

Relationship of soil properties to fractionation, bioavailability and mobility of lead and zinc in soil

N. Finžgar¹, P. Tlustoš², D. Leštan¹

¹*Biotechnical Faculty, University of Ljubljana, Slovenia*

²*Faculty of Agrobiological Sciences, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic*

ABSTRACT

Sequential extractions, metal uptake by *Taraxacum officinale*, Ruby's physiologically based extraction test (PBET) and toxicity characteristic leaching procedure (TCLP), were used to assess the risk of Pb and Zn in contaminated soils, and to determine relationships among soil characteristics, heavy metals soil fractionation, bioavailability and leachability. Regression analysis using linear and 2nd order polynomial models indicated relationships between Pb and Zn contamination and soil properties, although of small significance ($P < 0.05$). Statistically highly significant correlations ($P < 0.001$) were obtained using multiple regression analysis. A correlation between soil cation exchange capacity (CEC) and soil organic matter and clay content was expected. The proportion of Pb in the PBET intestinal phase correlated with total soil Pb and Pb bound to soil oxides and the organic matter fraction. The leachable Pb, extracted with TCLP, correlated with the Pb bound to carbonates and soil organic matter content ($R^2 = 69\%$). No highly significant correlations ($P < 0.001$) for Zn with soil properties or Zn fractionation were obtained using multiple regression.

Keywords: soil contamination; soil characteristics; potentially toxic metals; risk assessment

An unfortunate by-product of industrialization, urbanization, and modern agronomic practices has been the contamination of soil with potentially toxic metals (PTMs). Warnings and critical limits and clean-up action levels are often based on total PTM content. However, total soil PTM concentrations are poor predictors of toxicity to terrestrial organisms because they do not take into account soil characteristics that influence PTM bioavailability.

The routes of exposure to soil PTMs include inhalation of contaminated soil particles, direct soil ingestion (usually by children), ingestion of food produced on contaminated soil, or drinking water polluted with PTMs. Quantifying the exposure of these pathways requires information on the bioaccessibility and bioavailability of PTMs. Maiz et al. (2000) reported that PTMs lability and bio-

availability in polluted soils was highly dependent on the fractionation of PTMs in soils. To assess PTMs lability, sequential extraction analysis remains a widely used procedure despite some difficulties, such as sensitivity to procedural variables, limited selectivity of extractants, re-adsorption of PTMs at different phases during extraction, and overload of the chemical system if the content of PTMs is too high (Ramos et al. 1994, Chlopecka et al. 1996). This technique consists of submitting the soil to the successive action of a series of reagents with different chemical properties. Being capable of creating weak acidic conditions, or changing the redox potential of the solution or oxidizing the complexing agents, these reagents extract fractions of PTMs linked to specific forms. Several sequential extraction schemes have been designed for the determination of the forms of metals in soil.

Supported by the Slovenian Research Agency, Project No. 0481 P4-0085, and by the Czech Science Foundation and the Slovenian Research Agency, Project No. BI-CZ/06-07-007.

The accumulation of PTMs in indicator plants can be used to assess the extent of metal contamination and the potential exposure risk to indigenous biota. Common dandelion (*Taraxacum officinale*) has often been used as a PTM indicator plant species because of its abundance and wide distribution at different latitudes and altitudes, and ability to tolerate a wide range of environmental conditions (Keane et al. 2001). A number of studies investigating the suitability of dandelions for monitoring metal pollution sites (Cook et al. 1994, Marr et al. 1999) yielded confounding results regarding the extent to which PTM concentrations in dandelion tissue correspond to those in the environment. As stated by Keane et al. (2001) the factors affecting metals absorption from the soil by dandelions are complex and in addition to soil metal concentration, other soil, plant and environmental factors affect plant uptake.

The oral availability of metals can be assessed using animal models *in vivo* (immature swines and rats) as human surrogates. These models provide valuable contaminant bioavailability data but have the disadvantage of being time-consuming, expensive, requiring weeks or months to complete, and requiring highly specialized personnel (Basta and Gradwohl 2000). In order to evaluate Pb (and As) oral bioavailability (bioaccessibility) when animal study results are not available Ruby et al. (1993) developed a physiologically based extraction test (PBET). This is an *in vitro* test and incorporates gastrointestinal tract parameters representative of a human (stomach and small intestinal pH and chemistry, soil: solution ratio, mixing). The PBET is humane and less expensive, and provides a quicker assessment of Pb oral bioavailability than *in vivo* animal tests. Using animal models has validated results from the PBET model.

Leaching and transport of PTMs in (sandy) soils is a potential threat for groundwater contamination and thus for human health. When the PTM concentration in soil is elevated, the toxicity characteristic leaching procedure (TCLP) can be used to access PTM leachability (US EPA 1995).

Fractionation of PTMs in soil, and hence their bioavailability and entrance to the food chain, and soil mobility are likely controlled by many types of reactions: (a) adsorption/desorption reactions due to chemical bond formation, complex formation, and ion-exchange are one class of reactions; (b) another reaction class is precipitation of PTMs, generally with anions such as phosphate, carbonate, sulphate and hydroxides; (c) penetration into the crystal structure of minerals and isomorphous

exchange with cations in the crystal lattice; (d) biological mobilization and immobilization of the PTMs in the soil (Levy et al. 1992). These reactions are presumably determined and constrained by soil properties: soil texture, content of organic matter, content and type of clay minerals and Al, Fe and Mn oxides; and prevailing physicochemical conditions in the soil: soil saturation, soil aeration, pH, and redox potential.

The aim of the study was to determine possible correlations among soil characteristics, Pb and Zn soil fractionation, and data obtained with a battery of tests comprising sequential extractions, Pb and Zn uptake by *T. officinale*, PBET and TCLP. Better understanding of these interactions could improve methods of human health and environmental risk assessment. Historical factors, such as intensive development of metallurgy and chemical industries, have resulted in pollution of some regions in Slovenia, most notably the Mežica Valley and Celje region. Soil samples from these two regions were used in our study.

MATERIAL AND METHODS

Soil samples (0–5 cm) were collected from 12 locations in the Mežica Valley and 6 locations in the Celje region, Slovenia. Sampling locations were gardens, farmlands and permanent grasslands. The Mežica Valley has been exposed to more than three hundred years of active lead mining and smelting. Lead-sulfide (galena) was the predominant ore. Soils in the valley, including 6600 ha of agricultural land, are polluted especially with Pb, but also with Zn. In 1990, lead ore mining and smelting stopped and recycling old car batteries started. At present there are two main sources of pollution: the lead smelter in Žerjav and the landfill at Glančnik, where scoria and plastic parts of batteries are deposited. Sampling locations in the Mežica Valley are shown in Figure 1.

The Celje region lies in the middle of Slovenia. It is a topographic basin surrounded by Alpine foothills in which various industries are located: zinc smelter, brickwork and steel mills. Temperature inversions within the basin are common and they spread emissions from industrial sources over the basin. Sampling locations in the Celje region are shown in Figure 2.

Soil pH was measured in a 1/2.5 (v/v) ratio of soil and 0.01M CaCl₂ solution suspension. Soil samples were analysed for organic matter by Walkley-Black titrations, cation exchange capacity by ammonium

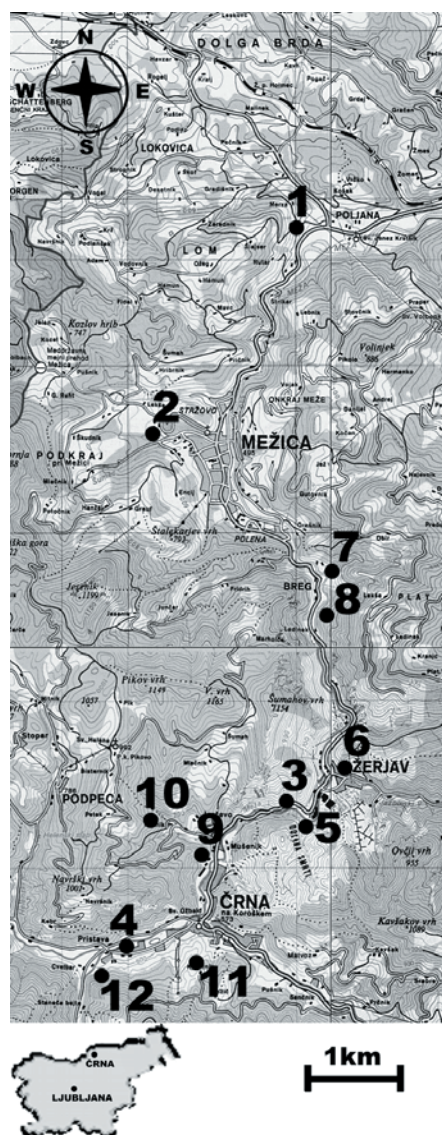


Figure 1. Map of the Mežica Valley showing sampling locations 1–12 and the position of the Mežica region in Slovenia

acetate and the Mehlich method, soil texture by the pipette method, easily extractable P was determined colorimetrically according to the Egner-Doming method, and carbonates manometrically after soil reaction with HCl (Kalra and Maynard 1992).

A modified analytical procedure according to Tessier et al. (1979) was used to determine fractionation of Pb and Zn into six fractions. The fraction soluble in soil solution was obtained by extraction of 1 g of air-dry soil, sieved to 150 μm , with 10 ml of deionised water for 1 hour. The fraction exchangeable from soil colloids to the soil solution was extracted from the residual soil sample with 10 ml of 1M MgNO_3 for 2 hours. MgNO_3 displaces ions electrostatically bound in

the soil matrix. The fraction bound to carbonates was extracted with 10 ml of 1M NH_4OAc (pH 5) for 5 hours. NH_4OAc solubilises carbonates (calcite, dolomite) and releases entrapped metals. The fraction bound to Fe and Mn oxides was extracted with 20 ml of 0.1M $\text{NH}_2\text{OH} \times \text{HCl}$ (pH 2) for 12 hours. $\text{NH}_2\text{OH} \times \text{HCl}$ reduces Fe and Mn oxides to soluble forms. The fraction bound to organic matter was obtained after heating the soil suspension in 3 ml of 0.02M HNO_3 and 5 ml of 30% H_2O_2 for 3 hours at 85°C, followed by extraction with 15 ml of 1M NH_4OAc for 3 hours. HNO_3 and H_2O_2 oxidise organic matter and solubilise sulphides. Oxidised organic matter releases complexed, adsorbed and chelated metals. The last fraction for completion of the mass balance was obtained after digestion of the soil sample with *aqua regia*. *Aqua regia* solubilises silicates and other materials. Determinations were all done in triplicate. Standard deviations of determinations of Pb and Zn recovered after each sequence of sequential extraction were lower than 10%. The final fractional recovery of Pb and Zn was calculated after summing the recoveries of all six steps of sequential extractions, and the error of final recovery therefore increased accordingly. This also presumably explains the range of recoveries, varying from 78.0 to 106.7% for Pb and from 89.0 to 122.9% for Zn.

Lead and Zn phytoavailability was assessed by dandelion (*Taraxacum officinale*) in four replicates. Plants were grown from seeds on tested soils. After germination, the shoots were transferred into 150 ml pots (1 shoot per pot) and grown in a greenhouse for 12 weeks, to avoid the effect of environmental factors on Pb and Zn plant uptake. Above-ground parts of the plants were cut, washed, dried, ground and prepared for Pb and Zn determination as a combined sample of all four replicates.

Lead oral bioavailability (bioaccessibility) was determined using PBET (Ruby et al. 1996). The stomach phase of PBET was simulated by adding 1.25 g pepsin, 0.50 g citrate, 0.50 g malate, 420 μl lactic acid and 500 μl acetic acid to 1 l water and pH adjusted to 2.50 ± 0.05 using 12N HCl. A 0.4 g soil sample (sieved through a 250 μm sieve) was mixed in a 250 ml polypropylene vessel with 40 ml of the simulated stomach solution. The contents of the vessel were agitated by bubbling water-saturated argon gas at a flow rate of 20 l/h. The vessel was suspended in a constant temperature bath at 37°C. Samples (2 ml each) were collected after 60 min, centrifuged and decanted. After

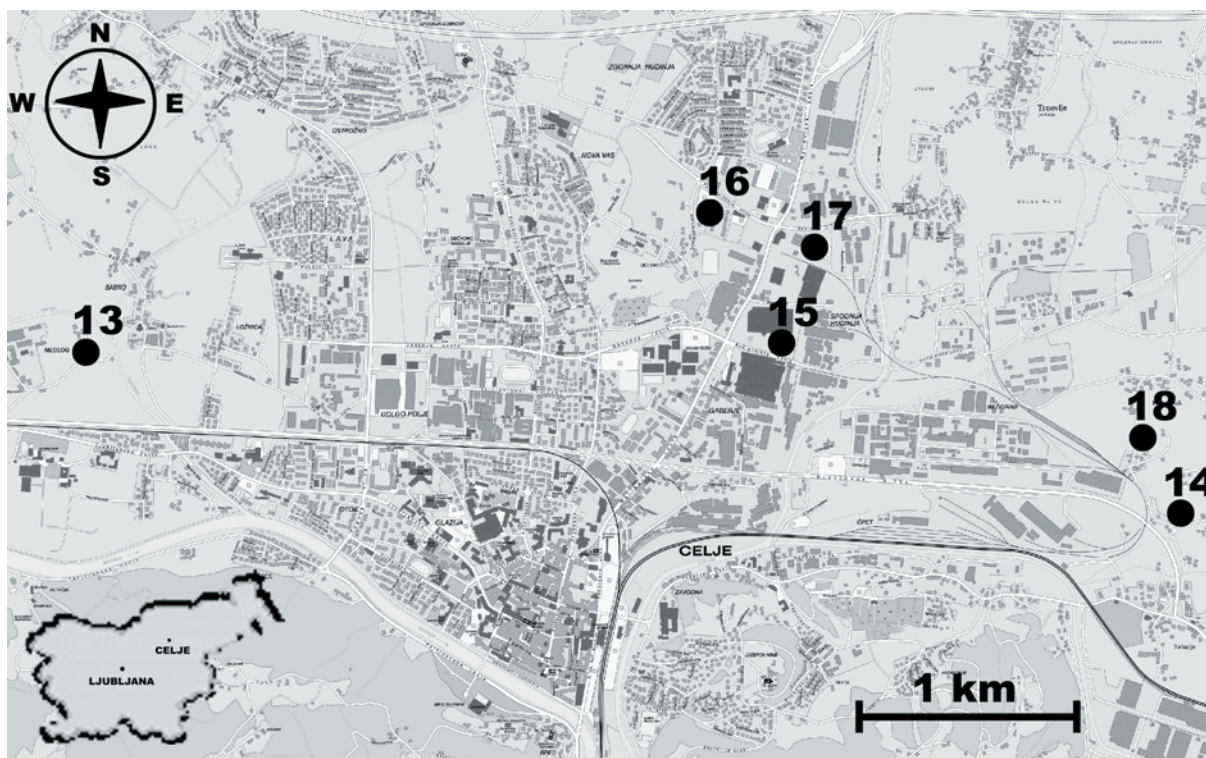


Figure 2. Map of the Celje region showing sampling locations 13–18 and the position of the Celje region in Slovenia

1 h, the flask contents were titrated to pH 7 using a dialysis bag (8000 MWCO, Spectra/Por cellulose ester tubing) containing 1 g of NaHCO_3 and 2 ml of water. 20 mg Pancreatin and 70 mg bile extract were added. Samples (2 ml) were obtained from small intestinal incubation for 1 h after the reaction flask had reached equilibrium at pH 7, been centrifuged, decanted and stored in cold storage (5°C) for further analysis. PBET was conducted in triplicate.

The mobility and leachability of Pb and Zn in soils was determined using TCLP analyses (US EPA 1995), conducted in triplicate. The procedure involves shaking a 10 g soil sample in 200 ml of 0.0992M acetic acid and 0.0643M NaOH with a pH of 4.93 ± 0.05 , for 18 h on a rotary shaker at about 300 rpm. At the end of the reaction period, the contents were filtered (Whatman No. 41 filter paper), acidified with concentrated HNO_3 to $\text{pH} < 2$ and stored in cold storage (5°C) for PTMs determination.

Soil samples were ground in an agate mill, digested in *aqua regia* and analysed by flame atomic absorption spectrophotometer (AAS). Leaves of the indicator plant, *T. officinale*, were washed with deionised water. Combined plant samples were dried and ground in a titanium mill and 0.3 g of plant biomass was digested in 65% HNO_3 in

a microwave oven, and analysed by AAS. PTMs in extracts (sequential extractions, PBET, TCLP) were determined by AAS directly. A standard reference material (BCR 601 and BCR 141R, Community Bureau of Reference, Brussels, Belgium; for plant and soil) was used in the digestion and analysis as a part of the QA/QC protocol. Reagent blank and analytical duplicates were also used where appropriate, to ensure accuracy and precision in the analysis.

Two-parameter and multiple regression analysis was performed to detect correlations among soil properties, Pb and Zn soil fractionation, and Pb and Zn bioavailability and leachability, using the computer program Statgraphics 4.0 for Windows.

RESULTS AND DISCUSSION

Soils from the Mežica Valley (Table 1, locations 1–12) were anthropogenic colluvials on dolomite (grasslands, farmlands and kitchen gardens), and soils from the Celje region were alluvial sandy-loams (permanent grasslands, Table 1, locations 13–18). Values of the selected pedological parameters of soils differ considerably. pH values ranged from very acidic (3.7) in a soil sample (location 2)

Table 1. Selected properties of surface soil samples from 18 different locations in the Mežica Valley (locations 1–12) and in the Celje region (locations 13–18), Slovenia

Location	pH (CaCl ₂)	Organic matter (%)	Particle size distribution			CO ₃ ²⁻ (g/kg)	CEC (cmol/kg)	P (mg/kg)
			Sand (%)	Loam (%)	Clay (%)			
1	6.2	8.6	51.4	41.7	6.9	701.3	22.9	15.5
2	3.7	7.8	34.1	50.3	15.6	3.6	28.7	0.35
3	6.5	10.0	36.7	41.0	22.3	251.7	36.7	5.85
4	6.6	1.5	70.5	20.2	9.3	349.3	17.2	1.31
5	6.6	7.4	70.7	22.2	7.1	268.9	17.4	6.24
6	6.7	12.5	20.8	52.9	26.3	317.6	43.4	0.57
7	6.6	6.6	25.4	64.5	10.1	664.7	26.0	36.9
8	6.3	8.7	37.9	50.2	11.9	139.8	31.8	76.7
9	6.8	8.5	29.1	35.0	35.9	280.9	30.8	6.89
10	6.9	10.0	11.0	66.4	22.6	259.8	39.8	1.66
11	6.5	10.2	59.4	31.3	9.3	37.2	27.2	8.55
12	6.7	6.6	57.4	32.1	10.5	16.0	19.5	0.61
13	7.3	5.1	41.9	42.6	15.5	113.8	27.5	2.92
14	7.0	4.4	20.9	59.8	19.3	45.6	27.1	5.06
15	6.9	9.9	36.0	49.6	14.4	190.2	30.3	10.8
16	6.2	2.3	10.5	71.6	17.9	1.1	18.0	0.26
17	6.5	4.5	32.5	48.0	19.5	10.4	23.8	3.10
18	6.6	6.4	15.4	68.4	16.2	17.8	26.9	18.2

from a grassland on a steep slope below conifer forest in the Mežica Valley (which explains the development of acidity and very low carbonate content, 3.6 g/kg), to slightly above neutral (7.3). Metal solubility tends to increase at lower pH and adsorption reactions become more important than precipitation and complexation reactions. However, adsorption also tends to decrease with lowering pH, due to the competition of protons (H⁺) with PTM cations for adsorption sites on soil colloids. As a result, adsorption reactions of Pb are significant at soil pH 3–5, and of Zn at pH 5–6.5. Precipitation and complexation reactions of both Pb and Zn predominate at soil pH 6–7 (Rieuwerts et al. 1998). The content of soil organic matter varied from 1.5 to 12.5%. Soil organic matter retains metals by complexation, adsorption and ion exchange. The carboxylic groups of humus substances ionize as the pH rises and form more stable metal complexes. Pb generally tends to persist in the soil surface layers, although a significant quantity of Pb bound to the soluble part

of the soil organic matter can be transported with percolates through the soil profile (Rieuwerts et al. 1998). The texture of the soil samples also varied substantially. The clay fraction of the soil mineral phase adsorbs metals through ion exchange and specific adsorption. Adsorption is pH dependent, but this could vary for different types of clay minerals. The cation exchange capacity (CEC) varied from 17.2 to 43.4 cmol/kg. Cation exchange of PTMs and other metals depends on the density of ionic strength of the surfaces of soil colloids and on the relative charges of metal species in soil solution. The surface negative charge may be pH dependent (soil organic matter) or permanent (some clay minerals) (Evans 1989). The concentration of easily extractable P varied from 0.26 to 76.67 mg/kg in the kitchen garden soils (apparently fertilized) from the Mežica Valley (location 8). The concentration of soil phosphates may have an important effect on PTM bioavailability, due to the formation of sparingly soluble salts (Nriagu 1974).

Pb fractionation, phyto-, oral and leachability

Fractionation of Pb in soil samples is shown in Table 2. Total Pb concentrations in soils ranged from 56.3 to 9585.1 mg/kg. All soil samples exceeded the EU warning limit, and 12 of 18 samples exceeded the EU critical limit for Pb in soil of 50 and 300 mg/kg, respectively (Council Directive 86/278/EEC 1986). The average recovery of Pb after sequential extractions was 92.9%. Pb was mostly bound to soil organic matter (31.5–74.7% of the total soil Pb) and carbonates (5.0–67.1%) or remained in the residual form (5.9–23.8%). This distribution pattern of Pb in different soil fractions was similar to that reported after our previous investigations in the Mežica Valley and Celje region (Leštan and Grčman 2001, Leštan et al. 2003). A strong association of Pb with organic matter was also described by Kabata-Pendias

and Pendias (1992), based on the results of many investigators. Li and Thornton (2001) reported a significant association of Pb with the soil carbonate phase. Chlopecka et al. (1996) and Ramos et al. (1994), however, found most of the Pb to be bound to Fe and Mn oxide and the organic fraction. In contrast to these reports, the fraction of Pb bound to oxides was quite low for most of our soil samples (0.11–8.3%). As observed before by other authors (Ramos et al. 1994, Chlopecka et al. 1996, Maiz et al. 2000), a very small fraction of the total Pb was determined in the soil solution (0.05–0.57%) and exchangeable from the soil colloids to the soil solution (0.01–6.40%).

To assess phytoavailable Pb and Zn, we used the indicator plant *T. officinale*. The concentration of Pb in the leaves of *T. officinale* grown in contaminated soils ranged from 6.7 to 89.3 mg/kg (Table 3). This is comparable to the results of Keane et al. (2001), who measured from 0.5 to 45 mg/kg Pb

Table 2. Total Pb concentration and fractionation of Pb in soil samples from the Mežica Valley and in the Celje region

Location	Total Pb (mg/kg)	Fractionation of Pb (mg/kg)						Recovery (%)
		I. ^a	II. ^b	III. ^c	IV. ^d	V. ^e	VI. ^f	
1	840.5	1.08 ± 0.16	3.37 ± 0.98	297.2 ± 7.7	0.99 ± 0.45	264.6 ± 10.0	85.7 ± 1.0	78.0
2	423.6	0.92 ± 0.22	27.1 ± 0.8	125.2 ± 4.0	35.2 ± 1.2	171.6 ± 6.1	53.7 ± 5.1	97.8
3	437.2	0.75 ± 0.16	0.35 ± 0.26	97.7 ± 0.8	2.11 ± 0.39	279.3 ± 9.0	104.0 ± 4.6	110.7
4	3816	1.74 ± 0.77	5.19 ± 0.40	2560 ± 194	20.7 ± 2.0	962.5 ± 57.2	352.0 ± 39.0	102.3
5	9585	13.7 ± 5.0	12.0 ± 5.1	4445 ± 72	74.2 ± 1.9	4213 ± 131	606.0 ± 25.5	97.7
6	1662	2.72 ± 0.50	1.41 ± 0.42	464.5 ± 6.6	7.93 ± 1.37	1083 ± 2	173.7 ± 14.7	104.3
7	1540	1.86 ± 0.57	2.04 ± 1.24	504.0 ± 6.9	3.74 ± 0.18	550.7 ± 11.1	205.3 ± 2.5	82.3
8	4588	4.83 ± 0.55	3.17 ± 2.19	1017 ± 13	11.7 ± 1.6	3256 ± 20	272.0 ± 9.0	99.5
9	561.3	0.87 ± 0.13	0.19 ± 0.18	157.5 ± 1.8	3.57 ± 0.04	419.0 ± 7.3	59.0 ± 3.0	114.0
10	579.3	0.88 ± 0.29	0.68 ± 0.10	121.5 ± 0.5	1.61 ± 0.25	299.8 ± 5.1	68.7 ± 5.0	85.1
11	929.2	0.81 ± 0.26	1.75 ± 0.35	209.5 ± 7.9	4.11 ± 1.30	677.5 ± 8.6	97.3 ± 4.0	106.6
12	272.7	0.91 ± 0.27	2.87 ± 1.56	6.39 ± 0.08	1.59 ± 0.13	173.6 ± 1.6	50.3 ± 8.4	86.4
13	170.8	0.19 ± 0.06	0.04 ± 0.08	23.5 ± 0.5	0.18 ± 0.31	86.2 ± 2.9	30.9 ± 2.0	82.6
14	182.2	0.32 ± 0.16	0.16 ± 0.11	21.9 ± 0.8	1.81 ± 0.87	85.5 ± 16.7	37.6 ± 2.0	80.8
15	475.9	0.99 ± 0.07	0.33 ± 0.04	122.2 ± 7.4	1.73 ± 0.25	245.7 ± 10.9	44.0 ± 5.3	87.2
16	56.3	0.32 ± 0.06	0.06 ± 0.05	2.79 ± 0.15	4.33 ± 3.08	24.2 ± 2.9	12.9 ± 1.8	79.3
17	82.9	0.47 ± 0.03	0.31 ± 0.21	7.67 ± 0.27	1.27 ± 0.34	51.0 ± 3.4	13.4 ± 0.9	89.5
18	211.5	0.34 ± 0.04	0.02 ± 0.03	21.5 ± 0.1	2.79 ± 1.14	129.0 ± 2.7	32.9 ± 2.0	88.3

^ain soil solution, ^bexchangeable, ^cbound to carbonate, ^dbound to Fe and Mn oxides, ^ebound to organic matter, ^fresidual fraction, ^gresults presented as means of three replicates ± SD

Table 3. Uptake of Pb into the *Taraxacum officinale* and Pb extracted from soil by PBET and TCLP

Location	Pb in <i>T. officinale</i> (mg/kg)	PBET (mg/kg)		TCLP (mg/l)
		stomach phase	intestinal phase	
1	— ^a	124.7 ± 36.9 ^b	52.0 ± 16.0	0.46 ± 0.08
2	—	95.0 ± 19.6	33.1 ± 14.6	0.43 ± 0.04
3	56.2	51.6 ± 14.5	7.9 ± 1.5	0.09 ± 0.04
4	—	536.7 ± 105.2	302.0 ± 51.4	8.01 ± 0.64
5	89.3	1233.0 ± 227.2	937.3 ± 165.9	12.4 ± 1.5
6	44.8	192.7 ± 21.9	61.8 ± 3.4	1.16 ± 0.16
7	39.3	159.3 ± 10.7	86.3 ± 10.8	0.96 ± 0.37
8	26.9	361.4 ± 58.5	209.8 ± 36.3	1.97 ± 0.29
9	13.7	48.1 ± 7.5	16.8 ± 2.1	0.32 ± 0.15
10	—	38.6 ± 4.1	16.9 ± 1.9	0.14 ± 0.03
11	26.9	79.4 ± 15.4	51.3 ± 9.7	0.46 ± 0.05
12	14.6	30.1 ± 8.2	7.8 ± 1.9	0.11 ± 0.02
13	—	15.3 ± 2.6	4.9 ± 1.7	0.11 ± 0.02
14	20.2	15.4 ± 1.4	1.4 ± 1.3	0.11 ± 0.01
15	6.7	50.6 ± 5.8	11.6 ± 1.2	0.05 ± 0.04
16	—	8.1 ± 4.8	5.1 ± 1.3	0.07 ± 0.06
17	11.2	8.2 ± 0.5	4.1 ± 1.3	0.06 ± 0.02
18	9.9	22.3 ± 4.2	5.5 ± 0.5	0.15 ± 0.04

^aPb plant concentration was not determined (less than 0.3 g of biomass in the combined sample), ^bresults presented as means of three replicates ± SD

in leaf samples of *T. officinale* grown on soil with 3.12 to 662 mg/kg Pb.

Ingestion is the major pathway of exposure to Pb in soils and dust. Oral bioavailability (bioaccessibility) of Pb was determined *in vitro* using Ruby's PBET test. The majority of Pb in our soil samples was not bioaccessible: the PBET stomach phase extracted from 6.7 to 23.0% of total initial soil Pb and the PBET intestinal phases extracted even less, from 0.75 to 7.9% of initial soil Pb. Consequently, the concentration of orally available Pb soluble in the stomach phase ranged from 8.1 ± 4.8 to 1233.0 ± 227.2 mg/kg, and Pb available from the intestinal phase from 1.4 ± 1.3 to 937.3 ± 165.9 mg/kg (Table 3). The highest Pb concentration soluble in stomach and intestinal phases was found in soil from location 5 (former farmland). High Pb bioaccessibility (361.4 ± 58.5 and 209.8 ± 36.3 mg Pb/kg in stomach and intestinal phases, respectively) was found, quite alarmingly, also in a soil sample taken from a kitchen garden (Table 3, location 8).

Pb leachability assessed by TCLP indicated a range of Pb concentrations in the extracts of contaminated soil from 0.05 ± 0.04 to 12.4 ± 1.5 mg/l (Table 3). In soil samples from locations 4 and 5, the concentration of Pb in TCLP leachate exceeded 5 mg/l, the TCLP regulatory limit for materials considered to be hazardous waste.

Zn fractionation, phyto- and leachability

Total Zn concentrations in soils ranged from 171.0 to 15 828 mg/kg (Table 4). All soil samples exceeded the warning and critical limits for Zn in soil, set at 150 and 300 mg/kg (Council Directive 86/278/EEC 1986). Average recovery of Zn after sequential extractions was 101.8%. The residual Zn fraction (17.4–86.6), fraction of Zn bound to organic matter (8.2–44.9%) and fraction of Zn bound to soil carbonates (2.5–44.7%) predominated (Table 4). A relatively small fraction of total Zn was determined in the soil solution (0.03–0.59%)

Table 4. Total Zn concentration and fractionation of Zn in soil samples from the Mežica Valley and in the Celje region

Location	Total Zn (mg/kg)	Fractionation of Zn (mg/kg)						Recovery (%)
		I. ^a	II. ^b	III. ^c	IV. ^d	V. ^e	VI. ^f	
1	373.7	0.40 ± 0.09 ^g	2.85 ± 0.27	155.4 ± 2.1	7.04 ± 0.13	139.0 ± 2.3	154.5 ± 4.5	122.9
2	181.6	0.15 ± 0.02	18.8 ± 0.6	4.46 ± 0.13	4.43 ± 0.09	33.3 ± 4.1	154.6 ± 4.3	118.8
3	804.7	0.69 ± 0.07	1.86 ± 0.07	112.1 ± 3.6	11.4 ± 0.2	241.4 ± 3.9	438.3 ± 20.6	100.1
4	4222	1.06 ± 0.18	14.0 ± 0.4	822.7 ± 35.6	201.0 ± 15.2	1233 ± 15	1775 ± 34	95.8
5	15828	5.88 ± 2.18	20.3 ± 2.0	7073 ± 646	1099 ± 16	4265 ± 200	3555 ± 154	101.2
6	971.8	1.24 ± 0.30	1.59 ± 0.33	90.0 ± 1.2	18.2 ± 0.1	213.7 ± 6.9	658.7 ± 29.0	101.2
7	676.0	0.77 ± 0.20	5.40 ± 0.03	132.6 ± 4.0	17.2 ± 0.3	218.7 ± 13.0	383.2 ± 11.3	112.1
8	1449	1.12 ± 0.17	7.48 ± 0.03	180.3 ± 5.8	38.4 ± 3.9	468.2 ± 14.7	741.0 ± 29.5	99.2
9	370.5	0.44 ± 0.13	0.17 ± 0.01	10.5 ± 0.7	0.09 ± 0.05	30.4 ± 1.9	320.9 ± 7.8	97.8
10	204.7	0.14 ± 0.08	0.16 ± 0.04	10.6 ± 1.0	1.04 ± 0.09	55.2 ± 9.6	127.1 ± 8.8	94.9
11	829.2	0.52 ± 0.08	15.5 ± 1.0	61.6 ± 6.5	19.3 ± 1.2	230.0 ± 17.4	509.0 ± 9.2	100.8
12	171.0	0.23 ± 0.08	0.29 ± 0.06	8.04 ± 0.22	3.39 ± 0.31	36.2 ± 5.0	119.3 ± 1.8	97.9
13	799.5	1.47 ± 0.04	1.01 ± 0.06	116.7 ± 7.9	26.6 ± 2.0	288.2 ± 29.6	344.0 ± 42.9	97.3
14	1202	1.79 ± 0.28	1.69 ± 1.19	169.7 ± 6.1	74.1 ± 16.7	386.0 ± 14.5	486.3 ± 15.3	93.1
15	1564	3.23 ± 0.09	4.69 ± 0.19	307.7 ± 3.8	32.9 ± 1.1	549.5 ± 8.3	701.7 ± 11.4	102.2
16	163.3	0.96 ± 0.21	0.75 ± 0.27	5.86 ± 0.17	13.6 ± 1.0	24.1 ± 1.3	126.9 ± 9.6	105.5
17	516.2	1.38 ± 0.08	2.49 ± 0.07	74.0 ± 6.6	60.3 ± 7.2	231.7 ± 12.1	89.7 ± 3.3	89.0
18	1037	1.71 ± 0.03	1.80 ± 0.09	121.0 ± 8.4	59.7 ± 27.4	416.2 ± 13.0	469.0 ± 44.5	103.2

^ain soil solution, ^bexchangeable, ^cbound to carbonate, ^dbound to Fe and Mn oxides, ^ebound to organic matter, ^fresidual fraction, ^gresults presented as means of three replicates ± SD

and in the fraction of Zn bound to soil oxides (0.03–11.7%). In most soil samples, the fraction of Zn exchangeable from soil colloids was very low, from 0.07 to 1.9%, except for soil from location 2 with pH 3.7, where 10.4% of total Zn was determined in the fraction exchangeable from soil colloids. Similar Zn fractionation in soils of Mežica Valley and Celje region was reported earlier (Leštan and Grčman 2001, Leštan et al. 2003). Kabala and Singh (2001), and Rivero et al. (2000) also reported for agricultural calcareous soil that Zn was mostly concentrated in the residual fraction. Chlopecka et al. (1996), however, found most Zn bound to the residual fraction and also to soil oxides, where we determined very small proportions of Zn. Dvořák et al. (2003) analysed three types of loamy and clay-loamy soils and found Zn distributed over exchangeable, oxides, organic and residual fractions.

The concentration of Zn in the leaves of *T. officinale* was 2 to 20 times higher than the concentration of Pb (Table 5). Keane et al. (2001) also reported a higher Zn uptake, even though they picked *T. officinale* in soils that generally contained less Zn than Pb. Many plants are known to accumulate or even hyper-accumulate high amounts of Zn. Some hyperaccumulators, e.g., *Alyssum* and *Thlaspi* spp., can accumulate > 1% of Zn in their dry biomass (Baker and Walker 1990). There is only one reliable report of a Pb hyperaccumulator; Puschenreiter et al. (2001) reported that *Thlaspi goesingense* accumulated 2840 mg Pb/kg in shoots.

We did not measure Zn oral availability, since PBET has been developed and validated only for Pb and As. For Zn, the US EPA sets the TCLP regulatory limit for materials considered as hazardous waste at 250 mg/l. This limit was not exceeded in any of soil samples (Table 5).

Table 5. Uptake of Zn into the *Taraxacum officinale* and Zn extracted from soil by TCLP

Location	Zn in <i>T. officinale</i> (mg/kg)	TCLP (mg/l)
1	— ^a	1.25 ± 0.19 ^b
2	—	0.35 ± 0.05
3	112.7	1.02 ± 0.00
4	—	14.0 ± 1.5
5	276.0	21.3 ± 0.8
6	246.0	1.07 ± 0.20
7	191.6	1.84 ± 0.20
8	186.2	2.79 ± 0.21
9	104.5	0.22 ± 0.12
10	—	0.14 ± 0.02
11	171.8	1.35 ± 0.10
12	114.8	0.19 ± 0.04
13	—	0.67 ± 0.06
14	132.2	2.85 ± 0.47
15	132.7	1.57 ± 0.19
16	—	0.19 ± 0.05
17	161.7	1.41 ± 0.21
18	125.4	2.12 ± 0.12

^aless than 0.3 g of plant biomass in the combined sample,

^bresults presented as means of three replicates ± SD

Relationships among soil properties, PTMs fractionation, bio- and leachability

Three simple two-parameter regression models [linear ($y = a + bx$) and two 2nd order polynomial ($y = a + bx^2$ and $y = a + bx + cx^2$)] were used to study possible correlations between total soil Pb and Zn concentrations and selected measured soil properties, the distribution of Pb and Zn in soil fractionates (in % of total soil Pb and Zn), phytoavailable Pb and Zn using *T. officinale* as an indicator plant (expressed as a bioconcentration factor: the ratio of metal concentration in plant shoots to metal concentration in soil), bioaccessible Pb using the PBET test (expressed as % of total soil Pb available in stomach and intestinal phases), and leachable Pb and Zn using TCLP (expressed as % of the total soil Pb and Zn measured in soil leachate). Two-parameter regression equations that gave the best fit to the given pair of experimental data, with significance level (P) lower than 0.05,

are listed in Table 6. Most dependent variables were significantly ($P < 0.05$) correlated with two or more independent variables. These variables were considered in the multiple regression analysis. Linear, cubic and 2nd order polynomial relations of dependent variable to each of the independent variables were tested within the regression equation, and the model giving the best fits is shown in Table 6.

Cation exchange capacity is the function of soil pH and negatively charged positions on soil colloids and mainly measures the quantity of available sorption sites on clay and organic matter. While the correlation of CEC with soil pH was not significant for our data, strong positive separate correlations between CEC and organic matter and clay content were confirmed (Table 6). Multiple regression comprising both independent variables in a single model gave even a better fit ($P < 0.001$) and explained about 75% of the variance of CEC (Table 6). Francois et al. (2004) also obtained a significant positive correlation ($R^2 = 86\%$) between CEC and soil organic matter and clay content using a linear multiple regression model. Although carbonate content and pH in soils are considered to be closely linked, they were not significantly correlated, as reported by the same author. We considered other soil properties presented in Table 1 to be independent of one another.

No significant correlations were found between the proportion of Pb and Zn in the fraction of PTMs soluble in soil solution and measured soil properties. A possible explanation is a very small amount of Pb and Zn (less than 0.6% of total PTMs soil content) determined in this fraction, obtained after soil extraction with deionised water (Tables 2 and 4). The fraction of PTMs soluble in soil solution consists of free hydrated ions and water-soluble organic and inorganic complexes. They are the most mobile forms of PTMs in soil and therefore important in the soil risk assessment. A considerable number of studies tried to infer relationships between the mobility of PTMs and soil properties, based on regression analysis. Sauve et al. (1997), for example, reported that the concentration of dissolved Pb and activity of free Pb^{2+} ions were highly significantly correlated to soil pH and total Pb content. Carlon et al. (2004) applied multiple regression to literature data and obtained a soil pH-dependent regression equation of the soil-water distribution coefficient for Pb (ratio of sorbed PTM to dissolved PTM).

Soil pH is generally acknowledged to be an important regulating factor for metal partitioning

Table 6. Results of a two-parameter regression analysis ($P < 0.05$) correlating soil parameters, data on Pb and Zn fractionation, Pb and Zn uptake into *Taraxacum officinale*, Pb oral availability (PBET), and Pb and Zn soil mobility (TCLP)

	<i>P</i> value	<i>R</i> ² (%)	Model
CEC			
Organic matter	0.0001	59.82	$y = 18.76 + 0.14x^2$
Clay	0.0076	36.83	$y = 17.81 + 0.60x$
II. Exchangeable Pb			
pH	0.0000	95.75	$y = 30.23 - 8.77x + 0.64x^2$
II. Exchangeable Zn			
pH	0.0000	95.85	$y = 36.78 - 10.24x + 0.72x^2$
III. Pb bound to carbonates			
Carbonates	0.0014	47.99	$y = 14.96 + 0.52x$
Total Pb	0.0095	39.05	$y = 15.30 + 1.15 \cdot 10^{-2}x - 8.86 \cdot 10^{-7}x^2$
III. Zn bound to carbonates			
Carbonates	0.0331	25.38	$y = 8.86 + 0.26x$
Clay	0.0190	29.85	$y = 26.94 - 0.79x$
Total Zn	0.0005	54.43	$y = 10.29 + 2.20 \cdot 10^{-3}x$
IV. Pb bound to oxides			
pH	0.0015	47.83	$y = 18.29 - 2.58x$
IV. Zn bound to oxides			
Organic matter	0.0038	41.76	$y = 9.00 - 0.72x$
CEC	0.0305	26.04	$y = 9.92 - 0.22x$
V. Pb bound to organic matter			
Carbonates	0.0193	29.71	$y = 63.86 - 0.34x$
VI. Pb in residual fraction			
Organic matter	0.0429	23.20	$y = 23.18 - 1.11x$
Total Pb	0.0050	44.03	$y = 19.67 + 5.17 \cdot 10^{-3}x - 4.04 \cdot 10^{-7}x^2$
VI. Zn in residual fraction			
Clay	0.0188	25.54	$y = 42.42 + 3.30 \cdot 10^{-2}x^2$
Total Zn	0.0434	23.11	$y = 56.95 - 2.41 \cdot 10^{-3}x$
Pb uptake into <i>T. officinale</i>			
IV. Pb bound to oxides	0.0425	38.24	$y = 1.31 \cdot 10^{-2} + 5.73 \cdot 10^{-2}x$
VI. Pb in residual fraction	0.0046	60.94	$y = -3.37 \cdot 10^{-2} + 5.85 \cdot 10^{-3}x$
Zn uptake into <i>T. officinale</i>			
III. Zn bound to carbonates	0.0490	30.81	$y = 0.34 - 8.86 \cdot 10^{-3}x$
Pb in PBET stomach phase			
II. Exchangeable Pb	0.0001	62.41	$y = 10.25 + 1.92x$
IV. Pb bound to oxides	0.0023	45.08	$y = 9.93 + 0.89x$
V. Pb bound to organic matter	0.0070	37.38	$y = 21.14 - 0.17x$
Pb in PBET intestinal phase			
Clay	0.0497	21.98	$y = 7.33 - 0.16x$
CEC	0.0088	46.78	$y = 22.03 - 1.06x + 1.47 \cdot 10^{-2}x^2$
Total Pb	0.0192	29.77	$y = 3.81 + 5.97 \cdot 10^{-4}x$
III. Pb bound to carbonates	0.0210	24.59	$y = 3.52 + 1.31 \cdot 10^{-3}x^2$
IV. Pb bound to oxides	0.0326	25.48	$y = 3.94 + 0.47x$
V. Pb bound to organic matter	0.0077	36.71	$y = 11.55 - 0.12x$
TCLP leached Pb			
Organic matter	0.0009	55.77	$y = 4.48 - 0.74x + 3.85 \cdot 10^{-2}x^2$
CEC	0.0016	51.97	$y = 8.91 - 0.47x + 6.69 \cdot 10^{-3}x$
III. Pb bound to carbonates	0.0005	59.10	$y = 2.06 - 9.04 \cdot 10^{-2}x + 1.86 \cdot 10^{-3}x^2$
V. Pb bound to organic matter	0.0014	52.87	$y = 8.82 - 0.24x + 1.91 \cdot 10^{-3}x^2$
TCLP leached Zn			
Clay	0.0274	26.21	$y = 5.41 - 0.12x$
III. Zn bound to carbonates	0.0459	24.86	$y = 1.16 + 0.27x - 4.72 \cdot 10^{-3}x^2$
VI. Zn in residual fraction	0.0189	29.90	$y = 6.21 - 5.21 \cdot 10^{-2}x$

and fractionation. Janssen et al. (1997) reported that pH was in fact the only soil parameter of significance for partitioning Pb, Zn and other metals between the soil solution and soil solid phases. In our study, soil pH affected the proportion of Pb bound to soil oxides and, highly significantly ($P < 0.001$), the exchangeable Pb and Zn. More than 95% of the variability of Pb and Zn in the exchangeable fraction was explained by the variability of soil pH (Table 6). The association between metal adsorption on exchangeable surfaces of soil colloids and pH is partly due to the competition of H^+ for adsorption sites at low pH resulting in decreased metal adsorption (Rieuwerts et al. 1998). Pb and Zn adsorption on exchangeable sites should therefore increase with increasing soil pH, but we obtained a negative correlation between soil pH and the proportion of exchangeable Pb and Zn (Table 6). However, soil pH values were fairly uniform for most samples. Except for a very acidic soil from location 2, they were within 1 pH unit in all other soils (Table 1). When soil from location 2 was taken from the equations, statistically less significant correlations ($P > 0.05$) were found between soil pH and Pb and Zn soil fractionation. This indicates that soil pH from location 2 was the influential input in the regression analysis of data.

Except for soil pH, soils used in this study had a wide range and also adequate distribution of characteristics (Table 1) to determine the relationships among soil properties, Pb and Zn fractionation, availability and mobility. Soil pH was not used in further regression analysis.

As expected, the proportion of Pb and Zn bound to carbonates increased with soil carbonate content, and also with total Pb and Zn content (Table 6). Chlopecka et al. (1996) found no such correlation for Pb, while the proportion of Zn associated with the carbonate fraction also slightly increased with Zn soil concentration. The proportion of Zn bound to Fe and Mn oxides was lower in more organic soils with higher CEC (Table 6). The proportion of Pb bound to soil organic matter was lower in soils with a higher content of carbonates (Table 6) and, interestingly, not statistically significantly ($P < 0.05$) dependent on soil organic matter content. There were no significant ($P < 0.05$) correlations between the proportion of Zn bound to organic matter and soil properties. The proportion of Pb in the residual soil fraction increased, while the proportion of residual Zn decreased with the total concentration of Pb and Zn in soil (Table 6). Chlopecka et al. (1996) reported an opposite ten-

dency: the proportion of Pb in the residual fraction decreased and the proportion of Zn increased with the total metal concentration. Residual Pb and Zn were correlated also with soil organic matter and clay content, respectively (Table 6). Rivero et al. (2000) reported that the residual soil Zn significantly correlated with clay and carbonate content in soil, but not with total Zn content.

The uptake of Pb and Zn by the indicator plant, *T. officinale*, correlated with the proportion of Pb and Zn in non-labile fractions, generally considered being not available for plants (Table 6). *T. officinale* grows very poorly on some soils (for some samples the amount of biomass was not sufficient to allow PTM determination) irrespective of Pb and Zn contamination. This could indicate that other soil factors than those measured in our study (i.e. soil structure, availability of micro and macro nutrients) could influence not just plant growth, but possibly also Pb and Zn uptake. Nevertheless, it is difficult to explain the significant positive correlation between Pb plant uptake and proportion of Pb in the least available, residual soil fraction (Table 6). Pichtel et al. (2000), for example, reported a slight correlation ($P < 0.05$) between Pb uptake by *T. officinale* and phytoavailable fractions of Pb soluble in soil solution and exchangeable from soil colloids. Marr et al. (1999) found no statistically significant ($P < 0.05$) correlations among Pb and Zn uptake by *T. officinale*, PTM concentration in soils, and PTM soil fractionation. They reported only a significant reduction of Zn plant uptake in soils with a higher concentration of easily available phosphates, which we found statistically not significant. On the other hand, Keane et al. (2001) reported that Pb and Zn concentrations in leaves of *T. officinale* collected in the fall increased significantly ($P < 0.001$) as the soil levels of PTMs increased. In leaf samples collected in the spring, a significant correlation was found only for Zn.

The solubility of soil Pb in both phases of PBET was correlated with several Pb soil fractions (Table 6). The proportion of stomach and intestinal soluble Pb decreased with the proportion of Pb bound to soil organic matter and increased with the proportion of Pb bound to oxides, exchangeable Pb (Pb in the PBET stomach phase only), and Pb bound to carbonates (Pb in the PBET intestinal phase only). Of soil properties, Pb in PBET intestinal phase correlated with CEC, and somewhat less significantly also with soil clay and total Pb content (Table 6). The application of multiple regression to Pb intestinal phase data provided a highly significant regression equation

($P < 0.001$) dependent on the proportions of Pb bound to oxides and organic matter and on total soil Pb (Table 6). To test whether it is possible to use data from PTMs fractionation to predict the bioaccessibility of PTMs from incidental ingestion of contaminated soil, Basta and Gradwohl (2000) correlated Pb and Zn bioaccessibility, determined using PBET, and Pb and Zn soil fractionation determined with Potentially BioAvailable Sequential Extraction (PBASE). They found a statistically significant correlation ($P < 0.01$) between Pb soluble in the stomach phase of PBET and the following soil fractions: the fraction of acid-soluble Pb in weak surface complexes, the fraction of Pb in precipitates and in strong surface complexes, and Pb in the insoluble soil fraction. Pb in the intestinal phase of PBET correlated with the fraction of exchangeable and soluble Pb, the fraction of acid-soluble Pb in weak surface complexes, and Pb in the insoluble soil fraction of PBASE. Yang et al. (2003) reported that Pb availability in the PBET stomach phase decreased ($P < 0.02$) at higher soil pH. However, they used soil freshly spiked with Pb, in which Pb soil fractionation typical for older contaminations would not be expected.

The proportion of Pb leachable using TCLP was lower in soils with a higher content of organic matter and CEC, and lower than the proportion of Pb bound to organic matter and carbonates (Table 6). Multiple regression analysis yielded a statistically highly significant correlation ($P < 0.001$) of leachable Pb with soil organic matter and the fraction of Pb bound to carbonates (Table 6). At $P < 0.05$, leachable Zn (TCLP) correlated with soil clay content, and the proportions of Zn bound to oxides and in the residual fraction (Table 6). However, these correlations explained less than 30% of variability in leachable Zn.

Contaminated soils for this study were selected to span a wide range of soil properties and Pb and Zn concentrations. Substantial variations in the distribution of Pb and Zn among different soil fractions, phyto and oral availability and leachability were determined. However, using regression analysis, relatively few statistically highly significant ($P < 0.001$) correlations were found among the measured parameters. It is possible that Pb and Zn soil fractionation, bioavailability, mobility and leachability are partly controlled by soil properties, processes and conditions that were not measured and included in regression models. These might include: (i) Redox potential. Metals are more readily dissolved in waterlogged soils

with low redox potential (Rieuwerts et al. 1998). (ii) Presence of cations and anions. As total soil metal concentrations in soil solution increase, the fixation capacity of the solid soil phase becomes saturated and metals remain in soil solution. (iii) Presence of chelates. Plants have evolved several strategies for increasing the bioavailability of metallic micronutrients by production of metal chelating compounds (i.e. siderophores). The bioavailability of PTMs may be facilitated by soil microorganisms producing polycarboxylic organic acids and other chelating metabolites (White et al. 1997). PTMs can also be complexed and mobilized by small molecules of fulvic and humic acids comprising dissolved soil organic matter (Jordan et al. 1997). (iv) Other miscellaneous factors that were not taken into account: microbial activity and earthworm activity increase plant-available Pb, root activity, aggregation of Pb and Zn forms within silica, oxide or other mineral matrixes (Rieuwerts et al. 1998).

Nevertheless, using multiple regression analysis, we obtained a statistically highly significant relationship between Pb bioaccessibility and leachability (intestinal phase of PBET, TCLP), and soil properties and Pb fractionation. High correlations ($P < 0.001$) indicate that the main factors influencing Pb availability and leachability were taken into account. These factors could be important for building future models predicting the bioavailability, mobility and leachability of PTMs in soils. Such models could be of a great importance for the management of polluted soils and industrial sites.

REFERENCES

- Baker A.J.M., Walker P.L. (1990): Physiological and molecular aspects of tolerance in higher plants. In: Shaw A.J. (ed.): Heavy Metal Tolerance in Plants: Evolutionary Aspects. CRC Press, Boca Raton, USA: 155–177.
- Basta M., Gradwohl R. (2000): Estimation of Cd, Pb, and Zn bioavailability in smelter-contaminated soils by sequential extraction procedure. J. Soil. Contam., 9: 149–164.
- Carlson C., Dalla Valle M., Marcomini A. (2004): Regression model to predict water-soil heavy metal partition coefficients in risk assessment studies. Environ. Pollut., 127: 109–115.
- Chlopecka A., Bacon J.R., Wilson M.J., Kay J. (1996): Heavy metals in the environment. J. Environ. Qual., 25: 69–79.

- Cook C.M., Sgardelis S.P., Pantis J.D., Lanaras T. (1994): Concentrations of Pb, Zn, and Cu in *Taraxacum* spp. in relation to urban pollution. *Bull. Environ. Contam. Toxicol.*, 53: 204–210.
- Council Directive 86/278/EEC (1986): On the Protection of the Environment, and in Particular of the Soil, When Sewage Sludge is Used in Agriculture. EC Official J.: L181.
- Dvořák P., Tlustoš P., Száková J., Černý J., Balík J. (2003): Distribution of soil fractions of zinc and its uptake by potatoes, maize, wheat and barley after soil amendment by sludge and inorganic Zn salt. *Plant Soil Environ.*, 49: 203–212.
- Evans L.J. (1989): Chemistry of metal retention by soils. *Environ. Sci. Technol.*, 23: 1046–1056.
- Francois M., Dubourguier H.C., Li D., Douay F. (2004): Prediction of heavy metal solubility in agricultural topsoils around two smelters by the physico-chemical parameters of the soil. *Aquat. Sci.*, 66: 78–85.
- Janssen R.P.T., Peijnenburg W.J.G.M., Posthuma L., Van Den Hoop M.A.G.T. (1997): Equilibrium partitioning of heavy metals in Dutch field soils. 1. Relationship between metal partitioning coefficient and soil characteristics. *Environ. Toxicol. Chem.*, 16: 2470–2478.
- Jordan R.N., Yonge D.R., Hathhorn W.E. (1997): Enhanced mobility of Pb in the presence of dissolved natural organic matter. *J. Contam. Hydrol.*, 29: 59–80.
- Kabala C., Singh B.R. (2001): Fractionation and mobility of copper, lead and zinc in soil profiles in the vicinity of a copper smelter. *J. Environ. Qual.*, 30: 485–492.
- Kabata-Pendias A., Pendias H. (1992): *Trace Elements in Soils and Plants*. CRC Press, Boca Raton, USA.
- Kalra Y.P., Maynard D.G. (1992): *Methods Manual for Forest Soil and Plant Analysis*. Canadian Forest Service, Edmonton, Canada.
- Keane B., Collier M.H., Shann J.R., Rogstad S.H. (2001): Metal content of dandelion (*Taraxacum officinale*) leaves in relation to soil contamination and airborne particulate matter. *Sci. Total. Environ.*, 281: 63–78.
- Leštan D., Grčman H. (2001): Speciation of lead, zinc and cadmium in contaminated soils from Mežica Valley. *Zb. Bioteh. Fak.*, 77: 205–14.
- 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.
- Levy D.B., Barbarick K.A., Siemer E.G., Sommers L.E. (1992): Distribution and partitioning of trace metals in contaminated soil near Leadville, Colorado. *J. Environ. Qual.*, 21: 185–195.
- Li X., Thornton I. (2001): Chemical partitioning of trace and major elements in soils contaminated by mining and smelting activities. *Appl. Geochem.*, 16: 1693–1706.
- Maiz I., Arambarri I., Garcia R., Millan E. (2000): Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. *Environ. Pollut.*, 110: 3–9.
- Marr K., Fyles H., Hendershot W. (1999): Trace metals in Montreal urban soils and leaves of *Taraxacum officinale*. *Can. J. Soil. Sci.*, 79: 385–387.
- Nriagu J.O. (1974): Lead orthophosphates: IV. Formation and stability in the environment. *Geochim. Cosmochim. Acta.*, 38: 887–898.
- Pichtel J., Kuroiwa K., Sawyerr H.T. (2000): Distribution of Pb, Cd and Ba in soils and plants of two contaminated sites. *Environ. Pollut.*, 110: 171–178.
- 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.
- Ramos L., Hernandez L.M., Gonzales M.J. (1994): Sequential fractionation of copper, lead, cadmium and zinc in soil from or near Donana national park. *J. Environ. Qual.*, 23: 50–57.
- Rieuwerts J.S., Thornton I., Farago M.E., Ashmore M.R. (1998): Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. *Chem. Spec. Bioavail.*, 10: 61–75.
- Rivero V.C., Masedo M.D., De la Villa R.V. (2000): Effect of soil properties on zinc retention in agricultural soils. *Agrochimica*, 43: 46–54.
- 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.
- Sauve S., McBride M.B., Hendershot W.H. (1997): Speciation of lead in contaminated soils. *Environ. Pollut.*, 98: 149–155.
- Tessier A., Campbell P.G.C., Bisson M. (1979): Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.*, 51: 844–851.
- US EPA (1995): *Test Methods for Evaluation of Solid Waste* vol. IA. US Government Printing Office, Washington DC, USA: Parts 403, 503.
- White C., Sayer J.A., Gadd G.M. (1997): Microbial solubilization and immobilization of toxic metals: key

biochemical processes for treatment of contamination. FEMS Microbiol. Rev., 20: 503–516.

Yang J.-K., Barnett M.O., Jardine P.M., Brooks S.C. (2003): Factors controlling the bioaccessibility of

Arsenic (V) and Lead (II) in soil. Soil. Sedim. Contam., 12: 165–179.

Received on December 19, 2006

Corresponding author:

Neža Finžgar, dipl. Ing. University of Ljubljana, Biotechnical Faculty, Center for Soil and Environmental Science, Agronomy Department, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
phone: + 386 01 423 1161, fax: + 386 01 423 1088, e-mail: neza.finzgar@bf.uni-lj.si
