10, 20, 40, 200 and 400 mmol/kg EDTA and [S,S]-EDDS solution. The soil slurry was mixed vigorously at 250 rpm and 25°C, using a laboratory shaker, for 24 hours. The soil-washing extractants were obtained after centrifugation of the soil slurry at 4000 g for 30 min and stored in the cold for further analysis. Three replicates of each treatment were made.

In the larger scale experiments, a polypropylene container was filled with 1.5 kg of dry soil and 1.5 L EDTA solution (40 mmol/kg of soil). The soil slurry was mixed vigorously at 250 rpm and 25°C, using a laboratory shaker for 24 hours. The soil-washing extractants were obtained after overnight filtration of soil through a 0.2-mm plastic mesh and additional soil washing and filtration by pouring 500 ml of tap water over the filtered soil. Filtrates were collected in a sedimentation tube, allowed to clear up over 24 hours, sampled for soil-washing extractant, and stored in the cold for further analysis.

**Heap leaching.** Soil heap leaching was simulated in 15 cm diameter soil columns at room temperature, in four replicates. Air-dried soil was sieved through a 5-mm mesh sieve. 4.5 kg of soil was placed in a column 24 cm high, on top of a 6 cm high horizontal permeable reactive barrier constructed in the column bottom. The reactive barrier was composed of a mixture of vermiculite (15 g), soya meal (75 g), sawdust (75 g), and Slovakite (50 g). Plastic mesh ( $D = 4$  mm) was placed between the soil and the reactive barrier to separate them and, additionally ( $D = 0.2$  mm) at the bottom of the column to retain the soil. The soil was treated with 10 mmol/kg [S,S]-EDDS in 2300 ml tap water. The leachate exiting the column was collected in a plastic container placed under the column and immediately transferred with a peristaltic pump (flow rate 420 ml/hr) onto the top of the column, where it was reused as soil washing solution for further heap leaching. The progress of heap leaching was followed by measuring the Pb concentration in the leachate. We stopped heap leaching after 6 days, after no Pb was detected in the leachate. Pb was determined by AAS after filtration through Whatman No. 1 filter paper. The reactive barrier, saturated with the Pb, and the remediated soil were separated and probed for Pb concentration, as described below.

**Pb phytoavailability.** Lead phyto-availability in untreated soil and soils obtained after solidification of soil Pb with Slovakite, extraction of Pb from the soil slurry phase with 40 mmol/kg EDTA

(larger scale – 1.5 kg of soil), and heap leaching with 10 mmol/kg [S,S]-EDDS was assessed by *Thlaspi goesingense* bioassays in three replicates. Plants were grown from seeds on tested soils. After germination, the shoots were transferred into 150 ml pots with 100 g of soil (dry weight, 1 shoot per pot) and grown in a greenhouse for 12 weeks. Above-ground parts of the plants were cut and immediately prepared for Pb determination.

**Pb oral bioavailability.** Lead oral bioavailability in untreated soil and soils obtained after solidification of soil Pb with Slovakite, extraction of Pb from the soil slurry phase with 40 mmol/kg EDTA (larger scale – 1.5 kg of soil), heap leaching with 10 mmol/kg [S,S]-EDDS, and soil vitrification using microwaves, was determined in triplicate using PBET (Ruby et al. 1996).

**Pb toxicity characteristic leaching.** The mobility and leachability of Pb in untreated soil and soils obtained after solidification of soil Pb with Slovakite, extraction of Pb from the soil slurry phase with 40 mmol/kg EDTA (larger scale – 1.5 kg of soil), heap leaching with 10 mmol/kg [S,S]-EDDS, and soil vitrification using microwaves, was determined using TCLP analyses (US EPA, 1995), conducted in triplicate.

**Pb determination.** For the analysis of Pb in soil, soil samples were ground in an agate mill for 10 min and then passed through a 250 µm sieve. After digestion in *aqua regia*, flame Atomic Absorption Spectroscopy (AAS, Perkin Elmer 1100) was used for the determination of Pb concentrations. Shoot tissues of *T. goesingense* were collected and thoroughly washed with deionized water. They were dried to a constant weight and ground in a titanium centrifugal mill. Pb concentrations in plant tissue samples (250–300 mg dry weight) were determined using an acid (70% HNO<sub>3</sub>) dissolution technique with microwave heating and analysed by AAS. Pb concentrations in extractants and leachates, and TCLP and PBET solutions were determined directly by AAS.

Controls of the analytical procedure were performed using blanks and reference materials (BCR 60 and BCR 141R, Community Bureau of Reference, for plant and soil) that were treated identically to the experimental samples.

## RESULTS AND DISCUSSION

### Pb soil immobilization

In this study, we used a new, commercially available, composite sorbent Slovakite as a soil amendment for Pb solidification. According to the producer's disclaimer, Slovakite is a mixture of natural raw materials: dolomite, diatomite, smectite basaltic tuff, bentonite, alginite and zeolite. Before we decided to use Slovakite, we compared the effectiveness of Slovakite with commonly used phosphate Pb sorbent apatite. In sorption tests of Pb<sup>2+</sup> from deionised water, Slovakite outperformed apatite (Table 2). In these tests, we used vermiculite as a carrier material for Slovakite and apatite, since vermiculite has a high water retention capacity. However, vermiculite also exhibited a substantial Pb sorption property (Table 2), presumably due to its high positive surface charge (Bouabid et al. 1991). The sorption of Zn<sup>2+</sup> and Cu<sup>2+</sup> from the water solution by Slovakite in vermiculite was also higher than by apatite in vermiculite (data not shown).

Soil vitrification currently involves using electricity for soil heating, due to low soil electric conductivity. Sedhom et al. (1992) proposed microwave soil treatment for fixation of Cr within the soil matrix, through the interaction of heavy metal with “lossy” native minerals, but they did not suggest soil vitrification. However, materials in soil with dielectric properties adsorb microwave energy and soil becomes extremely hot. Dielectric soil heating can be increased further by inserting rods of graphite or iron (materials that

Table 2. Percentage of Pb<sup>2+</sup> sorbed from 20 and 100mM water solutions by vermiculite, vermiculite and Slovakite, and vermiculite and apatite, after single and after five passes of the Pb solution through beds with sorbents; means and standard deviations (*n* = 3) are presented

Sorbent	No. of passes			
	20mM Pb <sup>2+</sup>		100mM Pb <sup>2+</sup>	
	1	5	1	5
Vermiculite	51.94 ± 3.63	51.22 ± 1.05	10.64 ± 3.14	13.01 ± 4.24
Vermiculite + Slovakite	99.98 ± 0.03	99.99 ± 0.00	82.61 ± 3.28	96.24 ± 0.33
Vermiculite + apatite	70.39 ± 5.04	99.25 ± 0.26	40.14 ± 4.23	48.64 ± 8.13

are very strong absorbers of microwave energy). Abramovitch et al. (2003) proposed microwave energy as an alternative to soil vitrification using electricity. In their study, they microwaved soil extracted from organic matter, using graphite pencil rods as dielectric material. In our experiments, we used non-treated (except sieving) soil. The soil was vitrified after just a couple minutes in a microwave oven. The soil material left after vitrification is shown in Figure 1.

### Pb soil washing

One of the most studied and efficient remediation methods for Pb contaminated soils is soil washing as batch extraction of soil slurry in a reactor, with EDTA as a chelator. We simulated soil Pb extraction on two scales. In a small scale soil extraction experiment (using only 15 g of soil), we compared Pb removal using a benchmark chelator EDTA and a rather new chelator, [S,S]-EDDS, at various concentrations of chelators (Figure 2). EDTA was found to be more efficient, with an extraction efficiency significantly increasing up to a chelator concentration of 40 mmol/kg of soil, where  $35.9 \pm 0.2\%$  of initial total Pb was removed from the soil (Figure 2). This supremacy of EDTA over [S,S]-EDDS was expected, since the Pb-EDTA complex has a higher stability constant than Pb-[S,S]-EDDS complex ( $K_s$  18.0 and 12.7 at 25°C, and ionic strength 0.1, respectively) (Martel et al. 2003). 40 mmol/kg EDTA was used in a larger scale simulation of soil extraction (1.5 kg of soil). After

filtration of extractant from the soil and additional washing of the soil with clean tap water 57.6% of Pb was removed, more than in the small scale (15 g of soil) extraction experiment ( $35.9 \pm 0.2\%$ ). The difference in Pb removal can be attributed to additional soil washing with clean water subsequent to chelator treatment in the larger scale (1.5 kg of soil) extraction. We did not perform extra tests, but it is likely that further washing with clean water would remove even more Pb (probably complexed with EDTA) from the soil, at the expense of producing more wastewater. In practice, this could represent a serious drawback, since there are currently no simple, cost-effective means of recycling EDTA or removing Pb from waste washing solution (Kim and Ong 1999).

In contrast to soil extraction in the reactor, soil heap leaching could be considered to be a soil-friendly soil washing method, with a low impact on the soil structure. The heap leaching process is operationally simple. Heavy metal contaminated soil is excavated, screened and mounded on a pad. Heavy metals are removed by passing washing solution (usually containing chelator) through the soil using some type of liquid distribution system (sprinkler or drip irrigation system). The extractant is collected in a pregnant solution pit and processed to remove heavy metals (Hanson et al. 1992).

In this study, we introduced a new heap leaching method, which effectively addresses the problem of wastewater generation. We used [S,S]-EDDS as a chelator, in a concentration of 10 mmol/kg. [S,S]-EDDS is non-toxic, naturally present in the soil, commercially produced (Jaworska et al. 1999) and, most important, [S,S]-EDDS forms an easily biodegradable complex with Pb (VanDevivere et

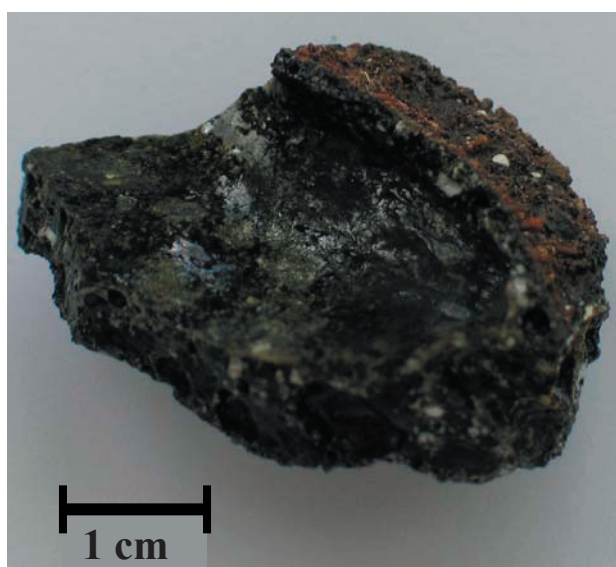


Figure 1. Glasslike vitrified soil material resulting from treatment of contaminated soil in a microwave oven

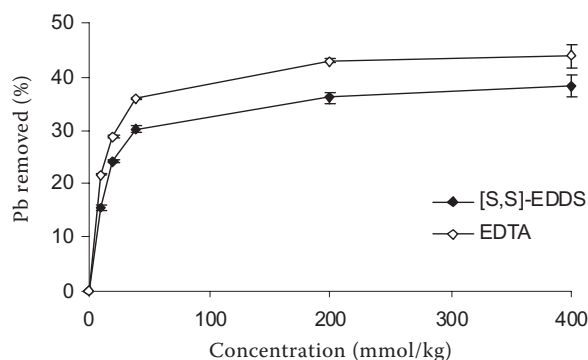


Figure 2. The percentage of total initial Pb extracted from the soil slurry with different concentrations of EDTA and [S,S]-EDDS; error bars represent standard deviation of the mean value ( $n = 3$ )

al. 2001). We recently demonstrated that, after soil washing with [S,S]-EDDS solution, Pb can be removed from the washing solution containing Pb-[S,S]-EDDS complexes by passing the washing solution through a permeable reactive barrier (Kos and Leštan 2003). The materials in the reactive barrier were substrates enabling enhanced microbial activity, which promoted efficient Pb-[S,S]-EDDS biodegradation, and adsorbents to solidify and retain released Pb. In the new heap leaching method, we constructed the horizontal permeable reactive barrier at the heap (column) bottom. The washing solution was treated, collected, and reused in a closed remediation loop (Figure 3). Figure 4 shows the concentration of Pb in the washing solution that ran off the barrier. After 6 days, no Pb was detected in the washing solution and we stopped the experiment. No Pb containing wastewater was generated.

The total amount of water used for heap leaching was approx. 135% of the field water retention capacity of the soil used. 17.8% of the initial Pb was removed from the soil and all leached Pb was accumulated in the barrier, which we easily separated from the soil. The distribution of Pb through the soil profile is shown in Figure 5. Soil extraction (small scale, 15 g of soil) with the same (10 mmol/kg) concentration of [S,S]-EDDS removed less,  $15.5 \pm 0.5\%$  Pb (Figure 2), presumably since, after separation of the extractant from the soil by centrifugation, the soil was not subsequently washed with clean water to remove retained Pb-[S,S]-EDDS complexes. On the other hand, the heap-leaching procedure was also not optimized. Soil extraction with 10 mmol/kg EDTA (small scale) removed  $21.7 \pm 0.2\%$  Pb (Figure 2). As discussed above, this higher efficiency could be attributed to the high chelating affinity of EDTA towards Pb.

### Pb phytoavailability

To assess phytoavailable Pb in the soil, we used the reported Pb hyperaccumulator *T. goesingense*. The concentration of Pb in the leaves of *T. goesingense* grown in soils before remediation was lower than in soils after Pb solidification with Slovakite, soil extraction with 40 mmol/kg EDTA (larger scale treatment, 1.5 kg of soil), and heap leaching with 10 mmol/kg [S,S]-EDDS (Table 3). Since soil extraction and heap leaching removed part of the Pb from the soil (57.6 and 17.8%, respectively), a higher Pb plant uptake after these treatments was probably due to the residual labile Pb-chelator complexes remaining in the soil. The literature on soil washing of Pb contaminated soil with chelators is extensive. Most of the studies, however, are concentrated on Pb soil removal and none of them report on the phytoavailability of Pb remaining in the soil.

Surprisingly, Slovakite soil amendment resulted in a more than 2-times higher Pb plant concentration compared to plants grown on untreated soil (Table 3). Pb phytoavailability (assessed by different plants) after Pb soil immobilization with various soil additives is well studied, although there are no reports on the use of Slovakite. In most studies, phosphate compounds (i.e. apatite, phosphate rock, triple-superphosphate) were used. The results are indecisive. For example: Zhu et al. (2004) reported that Pb concentrations in shoots and roots of two *Brassica* plants generally, but not always, decreased after soil treatment with different phosphate compounds. Geebelen et al. (2003) used lime, cyclonic ash and phosphate rock for Pb solidification and *Zea mays* as an indicator plant. They, too, reported that different soil amendments did not behave consistently across differ-

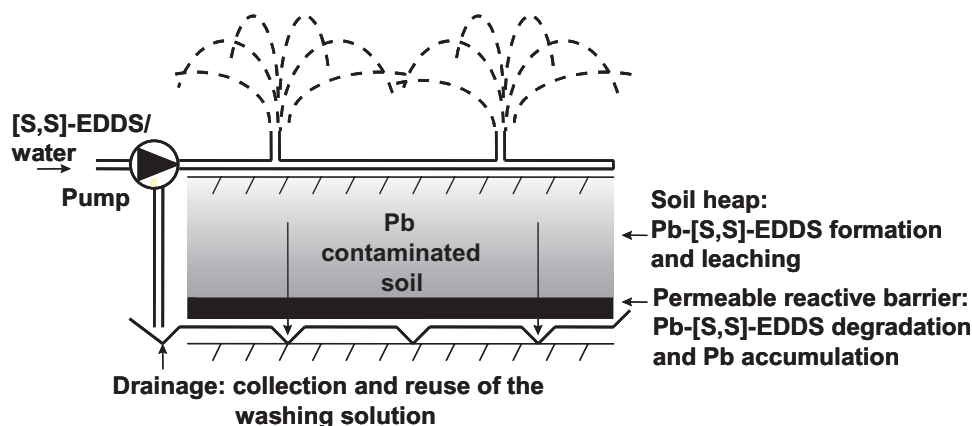


Figure 3. Proposed set up of heap washing of heavy metals contaminated soil with biodegradable chelator and a horizontal permeable reactive barrier

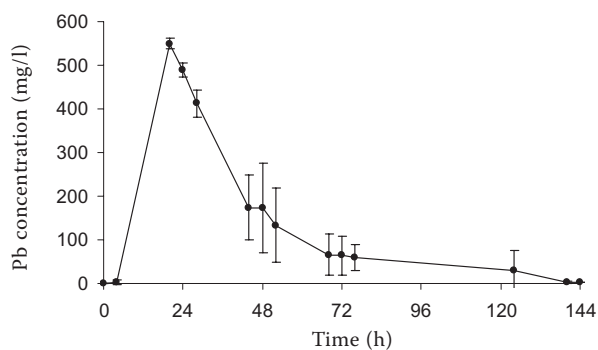


Figure 4. Concentration of Pb in soil washing solution during heap leaching with [S,S]-EDDS and a horizontal permeable reactive barrier; error bars represent standard deviation of the mean value ( $n = 4$ )

ent Pb contaminated soils. Laperche et al. (1997) reported that the addition of apatite significantly decreased the Pb content in shoot tissue of *Sorghum bicolor*, but increased the Pb content in the plant roots. Cao et al. (2003) reported similar results using *Stenotaphrum secundatum* as an indicator plant. Theodoratos et al. (2002) and Zwonitzer et al. (2003) observed no effect of phosphate soil addition on Pb accumulation by *Phaseolus vulgaris* and *Sorghum bicolor*.

The biomass of *T. goesingense* grown on Slovakite and chelator treated soil was lower compared to plants grown on untreated soil (Table 3). There were no visible symptoms of toxicity on plant leaves. However, it is possible that additives interacted with trace elements and nutrients in the soil; washing with chelators could remove essential elements from the soil, and adding Slovakite could make them less bioavailable. Theodoratos et al. (2002) also reported that soil treatment with Ca-phosphate had a negative effect on *Phaseolus vulgaris* growth, which was combined with a deficiency of Ca in the leaves. In contrast to these observations, Gebelen et al. (2003) reported that

soil amendments did not significantly decrease plant biomass production on any of the tested soils, and significantly increased plant biomass produced on the most polluted soil.

Puschenreiter et al. (2001) reported that *T. goesingense* accumulated 2840 mg Pb/kg in shoots, without any soil treatment. There are no other reliable reports on hyper-accumulator plant species for Pb under natural conditions. In our soil, the plant concentration of Pb did not exceed 200 mg/kg in any treatment. This might indicate that the hyper-accumulating capacity of *T. goesingense* is limited only to certain soil types. A possible explanation for low Pb plant uptake and inefficient Pb soil solidification with Slovakite are the small shares of water-soluble fraction of Pb and fraction of soil Pb exchangeable from soil colloids (Table 1). These fractions of soil Pb are probably available for both plant uptake and solidification.

### Pb oral availability

Oral bioavailability of Pb was determined *in vitro* using Ruby's tests (PBET) for predicting the bioavailability of metals from a solid matrix. The PBET model is designed to simulate the human gastrointestinal tract, which includes stomach and intestinal phases. Soil extraction with 40 mmol/kg EDTA (larger scale exp., 1.5 kg of soil) increased the concentration of Pb available in both the stomach and intestinal phases, while heap leaching with 10 mmol/kg [S,S]-EDDS increased only the concentration of Pb in the intestinal phase (Figure 6). After soil treatment with EDTA and [S,S]-EDDS, the percentage of residual soil Pb orally available in the stomach phase increased from an initial  $17.8 \pm 1.2\%$  in the original soil, to  $55.8 \pm 6.3\%$  and  $19.2 \pm 0.5\%$ , respectively. The increase in the intestinal phase was from  $4.9 \pm 1.4\%$  in the original soil, to  $50.1 \pm 5.0\%$  and  $7.0 \pm 0.5\%$  in EDTA and [S,S]-

Table 3. Concentration of Pb accumulated in above ground tissues and biomass dry weight of *Thlaspi goesingense* grown on Pb contaminated soil and soils after different remediation treatments; means and standard deviations ( $n = 3$ ) are presented

	Pb in <i>T. goesingense</i> (mg/kg)	<i>T. goesingense</i> biomass (g)
Original soil	$30.2 \pm 6.2$	$0.137 \pm 0.015$
Solidification with Slovakite*	69.8	0.060
Soil extraction with EDTA*	187.9	0.045
Heap leaching with [S,S]-EDDS*	103.8	0.106

\*combined sample of three replicates

EDDS treated soil, respectively. The higher Pb oral-availability after soil treatments with chelators was probably due to the Pb-chelator complexes retained in the soil, as discussed above for the increase of phytoavailable Pb. In heap leaching with [S,S]-EDDS, the soil was treated until no Pb was measured in the washing solution (Figure 4). As a result, only a moderate increase of Pb available in the intestinal phase was observed (Figure 6). A literature survey revealed no other studies on oral bioavailability of Pb (or of other metals) after soil washing with chelators (or with other extractants), to compare results with our data.

Slovakite amendment did not significantly effect Pb oral availability, compared to the original soil. In most other studies, Pb solidification resulted in a reduction of Pb oral availability. For example; Hettiarachchi et al. (2003) reported that triple superphosphate and phosphate rock soil amendments, alone or in combination with Mn oxide, significantly reduced Pb oral availability determined *in vivo* by the extent of Pb absorption into young rats and *in vitro* using the PBET test. However, Geebelen et al. (2003) reported that of different soil amendments (lime, cyclonic ash and phosphate rock) they used for Pb solidification,

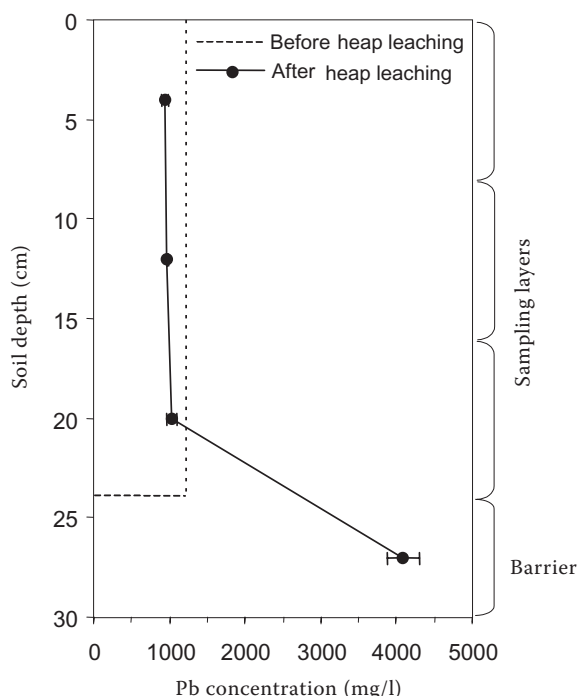


Figure 5. Pb concentration through the profile in soil columns with a horizontal permeable barrier before (dotted line) and after heap leaching with 10 mmol/kg [S,S]-EDDS (solid line); error bars represent standard deviation of the mean value ( $n = 4$ )

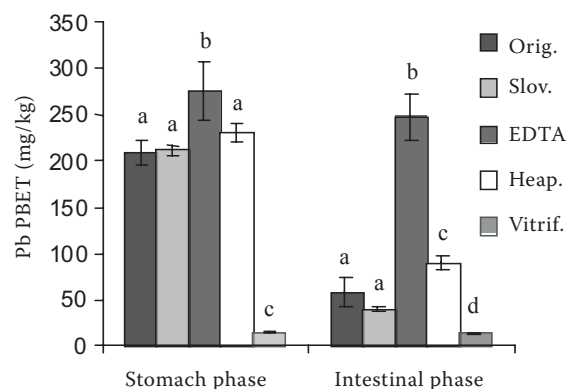


Figure 6. Oral bioavailability of Pb in stomach and intestinal phases, determined *in vitro* using Ruby's physiologically based extraction test, before and after soil treatment with different remediation technologies; error bars represent standard deviations ( $n = 3$ ); a, b, c denote statistically different treatments according to the Duncan test ( $P = 0.05$ )

only phosphate rock significantly reduced oral bioavailability, determined with the PBET test, but only in three of ten tested soils.

The only effective soil treatment was vitrification. It significantly reduced concentrations of soil Pb available in the stomach and intestinal phase from an initial  $208.8 \pm 13.8$  and  $57.5 \pm 16.4$  mg/kg, to  $14.4 \pm 0.6$  and  $13.3 \pm 1.2$  mg/kg, respectively (Figure 6).

### Pb mobility

In EU countries the warning and critical limits for Pb in soil are set at 50 and 300 mg/kg (Council Directive 86/278/EEC 1986). Pb concentration in the soil used in this study was 4-times higher than the critical limit. However, Pb mobility assessed by TCLP indicated only  $1.05 \pm 0.45$  mg Pb/l in the extract of the original soil. This is lower than the TCLP regulatory limit, set at 5 mg Pb/l by US EPA for materials considered as hazardous waste. It is probably due to this originally low Pb mobility, that Slovakite soil amendment, heap leaching with 10 mmol/kg [S,S]-EDDS, and even soil vitrification, did not statistically significantly reduce Pb in TCLP extract (Figure 7). Soil extraction with 40 mmol/kg EDTA (larger scale exp., 1.5 kg of soil) increased TCLP extracted Pb to  $14.39 \pm 2.30$  mg/l, well above the TCLP regulatory limit. The probable reason was, as already discussed above, Pb-EDTA complexes retained in the soil after treatment. Literature data indicate that treatment of Pb contaminated soil with different remedia-



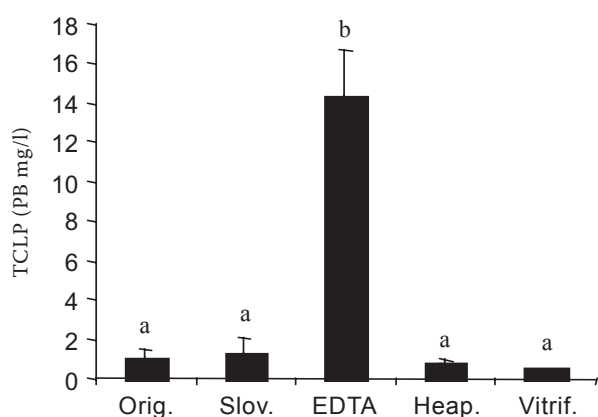


Figure 7. Pb mobility determined using TCLP and measured as the Pb concentration in the soil extract, before and after soil treatment with different remediation technologies; error bars represent standard deviations ( $n = 3$ ); a, b denote statistically different treatments according to the Duncan test ( $P = 0.05$ )

tion techniques in general successfully reduced Pb mobility, determined using TCLP. Theodoratos et al. (2002) reported that phosphate soil amendment in molar ratios  $\text{PO}_4^{3-}/\text{Pb}$  higher than 0.5 effectively reduced Pb in TCLP extract, while the same treatments failed to reduce Pb phytoavailability. Van Benschoten et al. (1997) reported that different Pb contaminated soils successfully passed TCLP limits after extraction with EDTA. However, they treated only small soil quantities and used a very high chelator solution:soil ratio (20:1), compared to the ratio 1:1 used in our study.

### Efficiency of tested remediation techniques

The results of our study indicate that, under the given conditions of all remediation methods tested in our study, only soil vitrification successfully reduced oral availability of Pb in the soil. Although Slovakite outperformed commonly used apatite as an absorbent of Pb from water solution, Slovakite soil amendment did not reduce Pb oral availability in the soil, and even increased Pb phytoavailability. Soil washing with chelators removed part of the Pb from the soil, but the share of phytoavailable Pb and orally available Pb increased in the treated soil. How much it is possible to reduce the concentration of bioavailable soil Pb by additional soil washing with clean water subsequent to chelator treatment remains to be studied. After soil treatment with a new heap leaching method using biodegradable chelator [S,S]-EDDS and a horizontal permeable reactive barrier, the resulting soil washing solu-

tion was Pb free. However, the phytoavailability of residual Pb in the soil treated in heap leaching was more than 3-times higher than in the original soil. Both the share and concentration of Pb orally available in the intestinal phase were also higher than in the original soil. This indicates that soil washing with chelators, effective in terms of heavy metal removal, might not always lead to bioavailable heavy metal stripping. The new heap leaching method offers advantages of soil washing in a closed remediation loop with no wastewater generation, lower requirements for process water than in current soil washing methods, and enables easy control over emissions. However, further studies with different types of heavy metal contaminated soils are needed to assess its feasibility as a remediation method.

### Acknowledgements

The authors are grateful to the Octel company for the gift of [S,S]-EDDS.

### REFERENCES

- Abramovitch R., ChangQing L., Hicks E., Sinard J. (2003): *In situ* remediation of soils contaminated with toxic metal ions using microwave energy. *Chemosphere*, 53: 1077–1085.
- Aydinalp C., Katkat A.V. (2004): The comparison of extraction methods for evaluating some heavy metals in polluted soils. *Plant Soil Environ.*, 50: 212–217.
- Bouabid R., Badraoui M., Bloom P.R. (1991): Potassium fixation and charge characteristics of soil clays. *Soil Sci. Soc. Am. J.*, 55: 1493–1498.
- Buelt J.R., Farnsworth R.K. (1991): *In situ* vitrification of soils containing various metals. *Nucl. Technol.*, 96: 178–184.
- Cao R.X., Ma L.Q., Chen M., Singh S.P., Harris W.G. (2003): Phosphate-induced metal immobilization in a contaminated site. *Environ. Pollut.*, 122: 19–28.
- Council Directive 86/278/EEC (1986): On the Protection of the Environment, and Particular of the Soil, When Sewage Sludge is used in Agriculture. EC Official J. L181.
- Geebelen W., Adriano D.C., van der Lelie D., Mench M., Carleer R., Clijsters H., Vangronsveld J. (2003): Selected bioavailability assays to test the efficacy of amendment-induced immobilization of lead in soils. *Plant Soil*, 249: 217–228.
- Hanson A.T., Samani Z., Dwyer B., Jacquez R. (1992): Heap leaching as a solvent-extraction technique for

- remediation of metals-contaminated soils. In: Sabatini D.A., Knox R.C. (eds.): Transport and remediation of subsurface contaminants, ACS Symp. Ser. No. 491. Am. Chem. Soc., Washington.
- Hettiarachchi G.M., Pierzynski G.M., Oehme F.W., Sonmez O., Ryan J.A. (2003): Treatment of contaminated soil with phosphorus and manganese oxide reduces lead absorption by Sprague-Dawley rats. *J. Environ. Qual.*, *32*: 1335–1345.
- Jaworska J.S., Schowanek D., Feijtel T.C.J. (1999): Environmental risk assessment for trisodium [S,S]-ethylene diamine disuccinate, a biodegradable chelator used in detergent application. *Chemosphere*, *38*: 3597–3625.
- Kim C., Ong S.-K. (1999): Recycling of lead-contaminated EDTA wastewater. *J. Hazard. Mater.*, *B69*: 273–286.
- Kos B., Leštan D. (2003): Induced phytoextraction/soil washing of lead using biodegradable chelator and permeable barriers. *Environ. Sci. Technol.*, *37*: 624–629.
- Laperche V., Logan T.J., Gaddam P., Traina S.J. (1997): Effect of apatite amendments on plant uptake of lead from contaminated soil. *Environ. Sci. Technol.*, *31*: 2745–2753.
- Leštan D., Grčman H., Zupan M., Bačac N. (2003): Relationship of soil properties to fractionation of Pb and Zn in soil and their uptake into *Plantago lanceolata*. *Soil Sedim. Contam.*, *12*: 507–522.
- Martell A.E., Smith R.M. (2003): NIST critically selected stability constants of metal complexes; Version 7.0. NIST, Gaithersburg.
- McGrath S., Zhao J.F. (2003): Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotech.*, *14*: 277–282.
- Peters R.W. (1999): Chelant extraction of heavy metals from contaminated soils. *J. Hazard. Mater.*, *66*: 151–210.
- Peters R.W., Shem L. (1992): Use of chelating-agents for remediation of heavy-metal contaminated soil. In: Vandegrift G.F., Reed D.T., Tasker I.R. (eds.): Environmental remediation removing organic and metal ion pollutants, ACS Symp. Ser. No. 509. Am. Chem. Soc., Washington.
- Puschenreiter M., Stoger G., Lombi E., Horak O., Wenzel W.W. (2001): Phytoextraction of heavy metal contaminated soils with *Thlaspi goesingense* and *Amaranthus hybridus*: Rhizosphere manipulation using EDTA and ammonium sulfate. *J. Plant Nutr. Soil Sci.*, *164*: 615–621.
- Reed B.E., Carriere P.C., Thompson J.C., Hatfield J.H. (1996): Electrokinetic (EK) remediation of a contaminated soil at several Pb concentrations and applied voltages. *J. Soil Contam.*, *5*: 95–120.
- Ruby M.V., Davis A., Link T.E., Schoof R., Chaney R.L., Freeman G.B., Bergstrom P. (1993): Development of an *in vitro* screening test to evaluate the *in vivo* bioaccessibility of ingested mine-waste lead. *Environ. Sci. Technol.*, *27*: 2870–2877.
- Ruby M.V., Davis A., Link T.E., Schoof R., Eberle S., Sellstone C.M. (1996): Estimation of lead and arsenic bioavailability using a physiologically based extraction test. *Environ. Sci. Technol.*, *30*: 422–430.
- Sedhom E., Daurman L., Ibrahim N., Windgasse G. (1992): Microwave treatment of hazardous wastes – fixation of chromium in soil. *J. Microwave Power E.E.*, *27*: 81–86.
- Theodoratos P., Papassiopi N., Xenidis A. (2002): Evaluation of monobasic calcium phosphate for the immobilisation of heavy metals in contaminated soils from Lavrion. *J. Hazard. Mater.*, *94*: 135–146.
- US EPA (1995): Test Methods for Evaluation of Solid Waste vol. IA, Laboratory Manual Physical/Chemical Methods, SW 846, 40 CFR Parts 403 and 503. 3<sup>rd</sup> ed. US Government Printing Office, Washington DC.
- Van Benschoten J.E., Matsamoto M.R., Young W.H. (1997): Evaluation and analysis of soil washing for seven lead-contaminated soils. *J. Environ. Eng.*, *123*: 217–224.
- VanDevivere P.C., Saveyn H., Verstraete W., Feijtel T.C.J., Schowanek D.R. (2001): Biodegradation of metal-[S,S]-EDDS complexes. *Environ. Sci. Technol.*, *35*: 1765–1770.
- Vysloužilová M., Tlustoš P., Száková J., Pavlíková D. (2003): As, Cd, Pb and Zn uptake by *Salix* spp. clones grown in soils enriched by high loads of these elements. *Plant Soil Environ.*, *49*: 191–196.
- Zhu Y.G., Chen S.B., Yang J.C. (2004): Effect of soil amendments on lead uptake by two vegetable crops from a lead-contaminated soil from Anhui, China. *Environ. Int.*, *30*: 351–356.
- Zwonitzer J.C., Pierzynski G.M., Hettiarachchi G.M. (2003): Effects of phosphorus addition on lead, cadmium, and zinc bioavailabilities in a metal-contaminated soil. *Water Air Soil Pollut.*, *143*: 193–209.

Received on May 16, 2005

---

*Corresponding author:*

Assoc. Prof. Domen Leštan, University of Ljubljana, Biotechnical Faculty, Agronomy Department, Jamnikarjeva 101, 1000 Ljubljana, Slovenia  
phone: + 386 1 423 1161, fax: +386 1 423 1088, e-mail: domen.lestan@bf.uni-lj.si

---