

# The effect of liming on cadmium, lead, and zinc uptake reduction by spring wheat grown in contaminated soil

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## ABSTRACT

For characterization of the ability of crops to reflect changing soil properties after the addition of ameliorative materials into the soil both pot and rhizobox experiments were provided. In the pot experiment, the influence of the addition of lime and limestone into contaminated Cambisol containing 7.14 mg Cd/kg, 2174 mg Pb/kg, and 270 mg Zn/kg on element availability for spring wheat was tested. The ameliorative materials were added into the pots containing 5 kg of soil in amount of 3 g CaO, and 5.36 g CaCO<sub>3</sub> per kg of the soil. Soil pH reached up to 7.3 in lime treatments compared to 5.7 in control soil. Mobile portion of soil elements (0.01 mol/l CaCl<sub>2</sub> extractable) dropped by 80% for Zn, 50% for Cd, and 20% for Pb, respectively. In both straw and grains of wheat reduced content of elements was observed in limed pots compared to the control ones. For a detailed characterization of the influence of root exudates on the strength of developed complexes in comparison with the bulk soil, short-term rhizobox experiment was set up under identical soil and lime treatments. Generally, the results of rhizobox experiment confirmed the findings from the pot experiment discussed above. Content of elements in shoots and roots of wheat dropped mainly in the case of Cd and Pb. Soil mobile portion of all three tested elements introduced clear depletion curve in control treatment, both limed treatments showed high stability of element complexes almost unaffected by wheat roots.

**Keywords:** liming; lime; limestone; immobilization; contaminated soil; cadmium; lead; zinc; pot experiment; rhizobox experiment

The immobilization of toxic elements in soil *via* alteration of one or more soil characteristics (soil pH, soil sorption complex, content and composition of soil humic substances and content of Fe/Mn oxides) can significantly minimize element uptake by crops planted in soils with elevated heavy metal content and improve quality of food production. Among the possible inorganic soil amendments liming has been applied for long time to increase soil pH and subsequently to decrease metal uptake by plants with respect to soil characteristics and behavior of individual elements in soil (Ciecko et al. 2004, Puschenreiter et al. 2005). A range of liming materials available include limestone (CaCO<sub>3</sub>), burnt lime (CaO), slaked lime [Ca(OH)<sub>2</sub>], dolomite [CaMg(CO<sub>3</sub>)<sub>2</sub>], and slag (CaSiO<sub>3</sub>), varying in their acid-neutralizing capacity of a liming material (Bolan and Duraisamy 2003). A decreasing mobility of elements such as cadmium, copper,

zinc, nickel, and lead in limed soil and effective decrease of their element uptake by several crops was intensively investigated in both pot and field conditions (Hooda et al. 1997, Krebs et al. 1998). Redistribution of zinc from plant-available to less mobile soil fractions was described in limestone treated soils (Davis-Carter and Shuman 1993) suggesting effective immobilization of this element. Relationships between physicochemical soil parameters and Pb mobility in soils were investigated by Aguilar et al. (2004) where CaCO<sub>3</sub> was determined as predominantly responsible for Pb retention in soils. The effects of liming on biological soil characteristics were already described. Liming increases biological activity of soil C leading to enhancement of microbial biomass activity in soil (Persson et al. 1989, Delorme et al. 2001). Changing bacterial diversity in limed soil was investigated by da Silva and Nahas (2002). Predictive models

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of element uptake by selected crops based upon a database of soil characteristics were also derived to optimize liming strategies (Hough et al. 2003). Liming strategies based on detailed long-term investigation of element mobility in soil and their uptake by plants as well as associated soil characteristics such as soil pH, sorption capacity and organic matter content were recommended by Blake and Goulding (2002).

Plant-availability of nutrients as well as potentially risk elements is predominantly driven by soil conditions in rhizosphere. The important role in this process play root exudates consisting of a mixture of organic acids, chelates, vitamins, amino acids, purines, nucleosides, inorganic ions ( $\text{HCO}_3^-$ ,  $\text{OH}^-$ ,  $\text{H}^+$ ), gas molecules ( $\text{CO}_2$ ,  $\text{H}_2$ ), and enzymes (Dakora and Phillips 2002, Schoettelndreier and Falkengren-Grerup 1999). Organic acids, such as malate, citrate and oxalate, have been proposed to be involved in many rhizosphere processes, including nutrient acquisition and metal detoxification, alleviation of anaerobic stress in the roots, mineral weathering and pathogen attraction. Mechanisms of the release of these compounds and their activity in soil (sorption, complex formation, decomposition by soil microflora) represent complex and not fully elucidated process (Jones 1998). In this context the differences among plant species and even among various varieties of spinach were reported (Keller and Romer 2001).

Statistically significant increases in cadmium accumulation of wheat plants grown on cadmium treated soils in the presence of increasing concentration of organic acids suggest the existence of Cd-organic acid interactions in the soil-plant system resulting into the formation of organically bound cadmium which is mobile and plant available (Nigam et al. 2000). Concentration of carboxylic acids and their composition in soil solution depends also on element and its concentration level. Similar conclusions can be inferred from the study of phytosiderophores, complex-forming substances released by the roots. The investigation of the effect of the zinc nutritional status on the rate of phytosiderophore release demonstrated that enhanced synthesis and release of phytosiderophores at deficient Zn supply is involved in Zn efficiency of wheat genotypes. It is suggested that the expression of Zn efficiency mechanism is causally related to phytosiderophore-mediated enhanced mobilization of Zn from sparingly soluble Zn pools and from adsorption sites, both in the rhizosphere and in plants (Cakmak et al. 1996). Shenker et al. (2001) observed increasing mobility of cadmium in soil

caused by fungal siderophore rhizoferrin (produced by *Rhizopus arrhizus*) whereas cadmium uptake by wheat and barley plants was not significantly affected. The influence of plant species and rhizosphere interactions among plant species sharing one pot on mobility and plant-availability of Cd, Zn, Cu, Pb, and Ni was documented by Gove et al. (2002). Plant roots and associated microorganisms can also alter rhizosphere pH via redox-coupled reactions. These various processes involved in root-mediated pH changes in the rhizosphere also depend on environmental constraints, especially nutritional constraints to which plants can respond. Soil pH itself and pH buffering capacity also have a dramatic influence on root-mediated pH changes (Hinsinger et al. 2003). Loosemore et al. (2004) demonstrated that pH decrease followed by mobilization of Zn in tobacco rhizosphere can result in underestimated amounts of exchangeable Zn if measured in bulk soil. Evidently, the evaluation differing between effects of pH alteration on mobility of toxic elements in bulk and rhizosphere soil can be helpful for understanding of behavior of toxic elements in soil-plant system.

In this experiment, the ability of crops to cope with changing soil properties after addition of liming materials into contaminated soil was investigated in both pot and rhizobox experiments. In pot experiment, the influence of the addition of lime and limestone into contaminated Cambisol on element availability for spring wheat was studied. For detailed characterization of the influence of root exudates on strength of developed complexes in comparison with the bulk soil, a short-term rhizobox experiment was set up under identical soil and lime treatments.

## MATERIAL AND METHODS

**Pot experiment.** Contaminated Cambisol containing 7.14 mg Cd/kg, 2174 mg Pb/kg, and 270 mg Zn/kg, characterized by pH 5.7, cation exchange capacity 147 mmol/kg, and content of oxidizable carbon ( $\text{C}_{\text{ox}}$ ) 1.9%, was investigated in the pot experiment. Soil was treated with 0.2 g N, 0.03 g P, and 0.08 g K per kg of the soil. The ameliorative liming materials were added into the pots before sowing in equal Ca amount corresponding with 3 g CaO and 5.36 g  $\text{CaCO}_3$  per kg of the soil. All additives corresponding to individual treatments were thoroughly mixed with the experimental soil and spring wheat cv. Aranka was planted. 5 kg of air-dry soil was used for individual pots and

studied treatments were run in four replications. Determination of available forms of nutrients in the soil after Mehlich III soil extraction procedure (Zbiral 2000) showed sufficient content of cations (62.2 mg K/kg, 68.5 mg Mg/kg, 1916 mg Ca/kg) and low P supply (21.9 mg/kg). The plants were regularly watered by deionized water and soil moisture was kept at 60% of its maximal water holding capacity. Plants were harvested in full maturity and after harvest the above ground biomass was separated to grain and straw, checked for fresh and dry biomass (dried at 60°C), grounded and analyzed.

**Rhizobox experiment.** Wheat plants were cultivated for 60 days in rhizoboxes (Fitz et al. 2003b) in the same soil treated by identical amount of lime, limestone, and nutrients as in the case of pot experiment. The experiment was carried out under controlled conditions at 16°C during the day and 5°C at night. For a determination of soil available metals the rhizosphere soil was cut without freezing into root-parallel sections regarding the distance from plant roots using a specially designed slicing device (Fitz et al. 2003a). Harvested biomass was separated to shoots and roots, dried, and homogenized as mentioned above.

**Analytical methods.** Plant samples were decomposed by dry ashing procedure as follows: An aliquot (~ 1 g) of the dried and powdered plant matter was weighed to 1 mg into a borosilicate glass test-tube and decomposed in a mixture of oxidizing gases ( $O_2 + O_3 + NO_x$ ) at 400°C for 10 hours in Dry Mode Mineralizer Apion (Tessek, Czech Republic). The ash was dissolved in 20 ml of 1.5%  $HNO_3$  (electronic grade purity, Analytika Ltd., Czech Republic) and kept in glass tubes until measurement (Miholová et al. 1993). Aliquots of the certified reference material BCR 281 Rye grass were mineralized under the same conditions for quality assurance of the total element contents in experimental plants. Total element concentrations in soils were determined before vegetation in the digests obtained by two-step decomposition as follows: 0.5 g of sample was decomposed by dry ashing in Apion Dry Mode Mineralizer; the ash was then decomposed in a mixture of  $HNO_3 + HF$ , evaporated to dryness at 160°C and dissolved in diluted *aqua regia* (Száková et al. 1999). For determination of mobile portions of elements in soil samples, aliquots of the fresh soil samples were extracted with 0.01 mol/l aqueous  $CaCl_2$  solution in ratio 1:10 (w/v) for 6 hours (Novozamsky et al. 1993), and with 0.43 mol/l  $CH_3COOH$  in ratio 1:40 (w/v) for 5 hours (Quevauviller et al. 1993).

The mixtures were then centrifuged for 10 min at 3000 rpm and kept at 4°C until measurement. The level of soil pH was determined in 0.01 mol/l aqueous  $CaCl_2$  [1:10 (w/v)] extract, as well. The total contents of cadmium, lead, and zinc in the seeds, straw, roots and shoots of wheat decomposed by dry ashing procedure and in soil extracts were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia). For the determination of calcium, magnesium and potassium in plant digests and soil extracts flame atomic absorption spectroscopy (VARIAN SpectrAA-300) was used.

**Statistics.** The plant yield and total element contents in plants and soil extracts from individual pots were evaluated by ANOVA (Statgraphics 5.1 plus) at the significance level  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

### Pot experiment

Soil pH reached up to 7.3 in lime treatment and to 7.0 in limestone treatment compared to pH 5.7 in the control soil confirming expectable effect of both applied ameliorative materials. Root exudation was not able to significantly change soil pH at limed treatments as well at the control one. The mobile portion of elements (0.01 mol/l  $CaCl_2$  extractable) dropped for Cd by 53% at lime and by 43% at limestone treatment, for Pb by 19% at lime and by 21% at limestone treatment, and for Zn by 78% at lime and by 82% at limestone treatments, respectively, in the bulk soil (Table 1). The significant reduction of mobile zinc and copper pools in compost amended by 5% of lime was also documented by Nissen et al. (2000) but Brown et al. (1997) reported an increased zinc mobility in soils with high rate of lime-stabilised biosolids explained by formation of labile zinc-fulvic acid complexes caused by their incorporation into the soil organic matter. Rate et al. (2004) explained the lower mobility of metals in limed soils by precipitation as hydroxides and carbonates, and increased adsorption to variably charged soil colloids. The mobility of numerous elements in soil solution as well as the plant-availability of these elements was investigated by Tyler and Olsson (2001) in pot experiment with *Agrostis capillaris* in the soil amended by  $CaCO_3$  to pH level in range 5.2–7.8. The concentrations of elements in the soil solution was usually negatively correlated with pH and positively related to plant element contents.

Table 1. The mobile portions of elements (mg/kg) and pH level determined in 0.01 mol/l CaCl<sub>2</sub> extract in soil from pot experiment according to individual soil treatments; the averages marked by the same letter did not significantly differ at  $\alpha = 0.05$  within individual columns

Treatment	Cd	Pb	Zn	pH
Control	0.379 ± 0.028 <sup>a</sup>	0.482 ± 0.043 <sup>a</sup>	0.489 ± 0.146 <sup>a</sup>	5.7 ± 0.1 <sup>a</sup>
Lime	0.178 ± 0.017 <sup>b</sup>	0.390 ± 0.063 <sup>a</sup>	0.108 ± 0.040 <sup>b</sup>	7.3 ± 0.0 <sup>c</sup>
Limestone	0.214 ± 0.011 <sup>b</sup>	0.379 ± 0.150 <sup>a</sup>	0.061 ± 0.009 <sup>b</sup>	7.0 ± 0.0 <sup>b</sup>

The decreased mobility of soil elements resulted in lower element uptake by wheat plants (Table 2). The element contents in wheat straw followed the pattern of mobile portions of elements in soil. However, the higher drop of straw element content was observed for zinc (68 and 64% at lime and limestone treatments, respectively) and for lead (57 and 61% at lime and limestone, respectively) whereas a milder effect was observed for cadmium (35 and 32%, respectively). In the case of wheat grain the decreased mobility of elements in soil suggesting lower phytotoxicity of these elements reflected significantly increased yield (by 28% at limestone, and even by 53% at lime treatment), as confirmed also by Wong et al. (2001) for above ground biomass of *Brassica chinensis*. On the other hand, Nissen et al. (2000) observed decreasing yield of ryegrass (*Lolium perenne*) growing in limed compost containing low levels of toxic elements. Thus, the decrease of element content in grain due to decreased element mobility in soil seems to be combined with the dilution effect of increasing grain yield. However, among the factors affecting the element uptake by wheat grain, the element itself seems to be the dominant one resulting in significant differences in the response of individual elements on liming in this experiment. The decrease of the element accumulation in wheat grain was the highest in the case of lead

(by 90% at lime and 73% at limestone treatment) followed by zinc (by 46, and 36%, respectively) and cadmium (by 39, and 26%, respectively). Nissen et al. (2000) confirmed the reduced removal of Cu and Zn by above-ground biomass of ryegrass by about 35% in soil amended by lime-treated compost. Puschenreiter et al. (2005) reviewed reduced concentrations of Cd and Zn in different crops grown at limed soils (lime rate in range 0.1–1%) from 12 to 56%. Among the number of elements determined by Tyler and Olsson (2001) in *Agrostis capillaris* in dependence of soil pH Cd and Pb were emphasized as closely negatively related to soil pH whereas for zinc no significant correlations were detected for both roots and shoots. The prolonged effect of lime application was observed by Tlustoš et al. (1995) in the case of spinach plants cultivated for four vegetation periods where cadmium and zinc contents in spinach biomass represented 20 and 25% of control treatment, respectively. Evidently, our pot experiment confirmed efficiency of liming for immobilization of Cd, Pb, and Zn in acid soil and limited uptake of these elements by spring wheat suggesting restricted impact of contaminated soil on crop production. Faster solubility of lime compared to limestone led to higher soil pH but the overall effect on element straw and grain accumulation was not significantly different between both treatments.

Table 2. The total contents of elements (mg/kg) and yield of dry biomass (g per pot) in wheat straw and grain samples from pot experiment according to individual soil treatments; the averages marked by the same letter did not significantly differ at  $\alpha = 0.05$  within individual columns

	Treatment	Cd	Pb	Zn	Yield
Straw	control	3.52 ± 0.58 <sup>a</sup>	21.6 ± 6.1 <sup>a</sup>	118 ± 18 <sup>a</sup>	40.5 ± 1.2 <sup>a, b</sup>
	lime	2.28 ± 0.42 <sup>b</sup>	9.30 ± 3.45 <sup>b</sup>	37.5 ± 8.5 <sup>b</sup>	38.1 ± 3.6 <sup>a</sup>
	limestone	2.39 ± 0.54 <sup>b</sup>	8.36 ± 0.54 <sup>b</sup>	42.4 ± 12.8 <sup>b</sup>	47.0 ± 7.6 <sup>b</sup>
Grain	control	2.24 ± 0.08 <sup>a</sup>	1.55 ± 0.58 <sup>a</sup>	86.5 ± 2.6 <sup>a</sup>	19.8 ± 1.0 <sup>a</sup>
	lime	1.36 ± 0.08 <sup>c</sup>	0.380 ± 0.037 <sup>b</sup>	46.7 ± 2.8 <sup>b</sup>	30.3 ± 2.3 <sup>c</sup>
	limestone	1.65 ± 0.22 <sup>b</sup>	0.413 ± 0.038 <sup>b</sup>	55.2 ± 10.6 <sup>b</sup>	25.3 ± 0.7 <sup>b</sup>

## Rhizobox experiment

The response of mobile portions of investigated elements depended on individual elements, soil treatment and extraction agent used for extraction of soil samples. The effects of root exudates on element mobility and pH level in soil is presented as depletion curves in Figures 1–4. Although the excretion of root exudates obviously lead to decreasing pH (Nigam et al. 2000, Loosemore et al. 2004), in our experiment soil pH in control sample decreased from 6.1 to 5.5 with increasing distance from the roots. Schoettelndreier and Falkengren-Grerup (1999) presented increasing rhizosphere pH compared to bulk soil where the increase correlated with the uptake of  $\text{NO}_3^-$  by the plants. Nitrate was also prevailing form of mineral N in our arable cambisol as well, and no significant changes of available and mobile major cations occurred within soil layers. The soil amendments reached the soil pH even higher compared to pot experiment varying in dependence on distance from roots from 7.4 to 7.6 at lime and from 7.2 to 7.3 at limestone treatment. Evidently the root exudates were able to slightly disturb the effect of liming in wheat rhizosphere. The 0.01 mol/l  $\text{CaCl}_2$  soil extract is able to release the element portion correlating with plant-available element pools. A depletion of plant-available potassium in rhizosphere soil was observed only in the control sample whereas in treated samples the potassium content was stable regardless of distance from root surface. Magnesium mobility was predominantly affected by soil treatment, where decreased significantly at limed variants, than by the distance from the roots. A significantly lower availability of all tested toxic elements was also found at treated soils in rhizobox experiment. Clear depletion curves were recognised for Cd and Zn, and in lesser extent for Pb in control soil as illustrated by Figure 2. Significant decrease of all three elements in treated samples in order  $\text{Zn} > \text{Cd} > \text{Pb}$  was comparable to the pot experiment. Short period growing wheat was not able to change metal availability at the distance from root surface at limed treatments. Loosemore et al. (2004) presented a lower concentration of exchangeable (EDTA extractable) Zn in the tobacco rhizosphere than in the control soil. They concluded that the changes of mobile Zn in the rhizosphere result from the competitive dynamics of pH change and Zn uptake induced by roots. The element pools extractable by 0.43 mol/l  $\text{CH}_3\text{COOH}$  can be characterized as exchangeable, relatively weakly bound but exceeding the plant-

available portion of elements. Expectably, the influence of the distance from root surface was less significantly demonstrated for both nutrient and toxic elements (Figures 3 and 4). However, the significant decrease of element mobility was detectable for lead, the element characterized by lowest mobility among the studied elements, and in lower extent for zinc.

While in pot experiment the soil amendments resulted in improved yield of wheat plants, especially grain, in rhizobox experiment the plants introduced limited growth of roots at limed treatment where root yield decreased by 54 and 33%. Shoot yield was unaffected at lime treatment and increased by 21% at limestone treatment similarly to the yield of wheat straw in pot experiment (Table 2). The element contents in roots and shoots of wheat reflected the changes in soil element mobility as in the case of pot experiment and also were not significantly related to the differences among growth parameters of individual variants (Table 3). For nutrients, significant decrease of Mg and Ca contents in roots treated by lime by 24 and 11% and limestone even by 49, and 62%, respectively was observed. In shoots, the Ca and Mg contents increased at lime (by 23, and 14%, respectively) and slightly decreased at limestone treatment (by 8 and 4%, respectively). Opposite results were observed by Tyler and Olsson (2001) in the case of Ca and Mg. For lead and cadmium the root concentrations dropped down by 40 and 24% at lime, and by 71 and 60% at limestone treatment, respectively. Shoot element contents dropped for Cd and Pb by 56 and 52% at lime, and even by 63 and 82% at limestone treatment, respectively. In the case of zinc different pattern contrasting to behavior of this element in soil was found. Root zinc content increased by 39 and 43% and shoot decreased by 57 and 62% at lime and limestone treatments, respectively. Confirmation of specific Zn behavior was limited by low biomass production. Because the differences in biomass yield as affected by individual soil treatments were less compared to the differences in element contents in roots and shoots of wheat, the trends in total element uptake by plants copied predominantly the element contents in plant biomass. Generally, the results of rhizobox experiment confirmed the findings from the pot experiment discussed above. However, with the respect of plant age different response of plant growth and contents of individual elements in plant biomass on lime treatment in pot and rhizobox experiment was occurred.

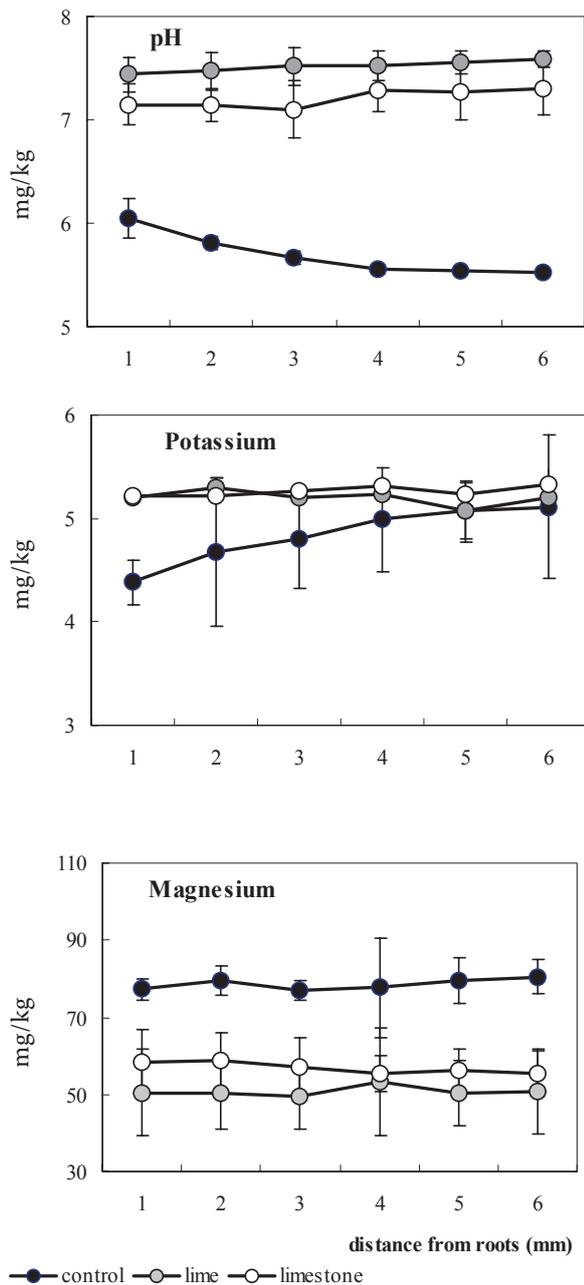


Figure 1. The plant-available portions of potassium and magnesium, and pH levels in rhizosphere soils determined in 0.01 mol/l  $\text{CaCl}_2$  extracts according to distance from wheat roots

The results confirming effective immobilization of Cd, Pb, and Zn in acid soil by liming in both pot and rhizobox experiment cannot be expected under wide scale of soil characteristics, especially soil pH. Bolan and Duraisamy (2003) determined increasing Cd uptake by mustard and sunflower at high level of  $\text{Ca}(\text{OH})_2$  explained as decreased  $\text{Cd}^{2+}$  adsorption resulting from increased  $\text{Ca}^{2+}$  competition. Also, enhanced mobilisation of met-

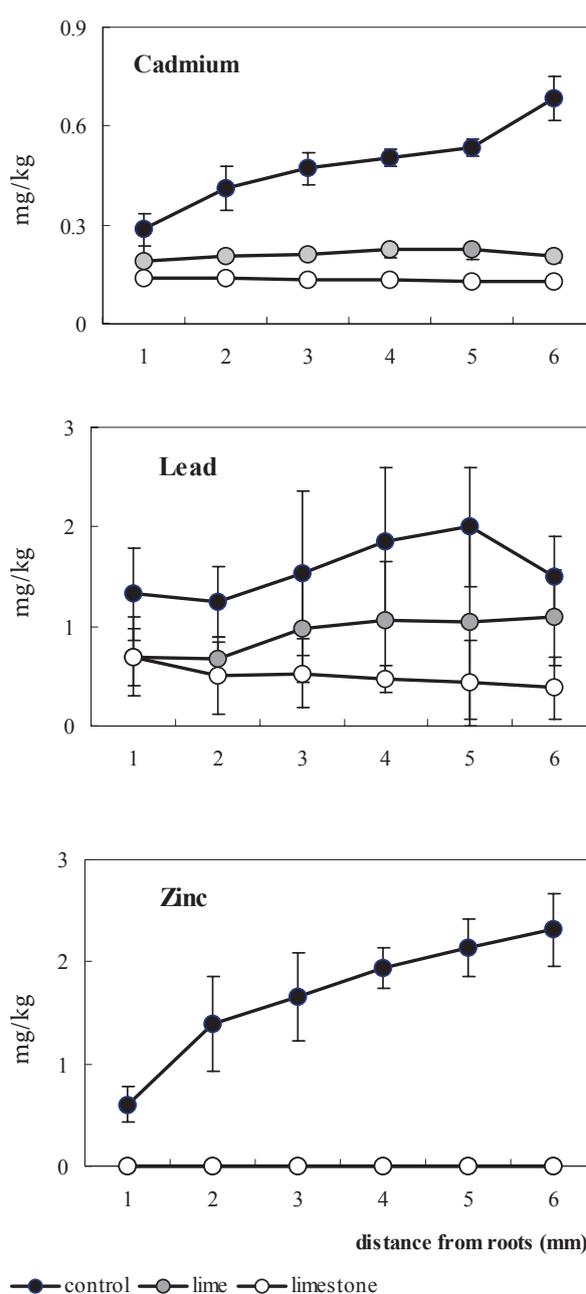


Figure 2. The plant-available portions of cadmium, lead and zinc in rhizosphere soils determined in 0.01 mol/l  $\text{CaCl}_2$  extracts according to distance from wheat roots

als in alkaline soils due to facilitated complexation with humic or organic acids was observed. These authors also summarized the mechanisms of pH-induced immobilisation of metals in soils as follows: i) an increase in pH in soils causes an increase of surface negative charge, resulting in an increase of cation adsorption, ii) an increase of soil pH can lead to formation of hydroxy cations having greater affinity for adsorption sites than just

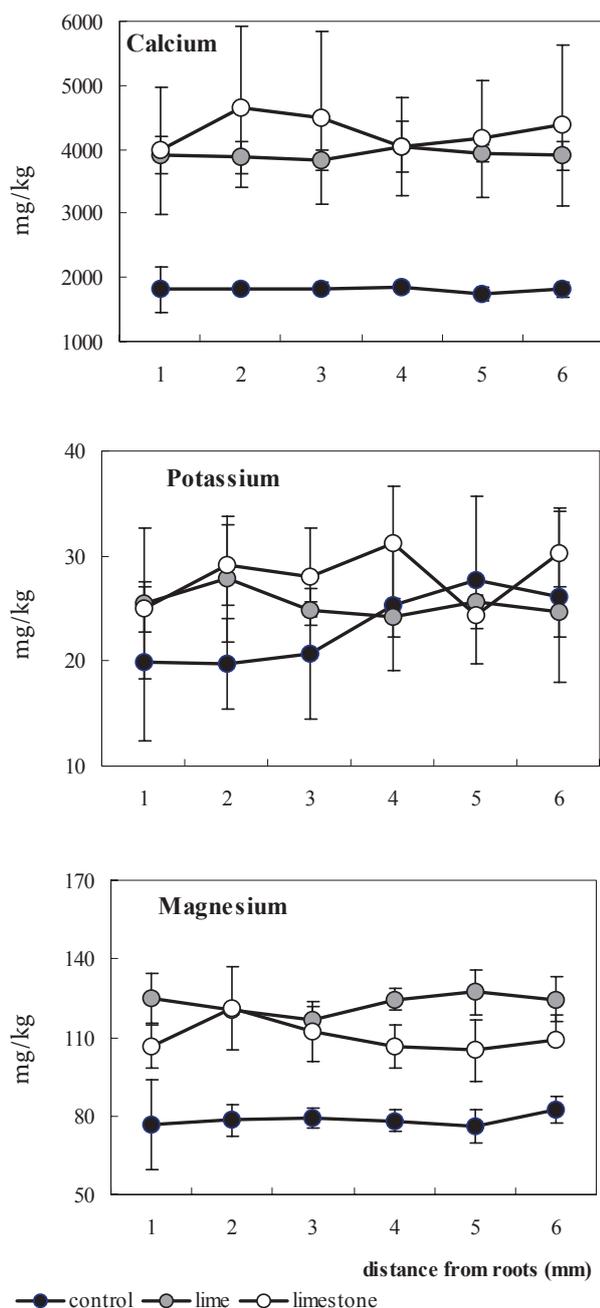


Figure 3. The exchangeable portions of calcium, potassium and magnesium in rhizosphere soils determined in 0.43 mol/l CH<sub>3</sub>COOH extracts according to distance from wheat roots

a metal cation, iii) precipitation of Cd as Cd(OH)<sub>2</sub> and its retention at pH > 10. Bolan et al. (2003) emphasized the importance of relative change of soil pH and Ca<sup>2+</sup> concentration in soil solution on liming effect on mobility and phytoavailability of heavy metals in soil. Thus, the application of liming measures for heavy metal immobilization in soil must be evaluated accordingly to specific soil characteristics at individual experimental

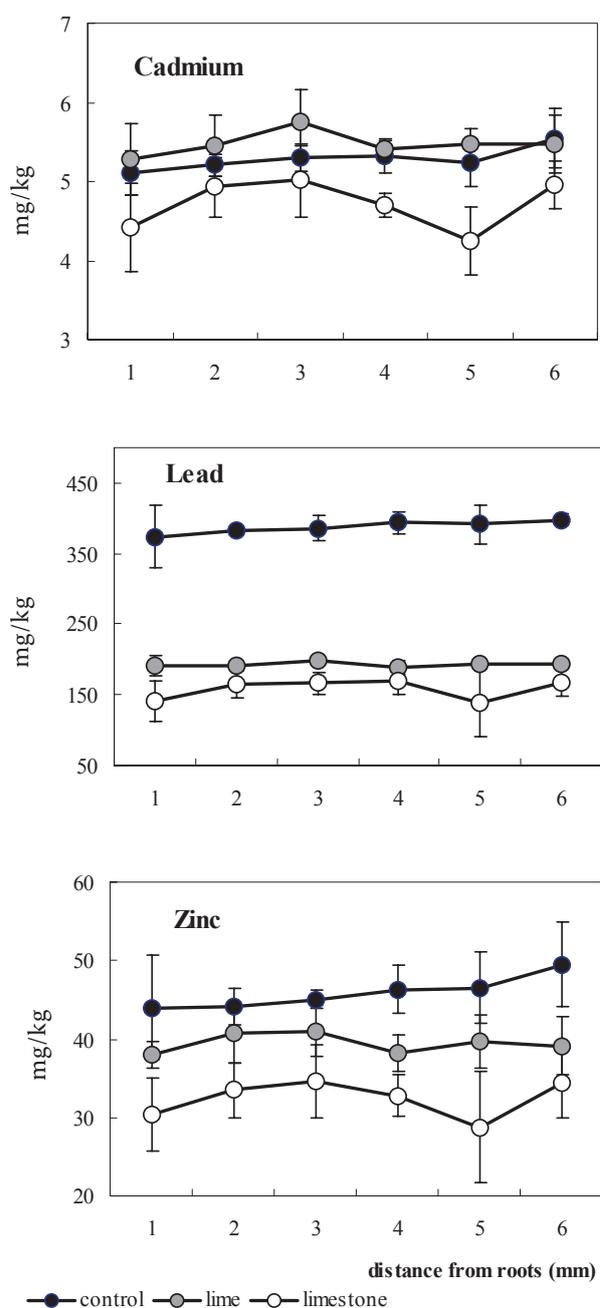


Figure 4. The exchangeable portions of cadmium, lead and zinc in rhizosphere soils determined in 0.43 mol/l CH<sub>3</sub>COOH extracts according to distance from wheat roots

sites. More experimental work will be necessary to complete elucidation of specialities of plant cultivation in rhizobox and more factors such as detailed characterization of root exudates, determination of free element ions in soil solution and determination of physicochemical and biological soil characteristics will be required for interpretation of the results from rhizobox experiments.

Table 3. The total contents of elements (mg/kg) and total yield of dry biomass (g) in wheat shoots and root samples from rhizobox experiment according to individual soil treatments; the averages marked by the same letter did not significantly differ at  $\alpha = 0.05$  within individual columns

	Treatment	Cd	Pb	Zn	Ca	K	Mg	Yield
Shoots	control	3.19 ± 0.40 <sup>b</sup>	6.33 ± 2.42 <sup>b</sup>	65.3 ± 5.4 <sup>b</sup>	9340 ± 170 <sup>a</sup>	5187 ± 184 <sup>b</sup>	2327 ± 143 <sup>a</sup>	1.68 <sup>**</sup>
	lime	1.40 ± 0.38 <sup>a</sup>	3.06 ± 0.84 <sup>a</sup>	27.8 ± 2.5 <sup>a</sup>	11503 ± 566 <sup>b</sup>	4281 ± 37 <sup>a</sup>	2644 ± 61 <sup>b</sup>	1.53 <sup>**</sup>
	limestone	1.19 ± 0.04 <sup>a</sup>	1.15 ± 0.24 <sup>a</sup>	25.0 ± 0.7 <sup>a</sup>	8589 ± 1208	5656 ± 162 <sup>c</sup>	2237 ± 128 <sup>a</sup>	2.03 <sup>**</sup>
Roots	control	14.9 <sup>*</sup>	317 <sup>*</sup>	98.4 <sup>*</sup>	18981 <sup>*</sup>	3154 <sup>*</sup>	1452 <sup>*</sup>	0.531 <sup>**</sup>
	lime	11.4 <sup>*</sup>	189 <sup>*</sup>	137 <sup>*</sup>	16990 <sup>*</sup>	3228 <sup>*</sup>	1108 <sup>*</sup>	0.242 <sup>**</sup>
	limestone	5.89 <sup>*</sup>	90.3 <sup>*</sup>	141 <sup>*</sup>	7213 <sup>*</sup>	3550 <sup>*</sup>	745 <sup>*</sup>	0.356 <sup>**</sup>

\*standard deviation not calculated for roots because of low sample mass

\*\*the samples were mixed together according to individual treatments to obtain representative amounts of samples for analysis

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