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The Role of Chloride Salts in Chemically Enhanced Phytoextraction of Heavy Metals From a Contaminated Agricultural Soil

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Chemically enhanced phytoextraction of heavy metals has proved in several cases to be a perspective and cost-effective method of soil remediation. The main drawback of this method continues to be the low mobility (and thus the bioavailability) of some heavy metals (e.g., lead [Pb]). The use of synthetic chelating agents, such as ethylenediaminetetraacetic acid (EDTA), significantly increased the mobility of heavy metals in soils and their translocation from the roots to the shoots of several plants (Blaylock et al., 1997; Huang et al., 1997). However, because of their high solubility and persistence in soils, chelates can possibly leach down the soil profile. Thus, they pose an important environmental risk for groundwater quality (Römkens et al., 2002). In addition, free EDTA and EDTA-heavy metal complexes are potentially toxic to plants (Vassil et al., 1998).

For these reasons, several alternatives to EDTA have been proposed. Among many, (*S*, *S*)-*N*, *N*'-ethylenediaminedisuccinic acid (EDDS), a biodegradable chelating agent, was introduced to minimize unwanted negative effects associated with the use of EDTA during phytoextraction experiments (Kos and Leštan, 2003). Xiong and Feng (2001) introduced chloride salts as another alternative for enhancing Pb accumulation in *Brassica pekinensis*. Highly soluble chloride salts dissociate in soil solution and thus provide (1) cations capable of exchanging the adsorbed heavy metals and (2) ligands needed to form water-soluble mobile complexes such as $CdCl^+$, $CdCl_3^-$, or $CdCl_4^{2-}$.

Other studies (Li et al., 1994; Smolders et al., 1998) also have proved the positive influence of chloride salts on heavy metal (especially Cd) accumulation in plants. The authors add that the use of chloride salts is preferred over the use of EDTA because of the lower costs and shorter dwell time of the formed soluble complexes in soils. Furthermore, the application of sodium chloride (NaCl) did not negatively influence plant biomass production to the extent that EDTA did (Xiong and Feng, 2001).

However, many authors have focused on soils artificially spiked with a single metal in a form that does not correspond to natural conditions (e.g., $Pb[NO_3]_2$ or $Cd[NO_3]_2$) (e.g., Begonia et al., 2003, 2004; Hovsepyan and Greipsson 2005; Xiong and Feng, 2001). In addition, the application of mobilizing agents to soils contaminated with multiple metals can lead to a reduction in plant biomass yields and the total amount of heavy metals phytoextracted due to the toxicity of other metals present at high concentrations in the soil solution (Chen and Cutright, 2001). The main objectives of this study were (1) to determine the effect of different chloride salt (NH_4Cl) concentrations on the mobility of cadmium (Cd), zinc (Zn), and Pb in a contaminated agricultural soil originating from the contaminated mining and smelting area of Příbram, Czech Republic, (2) to evaluate the phytoextraction efficiency of a hybrid poplar (*Populus nigra* × *Populus maximoviczii*) and maize (*Zea mays*) after the application of different NH_4Cl concentrations, (3) and to compare the effectiveness of NH_4Cl and EDTA in the phytoextraction process.

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Materials and Methods

The area of Příbram, Czech Republic, was chosen as a site severely contaminated by the smelting activities of a Pb smelter that has been in operation longer than 200 years. Samples of an agricultural soil in the area were taken from the arable layer (0–20 cm). The soil is classified as a Gleyic Cambisol. The samples used for soil characteristics determination, heavy metal contents, and incubation experiments were air dried, homogenized, and sieved (2-mm) before analyses. Soil samples used for pot experiments were air dried, homogenized, and passed through a 10-mm stainless sieve.

Total heavy metal (Cd, Zn, Pb) contents in the studied soil were determined using a combination of the dry-ashing procedure (Dry Mode Mineraliser Apion, Tessek, Czech Republic) and HNO_3-HF and *aqua regia* dissolution. The complete procedure is described elsewhere (Száková et al., 2000). The exchangeable and acid extractable fraction (''labile fraction'') of heavy metals was determined from the sequential extraction by Quevauviller (1998). For the evaluation of measurement precision and accuracy, the standard reference material, CRM Light Sandy Soil 7001 (Analytika Prague, Czech Republic), was used.

To assess the effect of different NH_4Cl (analytical grade, Fluka, Germany) concentrations on heavy metal mobility, incubation experiments for a 28-day period were performed at a constant temperature of 25°C. An aliquot part of air-dried soil (50 g) was placed into HNO_3-cleaned 250-mL polyethylene bottles. Next, 15 mL (~60% of the water field capacity) of the chloride solution (10, 20, and 30 mmol/kg of soil) was added to the soil. The control variant comprised 15 mL of deionized water. Water-extractable heavy metals and heavy metal contents in digests were analyzed using ICP-OES (Vista Pro, Varian, Australia).

Pot experiments were conducted in a controlled outdoor vegetation hall. Two hybrid poplar (*Populus nigra* × *Populus maximowiczii*) cuttings were planted and 10 maize (*Zea mays*) seeds sown in each pot containing 5 kg of air-dried and sieved (10 mm) soil. The pots were watered twice per day using only deionized water to maintain approximately 60% of the water holding capacity. The pots containing maize were thinned to four plants per pot after 2 weeks of growth. The applications of NH_4Cl (10, 20, and 30 mmol/kg of soil) and EDTA (3 mmol/kg of soil) were split into three doses to reduce potential phytotoxicity effects. The concentration of 3 mmol EDTA/kg was chosen because higher EDTA concentrations had negative effects on plant biomass production.

The first application was added to the plants 100 days after planting/sowing. The following applications were added after 10 and 20 days. The control treatment was included and consisted of deionized water. Each treatment was conducted in quadruplicates. The above-ground biomass was harvested 130 days after sowing and planting. Poplar leaves and stems were carefully separated. The harvested plant samples were washed carefully in deionized water, dried at 60°C to constant weight, and finely ground before decomposition. Plant samples were decomposed using the dry-ashing procedure (Száková et al., 2000) and analyzed using ICP-OES.

All statistical analyses were performed using analyses of variance (ANOVA) with a consequent Duncan test (software Statistica 6.0, StatSoft). The results were evaluated on the basis of homogeneous groups at a given significance level ($p < 0.05$).

Table 1 Basic physicochemical soil characteristics, total heavy metal contents, and heavy metal contents in the labile soil fraction

pH_{H20}	4.8
pH_{KCl}	3.9
CEC (cmol/kg)	11.3
TOC (%)	1.9
Clay content (%)	6.0
Total heavy metal contents (mg/kg) ($n = 3$)	
Cd	4.86 ± 0.33
Zn	266 ± 31
Pb	1360 ± 10
Heavy metal contents in the exchangeable and acid-extractable fraction (mg/kg) ($n = 3$)	
Cd	2.31 ± 0.08
Zn	35.9 ± 0.001
Pb	73.9 ± 1.5

CEC, cation exchange capacity; TOC, total organic carbon

Results and Discussion

Table 1 summarizes basic physical and chemical soil characteristics together with heavy metal contents. The relatively low pH value probably is attributable to the high contents of the smelter-derived SO_4^{2-} originating from the smelter chimney stack located nearby. Although this agricultural soil is extremely contaminated by Pb, only a small fraction of Pb is present in the ''labile'' fraction (exchangeable and acid extractable) of the soil (5.4%) (Table 1). This suggests that Pb is bound predominantly to the oxidizable (e.g., organic matter and sulphides) and reducible (iron [Fe], manganese [Mn], aluminum [Al] oxides and hydroxides) fraction of the soil (Ettler et al., 2005; Komárek et al., 2006). The amount of ''labile'' Cd is relatively high (up to 47%), suggesting a high mobility and potential bioavailability of Cd in the studied soil. The zinc

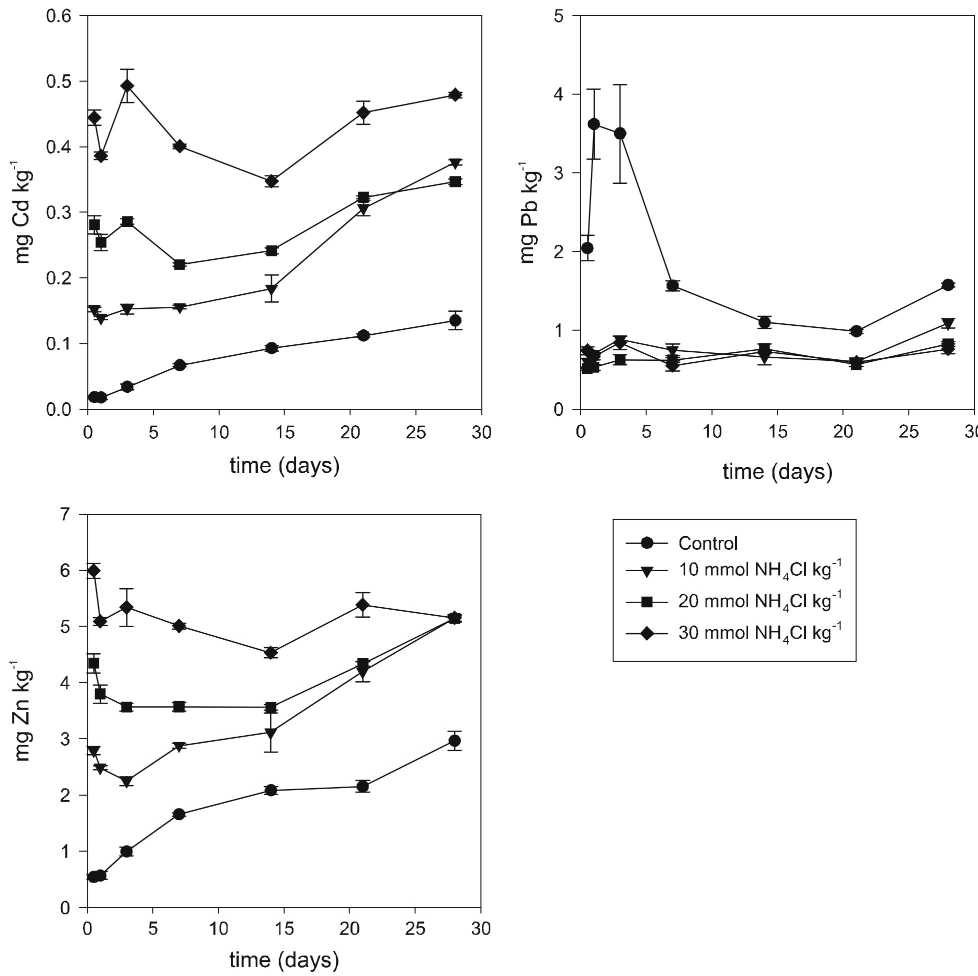

Fig. 1 Time evolution of water-soluble Cd, Zn, and Pb content in the studied soil after the NH_4Cl application. Data shown are means ± standard deviation (*n* = 3)

content in the exchangeable and acid-extractable fraction reaches 14%.

The application of NH_4Cl led to a significant increase in the water-soluble content of Cd and Zn in time, but did not increase the mobility of Pb. Whereas a higher NH_4Cl concentration led to a linear increase in the water-soluble Cd and Zn contents, increasing the NH_4Cl concentration did not have any significant effect on the solubility of Pb (Figure 1). This probably is attributable to the fact that Cl^- ions present in the soil solution form predominantly water-soluble complexes with Cd and Zn despite the higher concentration of Pb in the labile soil fraction.

Additionally, Pb present in smelter-impacted soils is strongly bound to clay minerals, complexed to soil organic matter, and bound to partially dissolved dust particles (containing lowly soluble $PbSO_4$) emitted from the smelter, and is therefore less available (Ettler et al., 2005). The increase of Pb contents in soil solution after the application of chloride salts observed by other authors (Xiong and Feng, 2001) was attributable to the different chemical form of Pb used (easily soluble $Pb[NO_3]_2$) and to a short period allowed by the authors for an equilibrium to be established between the added Pb and the mineral and organic phases of the soil. Therefore, in ''natural'' conditions, the extraction efficiency of NH_4Cl is limited due to the low amount of easily mobilizable Pb. In our experiment, deionized water was a slightly more effective Pb-mobilizing agent than NH_4Cl (Figure 1). We cannot fully explain this phenomenon.

The application of NH_4Cl did not significantly influence the biomass production of poplar plants. No severe phytotoxic symptoms, such as chlorosis or necrosis, were observed on any plant. Low biomass yields were caused not only by high heavy metal contents in the soil, but also to a great extent by the low soil pH (Tables 1 and 2). The highest NH_4Cl concentration (30 mmol/kg) led to a statistically significant decrease in maize biomass compared with the control condition, however. The addition of 3 mmol EDTA/kg did not have any significant effect on plant biomass production, except for the poplar leaves, whose biomass yields were increased by the addition of EDTA (Table 2). This is probably because the soil-applied EDTA helped the poplars acquire nutrients (especially Fe) lacking in the soil.

Table 2 Dry biomass yields and Cd, Zn, and Pb contents in dry biomass after the application of NH_4Cl and EDTA

Biomass (g/pot)	Control	10 mmol NH_4Cl/kg	20 mmol NH_4Cl/kg	30 mmol NH_4Cl/kg	3 mmol EDTA/kg
Poplar stems	$4.2^{a} \pm 1.1$	$3.9^{a} \pm 1.0$	$4.4^{a} \pm 1.4$	$3.6^{a} \pm 0.5$	$3.5^{a} \pm 0.2$
Poplar leaves	$8.1^{a} \pm 1.6$	$7.4^{a} \pm 1.0$	$7.3^{a} \pm 0.7$	$8.8^{a} \pm 1.1$	$9.8^{b} \pm 0.9$
Maize	$21.4^{b,c} \pm 0.8$	$22.5^{b,c} \pm 2.1$	$23.9^{c} \pm 0.9$	$17.8^{a} \pm 1.1$	$20.1^{a,b} \pm 2.1$
mg Cd/kg					
Poplar stems	$13.0^{a,b} \pm 2.0$	$14.1^{b} \pm 0.3$	$14.7^{b,c} \pm 1.8$	$16.5^{c} \pm 2.1$	$11.7^{a} \pm 0.7$
Poplar leaves	$25.2^{a} \pm 5.7$	$25.8^{a} \pm 3.7$	$26.9^{a} \pm 2.8$	$27.1^{a} \pm 3.7$	$27.4^{a} \pm 1.1$
Maize	$7.7^{a,b} \pm 2.0$	$7.3^{a} \pm 1.3$	$8.8^{a,b} \pm 2.0$	$9.5^{b} \pm 2.0$	$9.7^{b} \pm 1.8$
mg Zn/kg					
Poplar stems	$188^{a} \pm 23$	$190^{a} \pm 18$	$223^{b} \pm 33$	$202^{a,b} \pm 11$	$200^{a,b} \pm 12$
Poplar leaves	$181^{a} \pm 27$	$383^{b} \pm 15$	$406^{b} \pm 21$	$407^{b} \pm 12$	$383^{b} \pm 25$
Maize	$186^{a} \pm 29$	$161^{a} \pm 15$	$197^{a} \pm 29$	$202^{a} \pm 37$	$190^{a} \pm 10$
mg Pb/kg					
Poplar stems	$96.5^{a,b} \pm 7.9$	$94.6^{a} \pm 8.3$	$114^{c} \pm 19$	$106^{a,b,c} \pm 9$	$110^{b,c} \pm 4$
Poplar leaves	$33.3^{a} \pm 5.3$	$36.9^{a} \pm 8.0$	$38.3^{a} \pm 4.3$	$33.3^{a} \pm 4.7$	$157^{b} \pm 22$
Maize	$86.1^{a} \pm 15.5$	$84.6^{a} \pm 22.3$	$70.1^{a} \pm 6.7$	$91.2^{a} \pm 25.1$	$202^{b} \pm 43$

Note: Values shown are means ± standard deviation (n = 4). Data with the same index (a, b, c) are statistically similar within separate plants and plant parts

The addition of lower NH_4Cl concentrations (10 and 20 mmol/kg) did not result in a significant increase in Cd accumulation compared with the control condition in either maize or poplar. The only exception was an increase of Cd concentration in poplar twigs after the application of 30 mmol NH_4Cl/kg. Cadmium showed a high stems–leaves translocation rate in all the poplar plants (control and NH_4Cl/EDTA-amended plants), suggesting that both free and Cl-complexed Cd are highly mobile within plants (Table 2).

No statistically significant effect was observed after the addition of EDTA compared with the control condition in the case of Cd accumulation. An intensive Zn translocation from poplar stems to leaves was observed only after the addition of NH_4Cl and EDTA. This probably can be related to (1) the relative lower bioavailability of Zn in the studied soil compared with Cd, (2) the cation exchange between the NH_4^+ from the soil solution and soil-bound Zn^{2+} and the subsequent plant uptake of free Zn^{2+}, and/or (3) an intensive formation and uptake of water-soluble mobile Zn-Cl complexes (such as $ZnCl^+$) and Zn-EDTA complexes (Sarret et al., 2001).

The application of 3 mmol EDTA/kg gave results statistically similar to those of all NH_4Cl applications. In the case of maize, however, no significant effect of either NH_4Cl or EDTA on the uptake of Zn was observed. However, EDTA proved to be a strong Pb-chelating agent, thus increasing the plant uptake of Pb (Table 2). This is due to the high propensity of EDTA (log K = 17.9) to form water-soluble EDTA-Pb complexes. The application of EDTA led to an increased translocation rate within the plants (Epstein et al., 1999). However, NH_4Cl was not efficient in increasing plant Pb contents.

The efficiency of phytoextraction is limited not only by heavy metal contents in plant biomass, but also to a great extent by dry biomass yields obtained during the phytoextraction process. To determine the phytoextraction efficiency of NH_4Cl and to compare it with EDTA, the remediation factor (RF) for each treatment was calculated as shown by Vysloužilová et al. (2003):

$$RF = \frac{M_{plant} \times B_{plant}}{M_{soil} \times W_{soil}} \times 100,$$

where RF is the remediation factor (%), M_{plant} is the content of a heavy metal in the aboveground plant dry biomass (µg/g), B_{plant} is the dry weight of the aboveground plant biomass (g), M_{soil} is the total content of a heavy metal in soil (µg/g), and w_{soil} is the amount of soil in the pot (g). The RF reflects the amount of a heavy metal phytoextracted from the soil during one cropping season. The RFs obtained from the different treatments are summarized in Table 3.

The highest remediation factors were obtained for poplar and Cd (up to 1.27%). The application of 20 and 30 mmol NH_4Cl/kg positively influenced the Cd and Zn phytoextraction efficiency of both plants. In the case of poplars, the increase in NH_4Cl concentration was associated with an increase of RFs for Cd and Zn. Higher biomass yields after the application of 20 mmol NH_4Cl/kg compared with 30 mmol led to higher RFs for maize (Tables 2 and 3). This fact proved the importance of plant biomass yields in the phytoextraction process. Whereas NH_4Cl showed results comparable with those of EDTA for Cd and Zn, EDTA was much more efficient for Pb than

Table 3 Remediation factors (%) of poplar and maize after the application of NH_4Cl and EDTA

RF Cd	Control	10 mmol NH_4Cl/kg	20 mmol NH_4Cl/kg	30 mmol NH_4Cl/kg	3 mmol EDTA/kg
Poplar	1.06	1.01	1.07	1.23	1.27
Maize	0.68	0.68	0.87	0.70	0.80
RF Zn					
Poplar	0.17	0.27	0.30	0.32	0.33
Maize	0.30	0.27	0.35	0.27	0.29
RF Pb					
Poplar	0.01	0.01	0.01	0.01	0.03
Maize	0.03	0.03	0.02	0.02	0.06

NH_4Cl. However, the application of the chloride salt did not increase Pb phytoextraction efficiency (Table 3).

Chloride salts applied to a multimetal contaminated agricultural soil proved to be a successful phytoextraction-enhancing agent only for Zn and poplar (up to a twofold increase in Zn RF compared with the control condition). The calculated RFs showed that the addition of NH_4Cl did not significantly enhance the phytoextraction of Cd, except for 20 mmol/kg applied to maize and 30 mmol/kg applied to poplar. These increases, however, were not great enough to improve the efficiency of the phytoextraction process significantly with regard to the potential leaching risks. In the case of Cd and Zn, EDTA showed results similar to those for NH_4Cl. The RFs suggest that especially poplars could be used in Cd phytoextraction without any addition of mobilizing agents that could lead to unwanted leaching. Chloride salts were not effective at enhancing Pb phytoextraction efficiency. These results are, therefore, contradictory to those of Xiong and Feng (2001), in which equilibrium between the artificially added Pb and the soil constituents could not have been established because of the short time between the Pb addition and Pb phytoextraction. Chelating agents remain a more viable option for chemically enhanced phytoextraction of Pb from contaminated soils. All these facts show that chloride salts are unsuitable for a chemically enhanced phytoextraction of heavy metals from soils contaminated by multiple metals originating from the smelting industry.

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