Dolomite limestone application as a chemical immobilization of metal-contaminated soil

L. Trakal, M. Neuberg, P. Tlustoš, J. Száková, V. Tejnecký, O. Drábek

Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

ABSTRACT

In this experiment we proved an effect of dolomite limestone on chemical immobilization in soil contaminated by trace metals, namely Cd, Pb, and Zn. Primary, we set up lysimeter pot experiment to measure soil leaching without vegetation. Willow clone (*S.* × *smithiana* Willd) was cultivated in the second lysimeter pot as a new approach to monitor Pb, Cd and Zn leaching, which was affected by soil liming (used in 1% rate). At the time of both harvests, aboveground biomass increased significantly at the amended variant. After the second harvest aboveground biomass production increased by 80% in comparison with the first one, Cd and Zn concentration in biomass decreased 2-fold and 3-fold, respectively. Dolomite limestone as a process of liming: (i) restricted metals leaching from the soil substrate; (ii) reduced metal uptake by willow; and (iii) increased biomass production of willow. Liming also alleviated the plant stress imposed by risk elements resulting in better plant growth and lower levels of stress markers (total nitrogen content and the main amino acid metabolism parameters in the willow leaves) yet through different mechanisms.

Keywords: toxic metals; liming; willow; lysimeter pot; leaching

Chemical immobilization is a remediation method where inexpensive materials (e.g., fertilizer, waste products) are added to contaminated soil to reduce the solubility of metal contaminants. Compared with other remediation techniques, *in situ* chemical immobilization is less expensive and may provide a long-term remediation solution through the formation of low solubility minerals and/or precipitates (Basta and McGowen 2004). Because contaminant solubility is related to its mobility and bioavailability, chemical immobilization may reduce environmental risk (McGowen et al. 2001). Many studies were conducted in the last decade using chemical amendments (e.g. dolomite limestone) for chemical remediation of Pb, Cd, and/or Zn in contaminated soil (Chen et al. 2000).

Generally, the solubility of metals in the soil solution is greater at low pH and this increased solubility causes increased availability to plants. Large increases in soil pH caused by lime or fly ash addition decreased Cd, Zn and/or Pb uptake to plants, whereas in more studies concentrations of Cd and/or Pb in plants were generally unaffected by changes in soil pH (McLaughlin et al. 1994).

In the present paper we addressed question, if chosen fast growing willow clone (*S.* × *smithiana* Willd.) with high phytoextraction potential (Tlustoš et al. 2007) can be manipulated by an amendment of contaminated soil by liming. After dolomite limestone application we expected reduction of metals concentration in leachate and in willow as well.

The objectives of this study were to assess: (i) Cd, Zn, and Pb uptake by willow clone (*S.* × *smithiana* Willd.) from contaminated soil; and (ii) the effect of liming on Cd, Zn, and Pb content in willow and in leachate, respectively. We used lysimeter pot experiment as a new approach for impact of liming (by dolomite limestone) on leachate and willow uptake, respectively.

Additionally, metals also affect nitrogen uptake as they impair plasma membrane functionality, and they also affect nitrogen assimilation by blocking

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the cytosolic enzyme nitrate reductase (Kłobus et al. 2002). A mechanism by which many plants respond to and apparently detoxify toxic metals is the production of amino acids (Shah and Dubey 1998). These facts allow us to hypothesize that total nitrogen concentration and concentration of free amino acids will be increased in variant with dolomite limestone application. An additional aim of this study was to compare reached results more deeply.

MATERIAL AND METHODS

Experimental setup. As a new access to improve chemical immobilization by dolomite limestone application (1% rate), we used two types of lysimeter pots under greenhouse controlled conditions. First lysimeter pot experiment (10 cm high and 5 cm diameter) was set up to compare metal leaching from: (i) contaminated soil; and (ii) liming contaminated soil. Second lysimeter experiment was vessels (30 cm high and 20 cm diameter) that were also filled by contaminated soil and planted by chosen clone of willow during two vegetation periods to compare metal leaching on: (i) willows growing on contaminated soil; and (ii) willows growing on contaminated soil after liming.

Irrigation water was applied gravitationally, using drip emitters connected to individual Mariotte style reservoirs for each lysimeter of second experiment. First experiment was irrigated by steaming of constant volume. Leachate of both experiments was drained from the pots into the PE bottles, and samples were taken each week for analysis.

Experimental soil. Soil samples were taken from the arable layer (0–25 cm) of agriculturally used soil. Samples used for soil characteristics determination and metal contents, were air dried and homogenized. The contents of each selected metal were given as follows: Cd 9.37 ± 1.56, Pb 191 ± 211 and Zn 258 ± 82 mg/kg. The soil was classified as Gleyic Cambisol.

Plant material. A clone of the high biomass producing (*S.* × *smithiana* Willd., No. S-218) was used in the pot experiment for its high accumulation of selected metals (Vysloužilová et al. 2003). The willow plants in the lysimeter pots were harvested for the aboveground biomass after the first and second vegetation periods, whereas the root biomass was collected after the second period. The aboveground biomass and roots as well were dried at 6°C until they reached constant weight and were finely ground prior to decomposition.

Analytical methods. Soil pH was measured in suspension using a 1:1.25 (w/v) ratio of soil, deionized water, and 0.01 mol/L $CaCl_2$. Samples for soil CEC were prepared in suspension 1:50 (w/v) ratio of soil and 0.1 mol/L $BaCl_2$. The total concentration of Cd, Pb, and Zn in the soils was determined in the digests, and obtained by decomposition procedure (Száková et al. 2009). A certified reference material RM 7003 Silty Clay Loam was used for quality assurance of the results. For the determination of exchangeable concentrations of metals the soil samples were extracted with 0.11 mol/L CH_3COOH (Novozamsky et al. 1993) at a ratio of 1:20 (w/v). Plant samples were decomposed using the 'dry ashing' procedure (Miholová et al. 1993). The standard reference material DC73350 Leaves of Poplar (China National Analysis Centre for Iron and Steel) was used for evaluating measurement precision. These soil and plant analyses were performed using ICP-OES (Varian VistaPro, Mulgrave, Australia). Major inorganic anions (F^-, Cl^-, NO_2^-, Br^-, PO_4^{3-}, SO_4^{2-}, NO_3^-) were determined by means of ion-exchange chromatography with suppressed conductivity in the ion chromatogram ICS 90 (Dionex, Sunnyvale, USA). This was combined with available forms of soil nutrients (Ca, K, and Mg) with the Mehlich III soil extraction procedure (Zbíral 2000) and measured using the atomic absorption spectrometry with flame atomization (F-AAS, Varian 280FS, Mulgrave, Australia). The contents of the mineral N (N_{min}) and dissolved organic carbon (DOC) were determined in extractions of 0.01 mol/L $CaCl_2$ (Houba et al. 1986), using a colorimetric method on a SKALAR plus SYSTEM apparatus (Breda, the Netherhlands). The total organic carbon (TOC) was determined by wet oxidation with $K_2Cr_2O_7$ as well as measurement of the absorbance at 590 nm (Sims and Haby 1971). The total N level in plants was measured after wet decomposition by concentrated H_2SO_4 with an addition of mixed catalyst at 420°C, in a Kjeltec Auto 1030 Analyzer (Sollentura, Sweden). The determination of free amino acids was carried out by gas chromatography coupled with mass spectrometry detection using a HP 6890N/5975 instrument (Agilent Technologies, Wilmington, USA).

Statistical analyses. Data were subjected to an analysis of variance (ANOVA) to examine the effect of dolomite limestone application. A statistical analysis was conducted using the software Statistica for Windows version 7.0 (Statsoft, Inc., Tulsa, USA). The comparison of means was performed using the Tukey's honest significant difference (*HSD*) test at the significance level of ($P < 0.05$).

RESULTS AND DISCUSSION

Soil parameters. The utilized soil was analyzed after the first lysimeter pot experiment and their properties were compared with properties of initial soil. The pH values as well as basic characteristics of the soil are listed in Table 1. As it can be seen, an addition of dolomite limestone changed the pH values of soil. CEC of liming variant increased significantly in comparison with control variant, due to a well known fact, that limestone application increases the cation retention capacity of studied soil.

In comparison with initial concentration of available nutrients and major inorganic anions, their final concentrations were reduced due to both leaching and root uptake, respectively.

Exchangeable metal concentrations of Cd and Zn were reduced from initial 3.72 to 3.03/2.52 mg/kg and from 24.7 to 22.5/8.45 mg/kg, respectively, as a result of: (i) exhaustion unstable metal pool by willows; (ii) dolomite limestone presence; and (iii) higher unstable metal concentration in leachate in comparison with Pb (Singh et al. 1996). Lead exchangeable concentration remained unchanged (Table 1), because Cd and Zn are bioavailable in a much higher portion than Pb in soils (Chung et al. 2005).

Metal leaching. The leaching of metals from soil along with the utilization of treatment is presented both as a concentration in the outflow (Figure 1). Limed variant in the first lysimeter pot experiment showed (i) a significant reduction of cadmium and zinc soil leaching in comparison with control; (ii) lead significant reduction after 2 weeks, due to lead displacement onto carbonate complexes by cadmium and zinc as described by (Tesoriero and Pankow 1996).

Table 1. Chemical characterization of studied soil (initial and final)

	Initial soil	Final soil	
		control	1% liming
pH_{H_2O}	6.75	6.62	7.25
pH_{CaCl_2}	6.38	6.28	6.82
CEC ($mmol_{(+)}$/kg)	57.4	64.2	71.9
TOC (%)	2.75	3.20	2.70
DOC (mg/kg)	1045	632	631
N_{min} (mg/kg)	206	7.09	7.26
Available nutrients (mg/kg)			
Ca	2718	2451	10151
K	118	72.8	47.4
Mg	171	149	179
Major inorganic anions (mg/kg)			
F^-	8.23	5.61	4.04
Cl^-	29.8	13.8	13.9
$(NO_3)^-$	299	3.06	4.06
$(PO_4)^{3-}$	14.5	9.98	2.23
$(SO_4)^{2-}$	107	47.9	6.14
Exchangable metal concentration (mg/kg)			
Cd	3.72 ± 0.12^a	3.03 ± 0.05^b	2.52 ± 0.08^c
Pb	51.1 ± 3.0^a	49.6 ± 1.0^a	41.2 ± 0.2^b
Zn	24.7 ± 0.6^a	22.5 ± 0.3^b	8.45 ± 0.91^c

Data shown are means ± SD ($n = 5$). Data with the same index (in row) represent statistically identical values ($P < 0.05$)

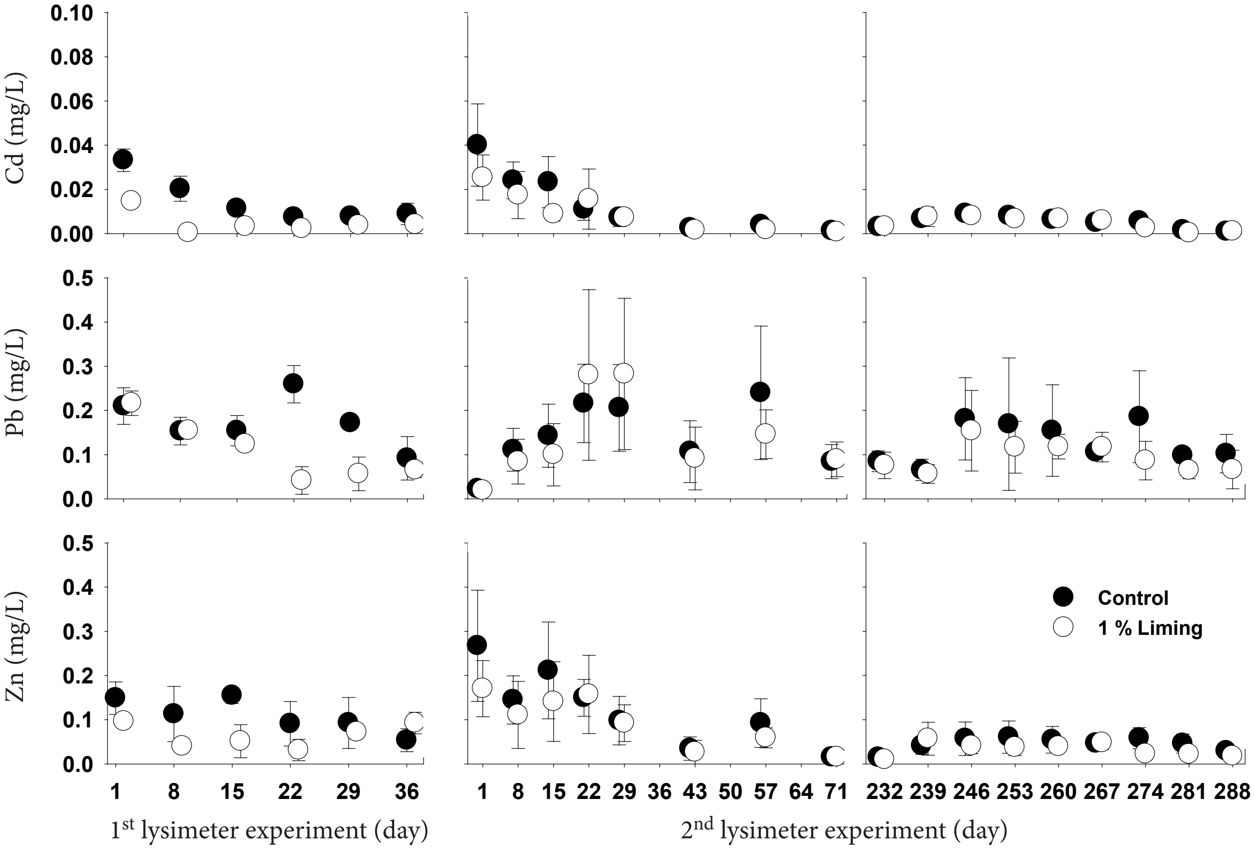

Figure 1. Cadmium, lead, and zinc leaching; the first and the second lysimeter experiment

After two vegetation periods of growth (second lysimeter experiment), the concentrations of Cd and Zn in the leachate decreased as compared to the initial concentrations, while Pb concentrations was not significantly changed, because of its important long-term stability in soil environments (Bataillard et al. 2003). Liming decreased the Cd and Zn concentration significantly compared to the control in first two weeks of experiment, when the metal concentration in leachate was 10-times higher than in the end.

Plant harvesting. A way how to evaluate metal tolerance in plants is to measure the survival rate in heavily toxic substrates or the reduction in growth rate (Lepp et al. 1997). Using this approach in this study, we observed that soil liming differ in willow response to metal contamination in term of growth. As it is apparent from the Table 2, increase in the biomass of plant organs (leaves and stems) in both vegetation periods was observed in limed group in comparison with non-treated clones under metal concentrations tested. At time of both harvests, control willow showed statistically significant reduction of total aboveground biomass (fresh and dry weight) compared to willow on liming soil, as a result of chemical immobilization and hence less metal concentration in biomass planted on liming soil (see below).

Table 2. Ecophysiological characteristics of willow (*S.* × *Smithiana* Willd.)

Aboveground biomass (g/pot)	Group		Statistical significance
	control	1% liming	
FW_{2009}	73.6 ± 8.8	91.5 ± 4.8	0.008
DW_{2009}	28.3 ± 2.6	31.7 ± 1.9	ns
FW_{2010}	132 ± 7	156 ± 4	< 0.001
DW_{2010}	47.9 ± 3.1	59.5 ± 3.5	0.001

FW – fresh weight; DW – dry weight. Data shown are means ± SD (*n* = 5)

Table 3. Metal contents in studied biomass (mg/kg) during two vegetation periods (second lysimeter experiment).

Biomass	Group		Statistical significance
	control	1% liming	
Leaves 2009			
Cd	106 ± 10	100 ± 13	ns
Pb	11.4 ± 1.1	9.48 ± 1.21	0.042
Zn	792 ± 60	688 ± 109	ns
Leaves 2010			
Cd	70.4 ± 19.2	57.3 ± 12.7	ns
Pb	9.14 ± 2.79	6.54 ± 1.29	0.021
Zn	282 ± 55	285 ± 63	ns
Stems 2009			
Cd	32.8 ± 3.6	28.2 ± 2.2	ns
Pb	9.65 ± 0.67	6.86 ± 0.83	0.001
Zn	137 ± 10	106 ± 7	0.001
Stems 2010			
Cd	21.9 ± 3.6	22.2 ± 5.0	ns
Pb	10.1 ± 2.3	5.33 ± 1.07	< 0.001
Zn	82.3 ± 12.0	72.9 ± 13.1	ns
Roots 2010			
Cd	23.7 ± 3.9	21.1 ± 3.2	ns
Pb	371 ± 66	454 ± 48	0.007
Zn	90.7 ± 13.8	90.9 ± 11.7	ns

Data shown are means ± SD (n = 5); ns – not significant

We observed statistically significant increase of root biomass after soil liming; it is in agreement with the fact that fine root growth was stimulated by liming (Bakker et al. 1999).

Metal concentration in biomass. Cadmium and zinc concentrations in the aboveground biomass of the selected willow clone (*S.* × *smithiana* Willd.), harvested during the first vegetation period showed rather high cadmium and zinc allocation into leaves, and 3-time less into stems. On the other hand, during the second harvest Cd and Zn allocation into aboveground organs had 5% reduction (Table 3). This corresponds to the preferential storage of Cd and Zn by willows in shoots and leaves in contaminated soils (Hammer et al. 2003). Dolomite limestone application had a positive effect on chemical immobilization of zinc ($P < 0.05$). Although cadmium levels in plant biomass were not significantly affected, the Cd concentrations tended to decrease, as well. Basta and McGoven (2004) discovered, that limestone treatment reduced Cd and Zn compared to the respective totals for each element eluted from the untreated soil.

Lead, which is a major contaminant, is notorious for its lack of soil mobility; this is primarily due to metal precipitation as insoluble phosphates, carbonates, and (hydr)oxides (Blaylock and Huang 1999). Many plants such as willows accumulate Pb in the roots; 95% of lead is accumulated in its roots (Begonia et al. 1998). However, rates of translocation to the shoots are very low, so lead concentrations in the aboveground biomass (both vegetation periods) was 10 and 7 mg/kg, respectively; in roots it was 370 and 450 mg/kg, respectively (Table 3). In support of this, Blaylock and Huang (1999) concluded that the limiting step for Pb phytoextraction is the long-distance translocation from roots to shoots. Brennan and Shelley (1999) suggest from their results that the key parameters are the precipitation of lead as Pb-phosphate in roots (only Pb not precipitated is available for translocation to the shoots). Liming significantly reduced Pb concentration in the aboveground biomass, which is in agreement with the results of Basta and McGoven (2004) that limestone treatment reduced Pb eluted compared to the respective totals for each species eluted from the untreated soil. We observed significantly higher lead concentration in the willow roots treated with dolomite limestone, because metal concentrations decreased after liming (except Pb solubility), due to chemical and physical bounding of lime (Máthé-Gáspár and Anton 2005).

Nitrogen and free amino acids concentration in biomass. To evaluate whether willows were less stressed by decreasing metal concentrations, and whether the group using treatment was similarly affected in terms of nitrogen use efficiency (NUE), we measured the total nitrogen (N) content and the main amino acid metabolism parameters. The total nitrogen content decreased in control plant leaves (3.02 ± 0.03%), while in biomass after liming was found higher, (3.36 ± 0.02%), after the first vegetation period. These findings suggest interference between metal and nitrogen uptake. Nevertheless, the second vegetation period showed statistically identical values: control (1.76 ± 0.09%) and amended willows (1.82 ± 0.09%). Willows harvested during the second vegetation period showed reduction of the total nitrogen content, as a result of nitrogen exhaustion from soil during the first vegetation period. On the other hand no significant differences between control and liming variant point out that toxic metals were exhausted from soil during the first vegetation period (see above).

The total amino acids content (AA) significantly changed as a result of liming, the average content of total AA reached up to 144 ± 10 μmol/kg FW, in comparison with control plants 128 ± 10 μmol/kg FW. Results suggest that a mechanism by which many plants and algae respond to and apparently detoxify toxic metals is the production of free AA (Mehta and Gaur 1999).

We can summarize that the lysimeter pot monitoring as a new experimental approach showed us metal leaching reduction after liming process. Dolomite limestone application as a process of chemical immobilization caused considerably decreased mobility of Cd, Zn, and as well as Pb. Metal uptake efficiency of willow (*S.* × *smithiana* Willd.) was reduced after application of dolomite limestone, whereas biomass production was significantly enhanced. Cadmium and zinc were taken up and stored in willow stems and leaves, but on the other hand lead was accumulated purely in roots.

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Corresponding author:

Mgr. Lukáš Trakal, Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů,
Kamýcká 129, 165 21 Prague-Suchdol, Česká republika
phone: + 420 224 382 740, e-mail: trakal@af.czu.cz