Effects of Chelating Compounds on Mobilization and Phytoextraction of Copper and Lead in Contaminated Soils

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Six chelating compounds: ethylenediamine-tetraacetic acid (EDTA), ethylenediamine-N,N’-disuccinic acid (EDDS), tartaric acid, citric acid, glycine and histidine, were tested as potential agents to mobilize copper (Cu) and lead (Pb) from two soils polluted with the emissions from copper smelters. Copper was mobilized with the following efficiency: EDTA > citric and tartaric acids > histidine > EDDS and glycine, while Pb extractability followed the order: EDTA > EDDS >> tartaric and citric acid >> glycine and histidine. With respect to these results, EDTA and EDDS were chosen for a pot experiment on chelate-induced phytoextraction of Cu and Pb by maize (Zea mays). Chelates were applied at the rates of 0.2, 0.5, and 1.0 mmol kg\(^{-1}\), and this experiment was carried out at two different watering regimes. Both EDTA and EDDS caused significant increase of Cu uptake from soils, but its concentrations in biomass were far below those required for efficient soil remediation. Lead uptake was only slightly affected by chelate application. Losses of Cu from soil by leaching were much higher than those caused by plant uptake.

Keywords EDDS, EDTA, extraction, leaching, maize, remediation, soil

Introduction

Soil pollution with heavy metals is not a serious problem in Poland; however, in some sites such as surroundings of smelters, soils indicate high concentrations of metals. The areas surrounding copper smelters Legnica and Głogów (so called protection zones) were in the last decades of 20th century strongly contaminated with copper (Cu) and lead (Pb), and, to a lower extent, with several other metals (Karczewska 1996, Szerszen, Chodak, and Kabala 1999). There are sites within those zones, where metal concentrations in soils significantly exceed those defined as soil quality standards. According to Polish environmental law, soil remediation should remove excessive amounts of pollutants, and therefore, effective and environmental-friendly soil cleaning methods are needed.

Phytoextraction is recently considered as a potential cost-effective technology for in-situ remediation of heavy metal-contaminated soils. The literature provides several examples proving that mobility of metals in soil, their subsequent uptake by plants...
and translocation in shoots may be successfully enhanced by addition of synthetic chelates, such as ethylenediamine-tetraacetic acid (EDTA) (Blaylock and Huang 2000; Huang, Chen, and Cunningham 1997). Recent articles stress various disadvantages of chelate-assisted phytoextraction, indicating low metal extraction rates (Kayser et al. 2000; Puschenreiter et al. 2001), long persistence of EDTA in soil, and the risk of groundwater pollution (Lombi et al. 2001; Romkens et al. 2002; Wu et al. 2004; Meers et al. 2005a). Several strategies have already been suggested for increasing the efficiency of metal uptake and reducing the risk of environmental pollution. These effects may be achieved by minimizing the concentration of chelate used, dosage splitting (Fischer and Bipp 2002, Schmidt, 2003), the use of natural, easily biodegradable, compounds such as low molecular weight organic acids (LMWOA) (Lombi et al. 2001; Wu et al. 2003, Kos and Leštan 2004; Nascimento, Amarasiriwardena, and Xing 2006) or ethylenediamine-N, N’-disuccinic acid (EDDS) (Kos and Leštan 2003; Luo, Shen, and Li 2005, Luo et al. 2006; Meers et al. 2005b), improving soil sorption properties by addition of acrylamide hydrogels or vermiculite and apatite mixtures (Kos and Leštan 2003, 2004), application of slow-release coated EDTA granules (Li et al. 2005) or by recirculation of leachates (Madrid, Liphadzi, and Kirkham 2003).

Undoubtedly, the risk of metal leaching depends on soil sorption properties, on the dose of chelates, and on their persistence in the environment. In this article, the authors present the results of a greenhouse study in which the efficiency of induced phytoextraction for cleaning two soils polluted by copper smelters emissions, differing in their textures were evaluated. Copper and Pb phytoextraction by maize (Zea mays) were tested after application of two chelates: EDTA and EDDS. The efficiency of treatment and the risk of metal leaching were related to two various watering regimes.

**Material and Methods**

**Soil Origin and Properties**

Two soils with different textures, containing originally over 1000 mg kg\(^{-1}\) Cu, and 400 mg kg\(^{-1}\) Pb, were collected from two sites situated in the protection zones of copper smelters: Legnica and Głogów. In the preliminary tests of plant growth, performed as a mini-pot experiment, the authors did not obtain sufficient growth of plants and therefore decided to “dilute” high concentrations of metals, by mixing the soils with unpolluted loamy sand in the proportion 2:3, as if the surface soil layer had been ploughed and mixed with subsurface soil, poor in metals (Karczewska 1996). Two soil materials prepared in this way were used in an additional study, presented in this article. Soils L (pre-treated soil from Legnica) and G (from Głogów) contained 600 and 510 mg kg\(^{-1}\) Cu, and 120 and 140 mg kg\(^{-1}\) Pb, respectively. Basic properties of those soils, determined with standard methods (Tan 2005), are presented in Table 1. Total concentrations of Cu and Pb in the samples were measured after acid digestion in the mixture of concentrated nitric and perchloric acids (HNO\(_3\) + HClO\(_4\)).

**Extractability Tests**

In an introductory study, extractability of Cu and Pb by chelating agents was tested in a batch experiment. Various doses of EDTA, EDDS, tartaric acid, citric acid, glycine, and histidine were applied to extract metals from soils. Soil samples were shaken end-over-end (m/v: 1/10) for 6hs with the solution 0.1 mol L\(^{-1}\) calcium nitrate [Ca(NO\(_3\)]\(_2\)]
Effects of Chelates on Cu and Pb Mobilization

Table 1
Properties of soils used in the experiment

<table>
<thead>
<tr>
<th>Soil</th>
<th>Texture</th>
<th>Percentage of grains</th>
<th>C org</th>
<th>CEC</th>
<th>Total Cu</th>
<th>Total Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>Silty loam</td>
<td>&lt;0.02 mm</td>
<td>&lt;0.002 mm</td>
<td>%</td>
<td>pH</td>
<td>cmol(+/-) kg^-1</td>
</tr>
<tr>
<td>G</td>
<td>Sand</td>
<td>15</td>
<td>2</td>
<td>0.73</td>
<td>6.7</td>
<td>6.2</td>
</tr>
</tbody>
</table>

as a background electrolyte and chelating agents at concentrations: 0.5, 2.0, 5.0, 20, and 50 mmol L^{-1}. After filtering the suspensions, the extracts were analysed for Cu and Pb using flame atomic absorption spectrophotometry Philips PU 9100X AAS (Cambridge, UK). All analyses were performed in the Institute of Soil Science and Environmental Protection, University of Environmental and Life Science, Wroclaw.

To determine the effects of multiple application of the same chelate to soil, sequential extraction tests were performed, where extraction was repeated five times with the same extracting solution added at concentration of 2.0 mmol L^{-1}. Cu and Pb concentrations were determined in the extracts, and total amounts of metal extracted from soils in this way were calculated. Results of extractability tests were analyzed to choose two most suitable chelating agents and their concentrations for a pot experiment.

Pot Experiment

The experiment was carried out in a greenhouse in Pawłowice (suburbs of Wroclaw). Each of 112 plastic pots was filled with 5 kg of tested soil, placed on a 5 cm deep bottom gravelly layer, in which leachates were collected throughout the whole experiment. When necessary, the leachates were removed from the bottom zone with manual suction system, and analyzed for Cu and Pb. At the beginning of experiment, soil was moistened and fertilized. Maize (Zea mays L.) was used as experimental plant, as many authors recommend this species for phytoremediation, due to its high biomass production, easily harvested, and with superior capacity for heavy-metal tolerance and accumulation (Huang, Chen, and Cunningham 1997; Li et al. 2005; Luo, Shen, and Li 2005; Meers et al. 2005a).

With respect to the results of extractability tests, the two most efficient chelates, differing in their biodegradability, such as EDTA and EDDS, were chosen for a pot experiment to enhance metal uptake by plants. The chelates were spread onto soil surface at the rates of 0.2, 0.5, and 1.0 mmol kg^{-1} at the stage of plant pre-maturity. Control (0) plots, set separately for soil L and G, did not receive chelates. For rough assessment, the growth of maize (its biomass) on soils L and G was compared with that obtained in an independent parallel experiment carried out with the same maize variety, grown in unpolluted sandy loam soil with optimum conditions of watering and fertilization. This comparison was made to roughly evaluate toxicity effects of metals present in tested soils L and G.

The doses of chelates were split into two parts applied within two days, according to the suggestions from the literature (Fischer and Bipp 2002, Wenzel et al. 2003). After 10 days from first chelate application, plants were harvested, dried, and examined on Cu and Pb concentrations. Thereafter, the experiment was continued with two different watering regimes, simulating “normal” weather with occasional rain and “wet” with repeated
heavy rainfalls. In a wet regime, soil in the pots was leached with distilled water six times, at 2, 5, 14, 28, 50, and 100 days after chelate application, and in a normal watering regime, four times after 14, 28, 50, and 100 days. In between, soil in the pots was kept moisture to enable natural chelate biodegradation. The volume of leaching water was adjusted to the mass of soil in each pot and original water field capacity, and was calculated to obtain 200 ml of leachates in normal regime and 500 ml in wet regime. In fact, the volumes of water necessary to obtain leachates differed considerably among the pots (in the range 100–750 ml per pot), and apparently depended on maize growth, plant transpiration rates, and the features of root system developed by individual plants. Therefore, the volumes of leachates collected from the pots differed considerably, and from technical grounds were impossible to be measured precisely. Presented are only the ranges and mean values of Cu and Pb concentrations in leachates, as well as roughly estimated amounts of Cu and Pb leached.

The pot experiment was carried out in a randomized complete design, in four replicates. All treatments with chelate additions were compared with control (0) plot that did not receive chelates. For each treatment, the mean values, standard deviations (SD), and confidence ranges were calculated at the 0.05 probability level. Significance of differences between the means was checked by least significant difference (LSD) test. Statistical analysis was performed using Excel XP 2003 (Microsoft; Redmond, WA, USA).

Results

Extractability Tests

For both soils, EDTA proved to be the most efficient extractant of Cu and Pb, which was confirmed both in the tests with different dosages (Figure 1) and with sequential extraction (Table 2). In the sequential extractions, Cu was mobilized from both soils with the following efficiency: EDTA > citric and tartaric acids > histidine > EDDS and glycine. Pb extractability differed from that found for Cu, and followed the order: EDTA > EDDS >> tartaric and citric acid >> aminoacids (glycine and histidine). Simple extraction tests gave different orders of extractability, depending on the concentration of chelating solution. At low concentrations, citric and tartaric acids appeared to be much more efficient in mobilizing Cu than were EDDS and aminoacids. At the concentrations higher than 5 mmol L\(^{-1}\), the order of efficiency was different, with relative increase of EDDS efficiency. Aminoacids, such as glycine and histidine, appeared to be almost ineffective in extraction of Pb, and their ability to extract Cu from soils was also relatively low. Application of tartaric and citric acids caused strong acidification of soil, and this might be one of the main mechanisms of metal solubilization. Therefore, the authors decided that a more neutral extractant, like EDDS, would be chosen for the pot experiment rather than any of those that strongly affected the soil pH, such as citric or tartaric acids.

Pot Experiment

A pot experiment was performed with EDDS and EDTA applied in the rates of 0.2, 0.5, and 1.0 mmol kg\(^{-1}\). These rates were lower than those examined in the batch tests so to avoid intensive metal leaching from the upper soil layers, as the chelates were spread on soil surface without soil mixing. Such application would affect mainly the surface layer of soil in the pots, which might result in much more intensive metal mobilization from this layer than it would be expected from batch experiments. Further application of the next chelate rate was considered if there was no metal leaching from soils after the first application rate.
Effects of Chelates on Cu and Pb Mobilization

Figure 1. The amounts of Cu and Pb extracted from soils L and G with various chelating agents: EDTA, EDDS, histidine, glycine, citric acid, and tartaric acid, applied at the rates: 5, 20, 50, 100, and 500 mmol kg\(^{-1}\).

Table 2
Total amounts of metal extracted sequentially (with 5 extracting steps) from soils by various chelating agents applied as 2 mmol L\(^{-1}\) solutions, at soil: solution ratio 4 g: 40 mL. Each single rate of chelating agent corresponds to 20 mmol kg\(^{-1}\).

<table>
<thead>
<tr>
<th>Chelating agent</th>
<th>Soil L Cu</th>
<th>Soil L Pb</th>
<th>Soil G Cu</th>
<th>Soil G Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>422</td>
<td>98.0</td>
<td>450</td>
<td>94.8</td>
</tr>
<tr>
<td>EDDS</td>
<td>218</td>
<td>44.8</td>
<td>210</td>
<td>23.5</td>
</tr>
<tr>
<td>Histidine</td>
<td>283</td>
<td>2.0</td>
<td>294</td>
<td>2.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>181</td>
<td>5.0</td>
<td>216</td>
<td>7.7</td>
</tr>
<tr>
<td>Citric acid</td>
<td>291</td>
<td>9.4</td>
<td>328</td>
<td>14.4</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>271</td>
<td>10.3</td>
<td>336</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Maize Growth
The growth of maize was assessed as satisfactory, although poorer than in a parallel experiment carried out with unpolluted soil. The mean biomass of shoots in the pots with soil L was assessed as 26% lower, and with soil G as 48% lower, in comparison with the same variety of maize grown in unpolluted soil with optimum conditions of watering and fertilization (where the mean biomass was 62.0 grams dry matter (g d.m.) per pot).
Biomass of maize in the pot experiment. Presented are minimum and maximum values of 4 replicates (min and max) as well as calculated means and standard deviation values SD.

<table>
<thead>
<tr>
<th>Chelating agent</th>
<th>Soil L</th>
<th></th>
<th></th>
<th></th>
<th>Soil G</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>min</td>
<td>max</td>
<td>mean</td>
<td>SD</td>
<td>N</td>
<td>min</td>
<td>max</td>
</tr>
<tr>
<td>O (control)</td>
<td>8</td>
<td>36.2</td>
<td>58.9</td>
<td>46.0</td>
<td>8.2</td>
<td>8</td>
<td>25.1</td>
<td>36.9</td>
</tr>
<tr>
<td>EDTA</td>
<td>24</td>
<td>40.6</td>
<td>54.1</td>
<td>46.5</td>
<td>9.7</td>
<td>24</td>
<td>27.6</td>
<td>35.0</td>
</tr>
<tr>
<td>EDDS</td>
<td>24</td>
<td>37.8</td>
<td>53.0</td>
<td>44.9</td>
<td>12.8</td>
<td>24</td>
<td>19.6</td>
<td>29.7</td>
</tr>
</tbody>
</table>

In the case of plants grown in soil L, the mean biomass of shoots was 45.8 g d.m. per pot, and did not depend on the rate or kind of chelating agent applied. For soil G, the mean biomass of plants in the plots with EDDS (26.4 g d.m. per pot) was lower than in control plots (0 plots) and with EDTA (31.8 g d.m. per pot), but the difference was insignificant at \( P < 0.05 \) (Table 3). Throughout the experiment, the plants indicated some typical symptoms of Cu toxicity (Reichman 2002), such as interveinal foliar chlorosis and white lesions (Figure 2). These effects were much stronger in plants grown on soil G, and became particularly intensive after application of chelating agents, especially EDDS, at the highest dose. Some leaves of plants started to wilt one week after application of chelating solution.

**Metal Uptake**

Both chelating agents resulted in enhanced metal uptake in comparison with control plots (Figure 3). The mean concentrations of Cu and Pb in the shoots increased with increasing rate of chelating agents, which was particularly well expressed in the case of Cu uptake from soil G. The increase of metal concentrations in maize shoots, as compared...
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Figure 3. Cu and Pb concentrations in the maize shoots. The symbols: 0, 1, 2, and 3 stand for various rates of EDTA and EDDS applied to soil: 0, 0.2, 0.5, and 1.0 mmol kg\(^{-1}\). Illustrated are the mean values of 4 replicates; the error bars indicate confidence intervals (at \(P < 0.05\)).

to control plots, was significant in the case of higher doses of chelating agents (0.5 and 1.0 mmol kg\(^{-1}\)). Although EDTA was more effective in solubilization of metals in soil, EDDS appeared much more efficient in inducing Cu phytoextraction and accumulation of this metal in plant shoots. Similar results were described by other authors (Santos et al. 2006). The highest concentrations of Cu in plant shoots (96.1 mg kg\(^{-1}\) d.m.) were found in the plots with soil G and the highest rate of EDDS (1.0 mmol kg\(^{-1}\)). In the case of that particular soil (G), with sandy texture and low water retention capacity, the uptake of Cu after EDDS application, depended apparently on watering regime. Cu concentrations in plant shoots in EDDS plots with normal watering were significantly higher than in the plots with wet watering regime. This effect was observed for all rates of EDDS applied (see Figure 3). Similar effects, however, were not observed for EDTA. In the plots with loamy soil L, any significant effect of watering regime on Cu uptake by maize was not observed, either after application of EDTA or EDDS.

The highest concentrations of Cu in the plants grown on the soil L, with a mean value of 38.1 mmol kg\(^{-1}\) d.m., were obtained in the plots with the highest EDDS rate in wet watering scheme. In spite of the fact that Cu uptake and translocation to maize shoots increased considerably after chelate application, particularly with EDDS, the concentrations of Cu in biomass were still far from those required for efficient soil remediation, and apparently insufficient to obtain reasonable phytoextraction rates. The data on calculated reduction in metal concentrations in soils due to plant uptake are presented in the Table 4. The highest reduction of total Cu, obtained in the case of soil G with the EDDS at normal watering, remained as low as 0.51 mmol kg\(^{-1}\), for example below 0.1 % of original Cu concentration in soil. Much higher reduction in Cu concentration resulted from soil leaching, which will be further discussed in this article.

In all treatments, the concentrations of Pb in dry plant biomass were very low in comparison with Cu or with the data reported in the literature (Huang et al. 1997), and remained below 8.0 and 6.0 mmol kg\(^{-1}\) d.m. for soil G and L, respectively. The experiment did not confirm high ability of either EDTA or EDDS, applied in reasonably low rates, to enhance Pb phytoextraction from soils polluted by the emissions from copper smelters.
Table 4

Estimated decrease in soil concentration due to metal uptake by plants in the pot experiment, in control plots (0) and at maximum rates, for example, 1.0 mmol kg\(^{-1}\), of chelating agents (EDTA 3, EDDS 3)

<table>
<thead>
<tr>
<th>Chelating agent</th>
<th>Watering regime</th>
<th>Soil L</th>
<th>Soil G</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>normal</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>wet</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>EDTA 3</td>
<td>normal</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>wet</td>
<td>0.18</td>
<td>0.22</td>
</tr>
<tr>
<td>EDDS 3</td>
<td>normal</td>
<td>0.19</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>wet</td>
<td>0.34</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Risk of Metal Leaching

It was not possible to control and precisely measure the volume of leachates, and therefore only a rough estimation was made to assess total metal leaching from the pots. Mean concentrations of Cu and Pb in leachates, collected six times in wet regime, and four times in the normal watering scheme, are presented in the Table 5. For both soils and both chelating agents examined, the same general tendency was observed concerning metal concentrations in leachates. In wet watering scheme, Cu concentrations tended to increase slightly with time, and high differences occurred between replicates. In normal watering scheme,

Table 5

Concentrations in leachates and estimated loss of metals from soils by leaching in the pot experiment carried out with the lowest rates: 0.2 mmol kg\(^{-1}\) of chelators (EDTA 1, EDDS 1), and with their maximum rates: 1.0 mmol kg\(^{-1}\) (EDTA 3, EDDS 3)

<table>
<thead>
<tr>
<th>Chelator and rate</th>
<th>Water regime</th>
<th>Soil L</th>
<th>Soil G</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA 1</td>
<td>normal</td>
<td>24.4</td>
<td>21.3</td>
</tr>
<tr>
<td></td>
<td>wet</td>
<td>17.5</td>
<td>22.1</td>
</tr>
<tr>
<td>EDDS 1</td>
<td>normal</td>
<td>19.8</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>wet</td>
<td>18.0</td>
<td>13.4</td>
</tr>
<tr>
<td>EDTA 3</td>
<td>normal</td>
<td>101.2</td>
<td>118.4</td>
</tr>
<tr>
<td></td>
<td>wet</td>
<td>85.2</td>
<td>61.8</td>
</tr>
<tr>
<td>EDDS 3</td>
<td>normal</td>
<td>61.5</td>
<td>123.4</td>
</tr>
<tr>
<td></td>
<td>wet</td>
<td>92.2</td>
<td>104.4</td>
</tr>
</tbody>
</table>

Mean concentrations of metals in leachates, mg L\(^{-1}\)

Estimated loss from soil, mg kg\(^{-1}\)
Cu concentrations in the first leachates (collected 14 days after chelate application), were relatively low—much lower than in a wet scheme—but increased drastically in leachates collected after 28, 50, and 100 days, and exceeded 100 mg L\(^{-1}\) at higher rates of both chelating agents. Mean concentrations of Cu were higher in leachates from soil G than those from soil L. Estimated losses of Cu from soils caused by mechanism of leaching were in the wet plots higher than in normal watering plots, and in the case of the maximum EDDS rate were calculated as 31.2 mg kg\(^{-1}\) Cu lost from soil G and 27.7 mg kg\(^{-1}\) from soil L.

The concentrations of Pb in leachates were much lower than those of Cu, and in most pots remained below 1 mg L\(^{-1}\), with the exception of the highest EDTA rate, when they reached the value of 5 mg L\(^{-1}\). Maximum loss of Pb from soils due to leaching was estimated as 1.5 g kg\(^{-1}\) (Table 5).

**Discussion and Conclusions**

The EDDS proved to be more effective in inducing Cu uptake by maize than was EDTA. Apparently higher efficiency of Cu uptake by plants was obtained for the sandy soil (G) than for the silty loam (L). However, the concentrations of metals in maize shoots still remained much below those expected for successful phytoextraction. From the experimental data, the authors calculated that the amounts of metals leached from soils were much higher than those removed by plant uptake (Tables 4 and 5). This effect referred both to sandy soil G and to silty loam L. The mechanism of Cu leaching was, therefore, decisive in Cu removal from both soils.

The amounts of Cu leached from the sandy soil (G) were much higher than those leached from silty loam (L). Not surprisingly, those amounts were particularly high in wet weather conditions, simulated in this study by a wet watering scheme. Long lasting increase of metal solubility in both EDDS- and EDTA-treated soils indicated that chelated metal complexes remained persistent in soil environment, and that their leaching should be considered as an unavoidable effect of the treatment. Similar observations were also reported by other authors, such as Meers et al. (2005b). It seems very likely that even potentially biodegradable EDDS remained in soils in the form of stable complexes with toxic metals that appeared to be relatively resistant and not easily decomposed.

Further research on induced phytoextraction as a remediation method for soils polluted by the emissions from copper smelters should be continued, with focus on looking for other chelating agents and testing their optimum application rates. In particular, other easily biodegradable chelates, including aminoacids: glycine and histidine.

Notwithstanding several bibliographical reports recommending induced phytoextraction as a method for cleaning heavy-metal polluted soils, this study disproves the possibility of its successful and safe application for soils polluted by the emissions from copper smelters Głogów and Legnica.

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References


