The Effects of Plants on the Mobilization of Cu and Zn in Soil Columns

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Whereas metal effects on plants have been well studied, much less is known about plant effects on metal mobilization in soils. We investigated metal mobilization and speciation with a resolution of a few cm in soil columns planted with willows (Salix viminalis). In the presence of plants, the concentration profiles of dissolved organic carbon (DOC), major ions (e.g., Ca), Cu, and Zn exhibited more variation than in the absence of plants, and both smaller and larger concentrations were observed, indicative of strong local effects. The Cu concentration in the absence and presence of plants was controlled by DOC mobilization and could be described by the same relationship. Zn mobilization was controlled by DOC and Ca in the plant-free system and by pH and Ca in the presence of plants. Cu²⁺ and labile Zn were reasonably well predicted by the NICA-Donnan model and were influenced by the plant-induced changes of the soil solution composition. Plant uptake reduced the dissolved Zn concentration and transpiration reduced Cu and Zn leaching.

1. Introduction

Plant uptake and toxicity of trace metals have received much attention in the past (1). Comparatively few studies have studied the influence of plants on metal behavior and fate in soils. Roots are known to have a strong influence on the composition of the rhizosphere soil solution. This rhizosphere effect depends on plant species and soil factors, including root density and developmental stage (2) and the sorption capacity of the soil.

Plants affect the composition of the rhizosphere solution directly and indirectly. Direct changes are caused by the extraction of water and nutrients and by the exudation of protons, inorganic ions, and organic compounds. Complexation by root exudates may increase or decrease metal mobility, depending on the solubility of the complex (3). Organic acids such as citrate and oxalate, which are exuded by roots (3) and secreted by microorganisms (4), are able to form mobile complexes with metals such as Cu and Zn (5). Release of protons also acidifies the rhizosphere solution directly. Furthermore, root uptake can increase or decrease metal concentrations indirectly by uptake of competing ions in the rhizosphere (6). The extraction of soil water by roots not only leads to an equivalent reduction in the water flow and thus in solutes, but also to an increase in the concentrations of all solutes that are not taken up to the same extent. Other physical effects of roots on metal mobility consist in the formation of macropores that can act as preferential flow paths (7). Through biotic processes, effects on metal solubility may occur such as the stimulation of microbial activity by root exudates and root litter (8).

The main soil solution factors governing the mobility of metals are generally pH, dissolved organic carbon (DOC), and Ca (9, 10). For example, an increase in DOC and pH and a decrease in Ca concentration due to the root activity of a copper-tolerant grass reduced both free and dissolved Cu concentrations (11).

In pot experiments, plants have been found to increase the mobilization of metals (10, 12, 13). In column and field lysimeter experiments, in which effluents were collected either at the bottom (14, 15) or sampled by means of suction cups at various depths (16, 17), plants were found to cause both mobilizing and immobilizing effects. These were dependent on the plant species, the investigated metals, the soil conditions, and the experimental setup (11, 18).

The aim of this study was to investigate the influence of plants on the mobilization of Cu and Zn in a contaminated topsoil and the leaching of these metals into uncontaminated root-free subsoil. We performed column experiments under steady-state flow conditions with and without willow plants. Willow is a fast growing plant and known to take up high levels of metals (19). To observe the influence of the roots on the composition of the soil solution, solution samplers were installed at vertical distances of a few centimeters. We were specifically interested in the relationship between the mobilization of metals, DOC, organic acids, and major nutrient ions in the soil solution. Our hypotheses were (i) that plants increase total dissolved Cu, but decrease free Cu ion concentrations due to DOC mobilization and (ii) that they also decrease total and free Zn concentrations due to uptake, reduced concentrations of competing Ca (by plant uptake), and complexation of labile Zn by increased DOC.

2. Materials and Methods

2.1. Soils. The soil materials were sampled in 2004 from a lysimeter experiment that had been used to study the effects of metal pollution on young forest ecosystems (details in ref 20). The topsoil, a weak acid loam originating from an agricultural field (Luvisol), was pretreated with filter dust from a non-ferrous metal smelter containing Cu, Zn, Cd, and Pb in 2000 (details in refs 20 and 21) and built into the lysimeters. For our study, the polluted topsoil was mixed with unpolluted topsoil at a ratio of 1:1 to lower the metal concentrations. An acidic loamy sand subsoil from a Haplic Alisol and a calcareous sandy loam subsoil from a Calcaric Fluvisol were used as subsoils, originating from the same lysimeter study (20, 21). The soil properties are given in Table S1 (Supporting Information). The soils were sieved through 2 mm and kept moist at a temperature of 15 °C before use.

2.2. Column Setup. The column setup is shown in Figure S1 (Supporting Information). Four PVC columns of 62 cm length and 20 cm diameter were filled with 2 cm quartz sand (grain size <0.5 mm) at the bottom, 35 cm subsoil (two columns with calcareous and two with acidic subsoil), 23 cm polluted topsoil, and another 2 cm quartz sand. Topsoil and subsoil were separated by a polyamide mesh (mesh size 60 µm) fixed by tape and glue to the column wall. A 100 cm long suction cup (Rhizosphere Research Products, The Netherlands) was installed in a spiral at 59 cm depth in the sand layer to continuously collect the leachate. A PVC plate with
holes (diameter 6 mm) covered by a polyamide mesh (mesh size 200 μm) sealed the column bottoms. An irrigation tube (diameter 6 mm) made of polyethylene with holes every cm was placed on the surface of the sand layer in two circles (diameters of 8 and 15 cm). Fifty 50 cm-long suction cups (Rhizosphere Research Products, The Netherlands) were installed during the filling of the columns at 4, 10, 16, 18, 20, 22, 24, 26, 28, 30, 33, 38, 44, and 50 cm depth. A depth of 0 cm refers to the topsoil surface.

The columns were flushed with 640 mm reverse-osmosis water. They were placed on balances in a climate chamber (diameters of 8 and 15 cm). Fifteen 50 cm-long suction cups was placed on the surface of the sand layer in two circles (diameter 6 mm) covered by a polyamide mesh (mesh size 120 μm). The average concentration of Cu was 38 mg/kg in the topsoil or between the presence and absence of plants. Some roots grew into the surface sand layer and some also penetrated into the uppermost part of calcareous subsoil through a hole in the separating mesh. The average concentration of Zn in shoots was 2468 mg/kg in shoots and 1905 mg/kg in roots. The average dry weights were 20.1 g for shoots and 1.1 g for roots. Total metal uptake by the plants was 26.4 μmol Cu and 790.7 μmol Zn.

A 1 mL subsample was stabilized immediately after sampling with 30 μL 37% formaldehyde (Merck, for analysis). The organic acids citrate, malate, and oxalate and inorganic anions (bromide, chloride, NO3-, PO43-, and SO42-) were measured by ion chromatography ( Dionex DX-320). The detection limits for citrate, malate, and oxalate were 2 μM. The recovery was 92 ± 6.8% for the addition of 1.0–5.0 μM organic acids to a soil solution sample. The pH was measured by a Metrohm 713 pH meter. DOC was measured within 2 days after sampling using a total organic carbon analyzer (Shimadzu TOC-5000, Tokyo, Japan).

Total metals were measured by X-ray fluorescence (SPECTRO X-LAB 2000, Spectro, Germany). Metal concentrations in roots, shoots and sticks were measured with AAS after microwave digestion of plant tissue with 65% HNO3 and 30% H2O2.

2.5. Modeling. Cu2+ and labile Zn were calculated using the NICA-Donnan model in ECOSAT (Version 4.8). Parameter values for metal-ion binding by humic substances were taken from Milne et al. (24). The input of DOM was assumed to be twice the DOC concentration (estimated C content of DOM is 0.5) and to be composed of 40% humic acid, 40% fulvic acid, and 20% inert organic matter. With this distribution, optimal prediction for both Zn and Cu speciation was obtained. Alkalinity was calculated by the atmospheric CO2 pressure. For pH, total cations (Ca, Cu, K, Mg, Na, and Zn) and anions (Cl−, NO3-, PO43-, and SO42−), the measured values were used in the calculations. Labile Zn was calculated as the sum of Zn2+ and the weak inorganic Zn complexes with Cl−, CO32−, NO3-, SO42−, and OH−.

3. Results

3.1. Plants and Water Content. The root density at the end of the experiment was highest below the top layer of sand and above the topsoil–subsoil boundary at 15–22 cm (Figure S2, Supporting Information). Some roots grew into the surface sand layer and some also penetrated into the uppermost part of calcareous subsoil through a hole in the separating mesh. The average concentration of Cu was 38 mg/kg in shoots and 798 mg/kg in roots, while the Zn concentration was 2468 mg/kg in shoots and 1905 mg/kg in roots. The average dry weights were 20.1 g for shoots and 1.1 g for roots. Total metal uptake by the plants was 26.4 μmol Cu and 790.7 μmol Zn.

The average water content in the topsoil was 20.2 g/kg in the plant-free columns and 16.8 g/kg in the planted columns, 13.3 g/kg in the calcareous subsoil and 14.0 g/kg in the acidic subsoil. There was no difference in water content between the presence and absence of plants in subsoils (Figure S3, Supporting Information). The average discharge rates were 75 mL/day for 8 mm/day irrigation and 140 mL/day for 10 mm/day for the plant-free columns. For the planted columns, the average discharge rates dropped to zero during the same irrigation as the plant-free columns, recovered to 140 mL/day after 30 mm/day irrigation for a week and remained at 140 mL/day for 20 mm/day.

3.2. Soil Solution Depth Profiles. Figure 1 shows the profiles of pH, Ca, NO3−, DOC, Cu, and Zn in the soil solution over depth after 55 days of plant growth. The concentrations were much higher in the soil solution than in the irrigation water (150, 60, 30, and 32 times for Ca, NO3−, SO42−, and Mg, respectively). With increasing depth pH slightly decreased, while the concentration of DOC, metals and all other solutes increased. Across the topsoil–subsoil boundary, the composition of the soil solution changed abruptly. In particular, Zn and Cu concentrations dropped sharply within 2 cm. The concentrations of DOC, Ca, and NO3− did not show such abrupt changes as the metals and pH. Below the topsoil–subsoil boundary, Ca and NO3− increased with depth more
in the calcareous than in the acidic subsoil, particularly in the columns with plants.

The columns with plants showed similar concentration profiles as unplanted columns, but more scatter and deviations from the general pattern. Ca and NO$_3^-$ were generally lower in the presence of plants, whereas DOC was higher at a given depth and for a given soil type. NO$_3^-$ and DOC exhibited a prominent peak at a depth between 13 and 20 cm in the column with calcareous subsoil, while DOC decreased slightly at the same depth in the column with acidic subsoil. The Mg concentration profile was similar to that of Ca and the SO$_4^{2-}$ profile similar to that of NO$_3^-$ (Figure S4, Supporting Information).

Cu and DOC concentration profiles showed striking similarities. A similar peak as in DOC also occurred in the Cu concentration profile in the column with calcareous subsoil at 13–22 cm and a decrease at the same depth in the column with acidic subsoil. The Mg concentration profile was similar to that of Ca and the SO$_4^{2-}$ profile similar to that of NO$_3^-$ (Figure S4, Supporting Information).

In the presence of plants, DOC, dissolved Ca and NO$_3^-$ reached more than twice the concentration as without plants in the calcareous subsoil (Figure 1). In contrast, the absence or presence of plants appeared to have little effect on the concentrations of all solutes in the acidic subsoil.

Increasing the irrigation rates in the planted columns after day 55 had little effect on the composition of the topsoil solution (Figure S5, Supporting Information). DOC still exhibited a peak in the column with calcareous subsoil and a decrease in the column with acidic subsoil. Cu showed a behavior corresponding to DOC. Dissolved Zn was lower in the calcareous subsoil column with plants than without plants and also showed a peak at 13–20 cm depth. The concentrations of all parameters in the subsoil were similar in the presence and absence of plants.

No measurable concentrations of oxalate, citrate, and malate were ever detected except for two samples taken at 18 and 20 cm in the column with calcareous subsoil on day 55 after planting. In these samples, malate and oxalate concentrations were 0.217 and 0.201 mM at 18 cm and 0.163 and 0.185 mM at 20 cm depth, respectively. Coinciding with the DOC peaks mentioned before, the two acids thus made up 45 and 51% of the total DOC at these locations. In the next two samplings, at 68 and 75 days after planting, the two acids were no longer detected despite the presence of a high DOC peak.

The dissolved Cu and Zn concentration profiles of the two columns with plants during various phases of the experiment were compared to the profiles from a previous experiment before the willows were planted (data from ref 22) (Figure S6, Supporting Information), which confirms that the changes in the Cu and Zn profiles are indeed caused by the plants. This comparison also shows that the Cu and DOC peak persisted in the columns over long times during growth of the plants.

3.3. Correlation Analysis. In the absence of plants, all ion concentrations were positively correlated among each other and were all negatively correlated with pH (Table S2a, Supporting Information). In presence of plants, most correlations were weaker than those in the columns without plants (Table S2b, Supporting Information). Only the correlations between DOC and Cu and between Mg and Ca remained the same.
Applying multi-linear regression analysis to the topsoil data of the plant-free columns, dissolved Cu was found to be related to DOC by the following equation ($r^2 = 0.624$, $n = 42$, $p < 0.001$) where the numbers in parentheses represent the standard errors and the units are mol/L for Cu and kg/L for DOC.

$$\text{logCu} = 0.917(\pm 0.113) \log(\text{DOC}) - 1.236(\pm 0.549)$$

where the numbers in parentheses represent the standard errors and the units are mol/L for Cu and kg/L for DOC. Including pH and Ca did not improve the relationship. Figure 2a shows that the same relationship also predicted the dissolved Cu from DOC in the soil solution of the planted columns.

Including pH did not improve the relationship. Figure 2b shows that with this equation dissolved Zn can well be predicted in the absence of plants (open symbols). Applying this relationship to the concentration of dissolved Zn in the presence of plants (solid symbols) did not give a good prediction (Figure 2b). This corresponds to the lack of correlation between dissolved Zn and DOC in the presence of plants. The best prediction for dissolved Zn in the topsoil of the two planted columns (Figure 2c) was obtained with this equation ($r^2 = 0.362$, $n = 42$, $p < 0.01$):

$$\log Zn = 1.075(\pm 0.152) \log (\text{DOC}) + 0.268(\pm 0.169) \log (\text{Ca}) + 0.540(\pm 0.715)$$

In the presence of plants, Cu$^{2+}$ decreased with depth, exhibiting considerable scatter. Cu$^{2+}$ in the calcareous subsoil was lower than in the acidic subsoil and no significant effects from plants were found. Also labile Zn varied more in presence of plants and tended to be lower than in the absence of plants.

3.4. Cu$^{2+}$ and Labile Zn Concentrations. The shapes of the depth profiles of Cu$^{2+}$ and labile Zn in the topsoil were similar as those of the respective total dissolved concentrations when plants were absent (Figure 3a and c). In the presence of plants, Cu$^{2+}$ decreased with depth, exhibiting considerable scatter. Cu$^{2+}$ in the calcareous subsoil was lower than in the acidic subsoil and no significant effects from plants were found. Also labile Zn varied more in presence of plants and tended to be lower than in the absence of plants.
The percentage of Cu$^{2+}$ was 0.05–0.1% of total dissolved Cu in the topsoil without plants and less than 0.05% in calcareous subsoil (Figure 3b). In the acidic subsoil it reached up to 1.6%. The percentage of Cu$^{2+}$ was slightly lower in the planted than in the plant-free columns. The percentage of labile Zn varied from 40 to 70% in the topsoil and from 30 to 80% in the subsoil (Figure 3d).

### 3.5. Modeling Cu and Zn Speciation

Based on the measured soil solution composition, the model correctly predicted that pCu was, in general, lower in the presence of plants than in the absence of plants (Figure 4a). The pCu values predicted for the two samples with observed malate and oxalate were on average, higher than the corresponding measured values. The predicted labile Zn was, on average, 28% less than the measured values (Figure 4b). Good agreement between predicted and measured values was found for the samples in which malate and oxalate had been detected, assuming that Zn-malate and Zn-oxalate complexes were non-labile. In these samples, 4.3 and 7.5% of dissolved Cu and 34 and 32% of dissolved Zn, were bound to malate and oxalate, respectively.

### 3.6. Leaching of Metals

Table 1 shows the amounts of metals and DOC leached from the topsoil and subsoils during the whole plant experiment. The values were calculated by multiplying the respective solutes concentrations at the lowest sampling depth in the topsoil (22 cm depth) and the subsoil (54 cm depth) with the discharge rates. Root water extraction in the subsoil was assumed negligible, as no (or only very few) roots were present in the subsoil. The total volume of effluent from the columns with plants was 70% of that without plants.

In total, DOC and Cu leaching from the topsoil with plants was only half and Zn leaching only 1/3, as large as from the topsoil without plants. The reduction in Cu leaching was proportional to that of DOC. There was less leaching of DOC, Cu, and Zn from topsoil above calcareous than acidic subsoil in the presence of plants, but not in the absence of plants.

Metal leaching from the subsoil was much less than from the topsoil. The presence of plants reduced the leaching of DOC, Cu, and Zn from the subsoils in similar proportions as from the topsoil. Corresponding to the large concentration of DOC, Cu was much higher from the calcareous subsoil. On the other hand, more Zn leached from the acidic subsoil.

### 4. Discussion

#### 4.1. Direct Effects of Roots on Metals in Soil Solution

The concentration profiles in the planted columns were less regular than in the plant-free columns, indicating strong local effects. DOC showed a prominent peak in the lower part of the topsoil in one of the columns and a “negative” peak at the same depth in the other column. These peaks, however, were not found to correlate with root density. Both columns had a maximum in root density at 20 cm depth, but only one column had a DOC peak around that depth.

Plant water extraction directly influenced the amount of metal leaching because the water uptake by plants reduced the volume of leachate. In combination with the decreased concentrations of Zn and Cu at the lowest depth in the topsoil, this resulted in much less export of metals from the topsoil to the subsoil in the presence of plants. A similar situation was found in the subsoil. Although we found no difference in metal concentrations in the presence and absence of plants in the lower part of the subsoil, the leaching of metals was much smaller in the presence of plants.

The willows also took up large amounts of metals. The metal uptake by plants was 2 times larger than the leaching from topsoil to subsoil for Cu and about 30 times larger for Zn. The higher uptake explains the stronger plant effects on Zn than on Cu. This may also be the reason for the fact that the Cu concentration in the soil solution would be described with one single regression equation, while two different equations were obtained for Zn for the planted and plant-free columns.

#### 4.2. Indirect Effects of Roots on Metal Solubility

Although indirect effects such as increased root exudation, they should have been present in more samples.

Dissolved Cu was closely correlated to the DOC concentration, regardless of the absence or presence of plants. The influence of plants on Cu solubility can, therefore, be explained by the change in DOC concentration. Enhanced DOC concentration in the presence of plants can be linked to carbon input by root exudation and decomposition of dead roots. As in the majority of the samples, no organic acids were found, we believe that the increase in DOC was

### Table 1. Leaching of Metals and DOC from the Topsoil into the Subsoil and out of the Subsoils during 115 days

<table>
<thead>
<tr>
<th>Subsoil Type</th>
<th>Leaching from Topsoil</th>
<th>Leaching from Subsoil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acidic</td>
<td>Calcareous</td>
</tr>
<tr>
<td>Water (L)</td>
<td>no plants</td>
<td>17.4 (±1.4)</td>
</tr>
<tr>
<td>plants</td>
<td>202 (±10.1)</td>
<td>324 (±11.2)</td>
</tr>
<tr>
<td>DOC (mg)</td>
<td>no plants</td>
<td>192 (±9.3)</td>
</tr>
<tr>
<td>plants</td>
<td>109 (±5.1)</td>
<td></td>
</tr>
<tr>
<td>Cu (μmol)</td>
<td>no plants</td>
<td>32 (±3.1)</td>
</tr>
<tr>
<td>plants</td>
<td>16 (±1.8)</td>
<td>11 (±1.0)</td>
</tr>
<tr>
<td>Zn (μmol)</td>
<td>no plants</td>
<td>92 (±6.7)</td>
</tr>
<tr>
<td>plants</td>
<td>36 (±2.9)</td>
<td>22 (±1.9)</td>
</tr>
</tbody>
</table>
primarily due to the stimulation of microbial activity. The additional DOC in the presence of plants could have resulted thus also from increased decomposition of secondary soil organic matter.

In the absence of plants, Zn mobilization could be predicted from DOC and Ca. This suggests that Zn ions were mobilized through ion exchange by Ca ions in addition to competition with DOC. In the presence of plants, however, Zn was negatively correlated to Ca. We attribute this change to the depletion of Zn in the soil solution by root uptake. Ca may have been mobilized through root exudation, but not taken up to the same degree. Uptake may have depleted Zn in the soil solution, in particular, if we assume that a new equilibrium between solid phase and solution was not reached quickly enough. Cu uptake was much less than Zn uptake, explaining why no similar depletion effect was observed.

The plant effect on the speciation of Cu and Zn in soil solution could be reasonably well predicted, assuming that the metal-binding strength of DOC was the same in the presence and absence of plants, using the NICA-Donnan model. This may be an indication that the source of DOC was in general the same, independently of the presence of plants. This means that the DOC sampled from the planted columns was not primarily root-derived, but as in the plant-free columns, resulted from the microbial decomposition of older soil organic matter.

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Supporting Information Available

Two tables show the soil properties and the correlation matrix for DOC, Ca, Mg, SO\textsubscript{4}\textsuperscript{2}-, NO\textsubscript{3}-, Cu, and Zn. Six figures show the column setup, root density, depth profiles, and concentration profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(20) Nowack, B.; Rais, D.; Frey, B.; Menon, M.; Schulini, R. Influence of heavy metal contamination on soil parameters in a lysimeter experiment designed to evaluate phyto-stabilization by afforestation. For. Snow Landscape Res. 2006, 80 (2), 201–211.

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