Doctoral Thesis

Soil solution chemistry in a heavy metal contaminated forest model ecosystem

Author(s):
Rais, David

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Soil solution chemistry in a heavy metal contaminated forest model ecosystem

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DOCTOR OF SCIENCES

presented by
DAVID RAIS
Licence ès Sciences, University of Neuchâtel
born December 2, 1974
in Lausanne, Switzerland

accepted on the recommendation of
Prof. Dr. Rainer Schulin, examiner
Dr. Jörg Luster, co-examiner
PD. Dr. Bernd Nowack, co-examiner

2005
# Table of contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of contents</td>
<td>I</td>
</tr>
<tr>
<td>List of figures</td>
<td>V</td>
</tr>
<tr>
<td>List of tables</td>
<td>IX</td>
</tr>
<tr>
<td>Summary</td>
<td>XI</td>
</tr>
<tr>
<td>Zusammenfassung</td>
<td>XV</td>
</tr>
<tr>
<td><strong>CHAPTER 1</strong></td>
<td></td>
</tr>
<tr>
<td>General introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Heavy metals in soils</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Soil contamination by heavy metals in Europe and Switzerland</td>
<td>1</td>
</tr>
<tr>
<td>1.3 Mobility and bioavailability of metals in soils</td>
<td>3</td>
</tr>
<tr>
<td>1.4 Influence of plants on metal mobility and bioavailability</td>
<td>4</td>
</tr>
<tr>
<td>1.5 Impact of heavy metals on soil functions</td>
<td>5</td>
</tr>
<tr>
<td>1.6 Soil solution</td>
<td>5</td>
</tr>
<tr>
<td>1.7 Objectives of this thesis</td>
<td>6</td>
</tr>
<tr>
<td><strong>CHAPTER 2</strong></td>
<td></td>
</tr>
<tr>
<td>Sorption of trace metals by standard and micro suction cups in the absence and presence of dissolved organic carbon</td>
<td>9</td>
</tr>
<tr>
<td>2.1 Abstract</td>
<td>10</td>
</tr>
<tr>
<td>2.2 Introduction</td>
<td>11</td>
</tr>
<tr>
<td>2.3 Material and methods</td>
<td>13</td>
</tr>
<tr>
<td>2.3.1 Suction cups characteristics</td>
<td>13</td>
</tr>
<tr>
<td>a) Standard size suction cups</td>
<td>13</td>
</tr>
<tr>
<td>b) Micro suction cups</td>
<td>13</td>
</tr>
<tr>
<td>2.3.2 Laboratory tests</td>
<td>14</td>
</tr>
<tr>
<td>a) Standard size suction cups</td>
<td>14</td>
</tr>
<tr>
<td>b) Micro suction cups</td>
<td>16</td>
</tr>
<tr>
<td>2.3.3 Analytical methods</td>
<td>17</td>
</tr>
</tbody>
</table>
2.4 Results .................................................................................................................. 17

2.4.1 Cleaning of standard size suction cups ......................................................... 17

2.4.2 Cleaning of micro suction cups ................................................................. 18

   a) Sorption of heavy metals, major cations and anions by standard size suction cups at acidic pH .................................................. 18

   b) Sorption of heavy metals, major cations and anions by standard size suction cups at alkaline pH ................................................. 20

   c) Sorption of heavy metals, major cations and anions by micro suction cups at acidic pH .................................................. 22

   d) Sorption of heavy metals, major cations and anions by micro suction cups at alkaline pH ................................................. 24

   e) Sorption of DOC by standard size suction cups .................................. 26

   f) Sorption of DOC by micro suction cups .............................................. 26

2.5 Discussion ........................................................................................................... 27

   2.5.1 Heavy metal sorption in absence of dissolved organic carbon. .............. 27

   2.5.2 Sorption of dissolved organic carbon ......................................................... 28

   2.5.3 Heavy metal sorption in presence of dissolved organic carbon .............. 29

2.6 Conclusions ....................................................................................................... 30

2.7 Acknowledgements .............................................................................................. 31

CHAPTER 3

Heavy metal concentrations and speciation in the soil solution of a forest model ecosystem: Seasonal variability and plant influence ............................................. 33

3.1 Abstract ................................................................................................................. 34

3.2 Introduction ......................................................................................................... 34

3.3 Material and Methods .......................................................................................... 36

   3.3.1 Experimental set-up and treatments .................................................. 36

   3.3.2 Sampling and analysis of soil samples .............................................. 38

   3.3.3 Soil solution sampling .............................................................................. 38

   3.3.4 Chemical analysis of solution samples .............................................. 39

   3.3.5 Statistical analysis and speciation modelling ........................................ 40

3.4 Results ................................................................................................................. 41

   3.4.1 Heavy metal concentrations in top- and sub- soil .................................. 41

   3.4.2 Correlation between HM concentrations and other soil solution parameters 44
3.4.3 Metal speciation .................................................. 50
3.5 Discussion ......................................................... 51
  3.5.1 Metal solubility control in the topsoil .......................... 51
  3.5.2 Heavy metal concentrations in the contaminated topsoils: seasonal variations and plant influence ....................... 53
  3.5.3 Heavy metal concentrations in the subsoil ....................... 54
  3.5.4 Metal speciation in the contaminated topsoil .................... 55
3.6 Acknowledgements .................................................. 55

CHAPTER 4
Effects of heavy metals on dissolved organic matter, sulphate, phosphate, and nitrate in the soil solution of a model forest ecosystem .......... 57
4.1 Abstract .......................................................... 58
4.2 Introduction ........................................................ 58
4.3 Material and methods .............................................. 60
  4.3.1 Experimental set-up and treatments ............................ 60
  4.3.2 Sampling and analysis of the soil samples ....................... 62
  4.3.3 Soil Solution Sampling ........................................ 62
  4.3.4 Chemical analyses of solution samples ......................... 63
  4.3.5 Soil sampling in the planted topsoil for determination of soil biological parameters .............................................. 64
  4.3.6 Soil biological analyses ....................................... 64
     a) Microbial biomass C ............................................. 64
     b) Soil basal respiration .......................................... 64
     c) Acid phosphatase .............................................. 65
     d) Beta-glucosidase .............................................. 65
     e) Nitrification potential ....................................... 65
  4.3.7 Statistical analysis ............................................ 65
4.4 Results ............................................................ 66
  4.4.1 Heavy metals ................................................ 66
  4.4.2 Dissolved organic matter .................................... 66
  4.4.3 Sulphate ..................................................... 67
  4.4.4 Phosphate .................................................... 70
  4.4.5 Nitrate ....................................................... 70
# Table of contents

4.4.6 Soil biological parameters .................................................. 73
4.5 Discussion ............................................................................ 73
4.5.1 Dissolved organic matter ..................................................... 73
4.5.2 Dissolved organic matter: C/N ratio ..................................... 75
4.5.3 Sulphate ........................................................................... 76
4.5.4 Phosphate ......................................................................... 76
4.5.5 Nitrate ............................................................................. 77
4.6 Conclusions .......................................................................... 78
4.7 Acknowledgments .................................................................. 78

**CHAPTER 5**

General conclusions ..................................................................... 81
5.1 Sorption characteristics of different suction cup materials in function of the soil solution chemistry – Does DOC matter and what is the best suited suction cup material for trace metal sampling? ..................................................... 81
5.2 Do plants and seasonal variations in plant and microbial activity change the concentration and speciation of heavy metals in soil solution? .............................. 82
5.3 Use of soil solution parameters as indicators for the mineralisation potential of a whole soil compartment. Do heavy metals show detrimental effects and what is the influence of plants? ......................................................................................... 83
5.4 Outlook and open questions ................................................... 84

References .................................................................................. 87
Acknowledgements ...................................................................... 103
Curriculum Vitae .......................................................................... 105
List of figures

CHAPTER 1
General introduction .................................................. 1

Figure 1.1: Bioavailability processes in the soil-plant system........... 4
Figure 1.2: The soil-plant system showing the key components concerned
with the dynamics of heavy metals................................. 6
Figure 1.3: Soil water sampler used in this experiment.................. 7
Figure 1.4: View of a model ecosystem within the "From Cell to Tree" framework... 7

CHAPTER 2
Sorption of trace metals by standard and micro suction cups in the absence
and presence of dissolved organic carbon............................. 9

Figure 2.1: Cd concentrations in the percolate collected from different standard
size suction cups at pH 4.5............................................ 19
Figure 2.2: Cu concentrations in the percolate collected from different standard
size suction cups at pH 4.5............................................ 19
Figure 2.3: Cd concentrations in the percolate collected from different standard
size suction cups at pH 7.5............................................ 21
Figure 2.4: Cu concentrations in the percolate collected from different standard
size suction cups at pH 7.5............................................ 22
Figure 2.5: Cd concentrations in the percolate collected from different micro
suction cups at pH 4.5.................................................. 23
Figure 2.6: Cu concentrations in the percolate collected from different micro
suction cups at pH 4.5.................................................. 24
Figure 2.7: Cd concentrations in the percolate collected from different micro
suction cups at pH 8.0.................................................. 25
Figure 2.8: Cu concentrations in the percolate collected from different micro
suction cups at pH 8.0.................................................. 25
Figure 2.9: UV absorptions measured at 254 nm in the percolate collected
from different micro suction cups at pH 4.5 and 8.0................ 27
CHAPTER 3
Heavy metal concentrations and speciation in the soil solution of a forest model ecosystem: Seasonal variability and plant influence ...................... 33

Figure 3.1: Dissolved Cu concentrations in the metal contaminated and uncontaminated topsoil and the subsoils at 40 cm with and without plants................................................. 42

Figure 3.2: Dissolved Zn concentrations in the metal contaminated and uncontaminated topsoil and the subsoils at 40 cm with and without plants. .................................................. 42

Figure 3.3: Dissolved Cd concentrations in the metal contaminated and uncontaminated topsoil and the subsoils at 40 cm with and without plants ................................................. 43

Figure 3.4: Measured Cd concentrations in the soil solution of the metal-contaminated topsoil vs. predicted values. ................................. 43

Figure 3.5: DOC, pH and PO₄³⁻ concentrations in the soil solution of the planted and unplanted contaminated topsoils ................................................................. 48

Figure 3.6: Measured Cu concentrations in the soil solution of the metal-contaminated and uncontaminated topsoil vs. predicted values. .48

Figure 3.7: Measured Zn concentrations in the soil solution of the metal-contaminated topsoil vs. predicted values ................................. 49

Figure 3.8: Measured Cd concentrations in the soil solution of the metal-contaminated topsoil vs. predicted values ................................. 49

CHAPTER 4
Effects of heavy metals on dissolved organic matter, sulphate, phosphate, and nitrate in the soil solution of a model forest ecosystem. ............... 57

Figure 4.1: DOC concentrations in the calcareous subsoil and the corresponding topsoil, with and without plants. ................................. 68

Figure 4.2: DOC concentrations in the acidic subsoil and the corresponding topsoil, with and without plants. ................................. 68

Figure 4.3: SO₄²⁻ concentrations in the soil solution of the calcareous subsoil and the corresponding topsoil, with and without plants. ................................. 69

Figure 4.4: SO₄²⁻ concentrations in the soil solution of the acidic subsoil and the corresponding topsoil, with and without plants. ................................. 70

Figure 4.5: PO₄³⁻ concentrations in the soil solution of the topsoil above calcareous subsoil with and without plants. ................................. 71
List of figures

Figure 4.6: $\text{PO}_4^{3-}$ concentrations in the soil solution of the topsoil above acidic subsoil with and without plants. .......................... 71

Figure 4.7: $\text{NO}_3^-$ concentrations in the soil solution of the calcareous subsoil and the corresponding topsoil, with and without plants. .......................... 72

Figure 4.8: $\text{NO}_3^-$ concentrations in the soil solution of the acidic subsoil and the corresponding topsoil, with and without plants. .......................... 72
List of tables

CHAPTER 1
General introduction ...................................................... 1

Table 1.1: Guide, trigger and clean-up values in the ordinance relating to impacts on soils. .................................................. 2

CHAPTER 2
Sorption of trace metals by standard and micro suction cups in the absence and presence of dissolved organic carbon. ......................... 9

Table 2.1: Properties of the standard size suction cups materials. ............... 13

Table 2.2: Properties of the micro suction cups materials. .......................... 14

Table 2.3: Composition of the solutions used in the laboratory tests of suction cup materials. ................................................. 15

Table 2.4: Percentage of metal, anion and DOC recovery with standard suction cups in absence/presence of DOC. ................................. 20

Table 2.5: Qualitative evaluation of the general sorption characteristics of the different standard size suction cup materials. ......................... 21

Table 2.6: Qualitative evaluation of the general sorption characteristics of the different micro suction cups. .................................... 23

CHAPTER 3
Heavy metal concentrations and speciation in the soil solution of a forest model ecosystem: Seasonal variability and plant influence ............... 33

Table 3.1: Selected physical and chemical properties of the soils used in the model ecosystems (with plants) and plant-free soil columns. .......... 37

Table 3.2: Average heavy metal concentrations as well as pH and DOC concentrations in the subsoil solutions. .................................. 45

Table 3.3: Correlation matrix for heavy metals, pH, DOC as well as major cations and anions in the contaminated topsoil solutions. ............... 46

Table 3.4: Model equations to predict Cu, Zn and Cd concentrations in the soil solution of the planted and unplanted soil systems. ................. 47
Abstract

Table 3.5: Total soluble, free and labile metal concentrations as measured in the metal-contaminated topsoil in the presence and absence of plants. ................................................................. 50

Table 3.6: Results of solubility calculations performed with SOILCHEM for solutions mimicking the soil solutions in the topsoil for different \( p(\text{CO}_2) \) and pH as well as \( \text{PO}_4^{3-} \), Zn, and Cd concentrations. .......... 52

CHAPTER 4

Effects of heavy metals on dissolved organic matter, sulphate, phosphate, and nitrate in the soil solution of a model forest ecosystem. ................. 57

Table 4.1: Selected physical and chemical properties of the soils used in the model ecosystems and plant-free soil columns. ........................................ 61

Table 4.2: Concentration range of protons and heavy metals in the soil solution of planted and unplanted soil systems for CO/HM treatments. .......... 66

Table 4.3: Mean values of microbial biomass, microbial activity expressed as basal respiration, beta-glucosidase activity, nitrification potential, and acid phosphatase activity in the presence of plants. ............... 73
Summary

Excessive concentrations of heavy metals (HM) in the environment are of major concern for ecosystem and human health. The mobility and speciation of HM are of utmost importance with respect to their bioavailability and their potential to leach to the groundwater.

The objective of this thesis was to gain insight into the mobility and speciation of HM in a young model forest ecosystem planted on a HM contaminated agricultural topsoil, as influenced by plants and seasonal variations in plant and microbial activity. In addition, the effects of HM contamination on soil solution parameters were investigated in order to evaluate the response of a whole soil ecosystem to HM stress in terms of HM influence on the mineralisation potential of a soil and on nutrient uptake by plants.

As soil solution sensitively responds to soil-plant-microbe interactions, and acts as a transport medium, the mobility and bioavailability of HM as well as their effect on nutrient cycling were investigated by monitoring the soil solution chemistry.

In a first step, preliminary laboratory experiments were performed to test suction cup materials used as soil water samplers for their sorption characteristics under different conditions in terms of pH and dissolved organic carbon (DOC) concentrations. The objective was to determine the best suited material with respect to a minimal influence on the soil solution chemistry, especially HM. Dissolved organic carbon reduced metal sorption on cup binding sites in most cases, especially at alkaline pH because of the higher metal-organic complex stability and the increase of DOC solubility with pH. The sorption of DOC on the cup materials changed with its quality, with "new" DOC from leaf litter extract being more sorbed than "old" DOC from a mineral soil. Therefore, it is expected that "old" DOC is more effective than "new" DOC to reduce metal sorption by the cups. In general, nylon and polyvinyl alcohol (PVA) proved to be the best suited materials if trace metals are of interest, but at alkaline pH both sorbed Cu in absence of DOC and Pb in absence and presence of DOC. Teflon, borosilicate glass and ceramic are not suitable for the monitoring of trace metals in soil solution at alkaline pH.

In a second step, experiments were carried out in the lysimeter facility of the Swiss Federal Institute for Forest, Snow and Landscape Research WSL within the multidisciplinary framework "From Cell to Tree". In addition to the investigation of the HM dynamics in the soil and their effect on mineralisation processes, this framework
was set up to study water fluxes and to gain knowledge on the reactions of plants and associated organisms to the chronic influence of HM and rain acidity.

In a factorial design, topsoil contamination (with/without HM), subsoil type (acidic/calcareous) and plant cover (with/without plants) under "ambient rain" were considered. For this purpose, plant-free soil columns were set up as references.

Soil solution concentrations of Cu, Zn, and Cd were always much higher in the contaminated than in the uncontaminated topsoil. In the metal-contaminated topsoil, the concentrations of HM varied relatively little with time. While Cu was governed by complexation with DOC, Zn and Cd variations in the contaminated topsoils were negatively correlated with phosphate. Based on this, plant uptake of phosphate was assumed to be responsible for about two times larger Zn and Cd concentrations in the presence than in the absence of plants. On the other hand, Cu concentrations did not differ between planted and unplanted systems. However, smaller fractions of free Cu (Cu$^{2+}$) in the absence than in the presence of plants indicated a difference in the metal binding properties of DOC. In the acidic subsoil, Zn concentrations were somewhat larger below contaminated topsoil than below uncontaminated topsoil in the presence of plants, with maximum concentrations in winter and spring. In the calcareous subsoil, none of the heavy metals was increased below the contaminated topsoil.

The effects of HM contamination on soil solution parameters were studied in the same experimental factorial scheme as the mobility of HM. The DOC and sulphate concentrations in the planted topsoils were reduced by HM contamination. This reflected the lower microbial activity measured by biological parameters obtained from microbial assays. In the plant-free systems, there was no HM effect on DOC and sulphate concentrations, and the concentrations were at a similar level as in the planted contaminated topsoil. This suggests on one hand a base level of microbial activity which was insensitive to HM contamination. On the other hand, the stimulating effect of root exudation on mineralisation processes was strongly limited under HM stress. In addition to microbial activity, nitrate and phosphate concentrations were controlled by other factors. Plant uptake mainly governed nitrate concentrations in soil solution and effectively masked metal effects on the nitrification potential. Plant uptake and chemical effects determined phosphate concentrations. With and without plants, phosphate solubility was lower in the presence of HM. Plant uptake led to lower
phosphate concentrations compared to the plant-free reference in both the HM and control treatment.

The results from this study simulating the afforestation of a slightly acidic agricultural topsoil indicate that plants in general have only little influence on soil solution parameters in metal-contaminated soils. However, the free Cu fraction, total Zn, and total Cd in solution were larger in planted soils.
Zusammenfassung

Hohe Schwermetallkonzentrationen (SM) in der Umwelt sind ein Risiko für Ökosysteme und die Gesundheit des Menschen. Die Mobilität und die Spezifizierung der SM sind wichtige Kriterien im Hinblick auf deren Bioverfügbarkeit und die ihre mögliche Auswaschung ins Grundwasser.


Da die Bodenlösung empfindlich auf Wechselwirkungen zwischen Boden, Pflanzen und Mikroorganismen reagiert und sie gleichzeitig als Transportmittel dient, wurde die Mobilität und die Bioverfügbarkeit der SM sowie deren Einfluss auf den Nährstoffkreislauf anhand der chemischen Zusammensetzung der Bodenlösung untersucht.

In einem ersten Schritt wurden Vorversuche im Labor durchgeführt, um die Materialien von Saugkerzen, die für die Beprobung der Bodenlösung in Frage kamen, auf ihr Sorptionsverhalten bei verschiedenen pH-Werten und Konzentrationen an gelöstem organischen Kohlenstoff (DOC) zu testen. Es war das Ziel, das am besten geeignete Material zu finden, das einen minimalen Einfluss auf die chemischen Eigenschaften der Bodenlösung und besonders auf die SM aufweist. DOC verringerte in den meisten Fällen die Sorption von Metallen an den Bindungsstellen der Saugkerzen. Dieser Effekt war besonders gross bei alkalischem pH, weil unter diesen Bedingungen metall-organische Komplexe stabiler und DOC bessser loslich ist. Das Sorptionsverhalten des DOC an den Saugkerzen-Materialien veränderte sich mit der Qualität der DOC: "Neues" DOC in Streuextrakten wurde stärker sorbiert als "altens" DOC aus einem Mineralboden. Deshalb erwarten wir, dass „altes“ DOC die Metallsorption an Saugkerzen stärker verringert als "neues" DOC. Im Allgemeinen stellte sich heraus, dass Nylon und Polyvinyl-Alkohol (PVA) für die Untersuchung von
Zusammenfassung

metallischen Spurenelementen die am besten geeigneten Saugkerzen-Materialien sind. Jedoch sorbieren bei alkalischem pH beide Materialien Cu in Abwesenheit von DOC und Pb mit und ohne DOC. Teflon, Borosilikatglas und Keramik sind für die Untersuchung von metallischen Spurenelementen in Bodenlösungen bei alkalischem pH nicht geeignet.


Behandlungs-Variablen waren die Schwermetallbelastung des Oberbodens (mit/ohne SM), die Art des Unterbodens (sauer/kalkhaltig) und die Bepflanzung (mit/ohne Bepflanzung) unter neutrale Beregnung. Als pflanzenfreie Referenz wurden Bodensäulen eingerichtet.


Die Auswirkungen der SM-Belastung auf weitere Bodenlösungsparameter wurden mit den gleichen experimentellen Variablen untersucht wie die Mobilität der SM. Die DOC- und Sulfatkonzentrationen im bepflanzten Oberboden waren unter dem Einfluss
Chapter 1

General introduction

1.1 Heavy metals in soils

The term "heavy metals" refers to metals and metalloids having densities greater than 5 g cm\(^{-3}\) and is associated with pollution and toxicity when present in excess, although some of these elements are required by organisms in small doses (Adriano, 2001). For instance, Cu and Zn are essential in the metabolism of humans, plants and bacteria (Adriano, 2001; Blencowe and Morby, 2003; Cavet et al., 2003), but at high concentrations they exert toxic effects on cells (Baker and Walker, 1989).

Heavy metals in soils may be of natural or anthropogenic origin. Natural sources include vulcanic emissions, aeolian dusts and weathering of rocks (Ernst, 1998). However, the major contamination is usually of anthropogenic origin due to mining and smelting, application of metal-containing pesticides and fertilizers in agriculture, combustion of fossil fuel, waste disposal, as well as military activities (Alloway, 1995). Cu, Ni, Cd, Zn, Cr, Pb are the most important inorganic soil pollutants. Because soils have limited capacity to inactivate or to attenuate these inputs, heavy metals can persist in soil for very long times, with little decrease in their biological potency (Brookes, 1995). Ample evidence now exists that chemically degraded soil may not only imperil our life support system, i.e. the soil, but also may threaten the whole food chain and consequently humans (Adriano, 1995). In addition, soil degradation may impact economic development by limiting the marketing of agricultural products and reducing the profitability of the agricultural industry.

1.2 Soil contamination by heavy metals in Europe and Switzerland

It is estimated that in Western Europe, up to 1.4 Mio. sites are polluted, many of them with heavy metals such as Zn, Cd, Pb and Cu (McGrath et al., 2001). Accumulation of these metals continues as inputs exceed the outputs in many European soils.

A study of the Swiss Agency for the Environment Forests and Landscape (SAEFL)
in 1997 estimated that around 50'000 sites are contaminated with heavy metals in Switzerland (Elsenbeer et al., 1997). With the enforcement of the *Ordinance relating to Impacts on the Soil* (OIS 1998), Switzerland has implemented a three level evaluation scheme consisting of guide, trigger and clean-up values of metal contents in soils in order to evaluate risks arising from soil pollution to soil fertility and health of plants, animals and humans (Table 1.1).

**Table 1.1: Guide, trigger and clean-up values in the ordinance relating to impacts on soils (OIS, 1998).**

<table>
<thead>
<tr>
<th>Use of land</th>
<th>Cd</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
<td>s</td>
<td>t</td>
<td>s</td>
</tr>
<tr>
<td>Guide value</td>
<td>0.8</td>
<td>0.02</td>
<td>40</td>
<td>0.7</td>
</tr>
<tr>
<td>Food plant cultivation</td>
<td>2</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fodder plant cultivation</td>
<td>2</td>
<td>0.02</td>
<td>150</td>
<td>0.7</td>
</tr>
<tr>
<td>Direct cultivation</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clean-up value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agriculture and horticulture</td>
<td>30</td>
<td>0.1</td>
<td>1000</td>
<td>4</td>
</tr>
<tr>
<td>Domestic gardens and allotments</td>
<td>20</td>
<td>0.1</td>
<td>1000</td>
<td>4</td>
</tr>
<tr>
<td>Children playground</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

t = pseudo-total; s = soluble; all values are in mg metal kg⁻¹ of soil

Each of these values refers to a pseudo-total metal content (t, extracted with 2 M HNO₃) or a soluble fraction (s, extracted with 0.1 M NaNO₃) or to both. If one of these concentration exceeds the respective threshold, the soil is considered as contaminated. The guide values represent the tolerable levels above which soil fertility is no longer assured in the long run (Vollmer et al., 1997). The trigger values give levels above which authorities have to assume that harmful effects on biological toxicity to humans, animals or plants may be a problem and that more detailed investigations are necessary. Clean-up values give the levels of contamination of the soil above which a hazard is assumed to exist and action, either restriction of land use or soil remediation, is required (Vollmer et al., 1997).
1.3 Mobility and bioavailability of metals in soils

The solubility of heavy metals in soils is governed by pH, redox conditions, the ionic composition of the soil solution and the nature of the soil's solid phase (Alloway, 1995; Adriano, 2001). The solubility of most heavy metals increases with decreasing pH. Reducing conditions (decreasing and negative Eh (redox potential)) can lead to the dissolution of oxides and the subsequent liberation of sorbed, occluded and coprecipitated heavy metals into solution while on the other hand oxidising conditions can lead to the oxidation and dissolution of insoluble sulphide minerals. The complexation of sorbed metal species by soluble low molecular weight organic ligands in the soil solution such as dissolved organic carbon (DOC) can enhance the mobilisation of many heavy metals. Variations in ionic composition of the soil solution can influence the degree of competition for sorption sites, the formation of soluble or insoluble compounds depending on the elements in presence, and the ionic strength, the increase of which can result in reduced sorption and enhanced desorption from soil surface sites. Once soluble, heavy metals can become mobile and leach through the soil profile and eventually to the groundwater. The speciation of a metal, that refers to the distribution of this metal amongst its chemical species, also depends on the above mentioned soil factors.

Both the solubility and speciation of heavy metals govern the bioavailability and thus the toxicity of heavy metals to organisms. In order to be bioavailable, a metal needs to be mobile and to come in contact with the organism in a form in which it can be taken up and react with its metabolic machinery (Campbell, 1995) (Figure 1.1). According to the free-ion activity model (FIAM), metal uptake into cells is a function of the activity of the free metal ions in solution, independent of the total concentration or of other species (Brezonik et al., 1991). The validity of the model is supported in plant nutrition where plant response generally correlates best with the activity of the free, uncomplexed metal ions in solution (Parker et al., 1995).
Introduction

Bioavailability processes (A, B, B' and C)

<table>
<thead>
<tr>
<th>Metal interactions between phases (A)</th>
<th>Transport of metals to plant (B, B')</th>
<th>Passage across root membrane (C)</th>
<th>Circulation within plant, accumulation in target tissue and toxic effects (D)</th>
</tr>
</thead>
</table>

Figure 1.1: Bioavailability processes in the soil-plant system (from Adriano et al., 2004).

### 1.4 Influence of plants on metal mobility and bioavailability

Plants have an important influence on the physicochemical conditions that determine the mobility and the bioavailability of heavy metals in soil and as a consequence their impact on biota. Of particular importance are reactions that occur at the soil-root interface, i.e. in the rhizosphere. McLaughlin et al. (1998) specifically printed out the following factors and processes:

1. Plant uptake may reduce ion activity and desorb contaminants from surfaces,
2. Plant-induced changes in soil solution chemistry can affect sorption, e.g. by altering pH, Eh, ionic strength,
3. Plants excrete organic ligands that may increase or decrease the total concentration of contaminant in solution,
4. Living or dead plant materials in the rhizosphere can act as sorbing surfaces for contaminants,
5. Microbial activity, stimulated by plants, can also alter soil solution chemistry and, thus, affect contaminant behaviour.
Some plants can take up high amounts of heavy metals or release compounds that render these metals less soluble or even insoluble. For these reasons phytoremediation has been proposed as a cost-effective technology for the treatment of contaminated soil (Lasat et al., 2000).

1.5 Impact of heavy metals on soil functions

Plant roots and most soil organisms depend on energy derived from assimilates produced above ground by photosynthesis. The mineralisation of organic residues by bacteria and fungi, i.e. "microorganisms", releases inorganic nutrients such as nitrate, sulphate and phosphate that can then be utilized again by plants and other organisms. Microbial biomass and activity are generally much higher in the rhizosphere than in the bulk soil due to the much larger availability of substrates such as root exudates (Brimecombe et al., 2001). Because of their role in organic matter decomposition, microorganisms are of utmost importance for the fertility of soil as well as for the recycling of nutrient elements. Poisoning of soil microorganisms can thus have dramatic consequences for the functioning of the entire ecosystem. Soil contamination by heavy metals is one of the problems threatening soil fertility and element cycling most seriously in many areas. If present in sufficiently high concentrations, heavy metals have been found to reduce the size of the microbial populations, to destruct their community structure and to reduce their activity (Chander et al., 2002; Wang et al., 2004; Kandeler et al., 2000). Heavy metal pollution may therefore result in severe ecosystem disturbance.

1.6 Soil solution

The soil solution is the central pool of soil nutrients that links all other biotic and abiotic compartments in soil, in particular soil matrix, plants roots and microorganisms (Figure 1.2). It is, as a consequence, very dynamic and sensitively responds to changes in conditions affecting input and output fluxes. In view of this sensitivity, the present thesis standed out from the hypothesis that effects of soil metals on nutrient cycling should be visible immediately in changes in the soil solution composition. Thus, monitoring of soil solution chemistry should provide a good indicator of pollution effects long before many other effects become manifest.
Within the soil, soil solution is held largely under tension by capillary forces. This tension determines how the moisture moves in the soil and is referred to as "soil water tension". Tension lysimeters based on porous cups (Figure 1.3) are a type of sampler that allows water to be removed from the soil by creating a vacuum (negative pressure or suction) inside the sampler greater than the soil tension that holds the water in the capillary spaces. This establishes a hydraulic gradient for the water to flow through the porous cup and finally into the sampler. Tension lysimetry offers a convenient way to collect soil solution continuously at a given location with minimum disturbance.

1.7 Objectives of this thesis

This study was performed in the framework of the interdisciplinary project "From Cell to Tree". It was carried out at the Swiss Federal Research Institute WSL in Birmensdorf. The goal of the project was to study the effects of soil contamination by...
heavy metals on ecophysiological processes in young forest model ecosystems (Figure 1.4). The thesis presented here focuses on soil solution chemistry in order to better understand the mobility and speciation of heavy metals as influenced by plants. In addition, the effects of heavy metals on mineralisation of organic matter and nutrient recycling were investigated.

Figure 1.3: Soil water sampler used in this experiment

Figure 1.4: View of a model ecosystem within the "From Cell to Tree" framework.
In order to obtain reliable soil solution data a sampling method is required that does not change the composition of the sampled solution. In this study, tension lysimetry based on porous suction cups was chosen as a convenient method (see above). Sorption of metals and other components such as DOC and their release from the walls of the cups as well as clogging of the pores may however alter the composition of the sample. The first objective of this thesis was therefore to evaluate suitable materials for these soil water samplers. This evaluation is presented in Chapter 2.

Using the material that performed best in the previous evaluation, i.e. nylon suction cups, soil solution was sampled monthly from the model ecosystems mentioned above over 1 year. The solution samples were analysed for the heavy metals Cu, Zn, Cd and Pb, for the major inorganic cations and anions, and for DOC, dissolved organic nitrogen (DON) and pH. Using these data, the speciation of Cu and Zn was studied. The variation in the concentration and speciation of metals of the collected solution samples and its dependance on the presence of plants, the total concentration of metals in the topsoil and the type of subsoil is described and discussed in Chapter 3.

Chapter 4, finally, analyses the effects of the before-mentioned factors on the time course of DOC, DON and the mineralised ions of the macronutrients N, P and S in the topsoil and subsoil solution of the studied model ecosystems.
Chapter 2

Sorption of trace metals by standard and micro suction cups in the absence and presence of dissolved organic carbon

D. RAIS, B. NOWACK, R. SCHULIN, J. LUSTER

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2.1 Abstract

Both the bioavailability of a trace metal (TM) in a soil and the risk of leaching to the groundwater are linked to the metals concentration in the soil solution. Sampling soil solution by tension lysimetry with suction cups is a simple and established technique that is increasingly used for monitoring dissolved TM in soils. Of major concern, however, is the sorption of TM to the walls of the samplers. Metal sorption to different materials used in suction cups can vary widely, depending also on the chemistry of the soil solution such as pH and DOC content. We compared different standard and micro suction cups in the laboratory for their sorption characteristics with respect to the TM Cu, Zn, Cd and Pb at two pH values (4.5 and 7.5) in presence or absence of dissolved organic carbon (DOC). In addition, we investigated the sorption of DOC from diverse origins by cup materials. At both pH values, least sorption of the four metals was found for nylon and polyvinyl alcohol. Borosilicate glass, ceramic and Teflon were not found suitable for the monitoring of TM in soil solution at alkaline pH. At low pH, ceramic as well as Teflon strongly sorbed Cu and Pb. Major cations (Na, Mg, K, Ca) and anions (Cl⁻, NO₃⁻, SO₄²⁻) were not or very little sorbed by the different cup materials, except K, Ca and Mg by the borosilicate glass at pH 7.5. TM sorption by suction cups was greatly reduced in presence of DOC, especially at alkaline pH. "New" DOC from leaf litter extract was much more sorbed than "old" DOC of a mineral soil solution. Consequently, also more TM are expected to be sorbed in the presence of "new" DOC compared to "old" DOC. Based on our results we recommend suction cups made of nylon or polyvinyl alcohol (PVA) for trace metal sampling in mineral soil horizons. None of the tested materials gave satisfactory results with respect to the determination of Pb concentrations in alkaline solution.

Abbreviations: CEC, cation exchange capacity; DOC, dissolved organic carbon; DOM, dissolved organic matter; TM, trace metals; PTFE, poly(tetrafluorethene); PVA, polyvinyl alcohol.
2.2 Introduction

The soil solution is the medium linking soil matrix, plant roots and groundwater. Its chemical composition mirrors important soil and rhizosphere processes such as nutrient cycling, organic matter turnover or trace metal (TM) leaching. Tension lysimeters built from porous suction cups offer a convenient way to collect soil solution with minimum disturbance continuously over time at a given location. Sorption of the analytes of interest to the walls of the cups, pore clogging or release of components of the cup material may, however, interfere with the sampling process and alter the composition of the sampled solution as it is transferred from the soil into the sampler.

More and more, such cups are also used to monitor TM concentrations in soil solutions. Compared to major anions and cations, many TM are in general strongly sorbed onto mineral and organic surfaces. In addition, complexation by dissolved organic matter (DOM) may drastically change their interaction with the suction cup material.

Ceramic cups have a relatively high cation exchange capacity (CEC). Consequently they can adsorb large amounts of TM cations (Grossmann et al., 1990). They can also adsorb DOC and inorganic anions such as phosphate (Grossmann et al., 1990; Wenzel and Wieshammer, 1995; Wenzel et al., 1997). Under acidic conditions, ceramic cups have been found to release Na, Mg, Al, K, and Ca (Grover and Lamborn, 1970; Hansen and Harris, 1975; Litaor, 1988; Raulund-Rasmussen, 1989; Wenzel and Wieshammer, 1995).

Suction cups made of borosilicate glass were found to exhibit very little retention of DOC (Wessel-Bothe et al., 2000). Little is known about the sorption of TM by glass cups. McGuire et al. (1992) noted significant sorption of Cr, Co, Cd and Zn on fritted glass at pH 6.5. Koch and Grupe (1993) investigated the sorption of Mn, Ni, Cu, Zn, Cd and Pb by borosilicate glass cups at pH 4 and reported weak sorption of Cu and strong sorption of Pb.

Suction cups made from plastic materials have received much attention because of their low CEC compared to ceramic and glass cups. The two most commonly used types are Teflon and nylon cups. Teflon cups from Prenart ApS consist of a porous body made from Teflon (PTFE) into which silicate is mixed in order to reduce its hydrophobicity. Sorption of Cu, Zn, Cd and Pb by Teflon cups was found to be a problem for sampling low concentrations at pH > 4.5 despite the rather low CEC.
Sorption of trace metals by standard and micro suction cups

(Andersen et al., 2002). However, this study was performed at an ionic strength which was far above what is normally found in soil solutions.

Nylon had negligible effects on sampled TM concentrations between pH 3 and 5, and only slight effects at pH 6 (Wenzel and Wieshammer, 1995; Wenzel et al., 1997). Under alkaline conditions, little sorption of Zn and Cd but strong sorption of Cu and Pb were reported (Grossmann et al., 1990; Grossmann and Udluft, 1991).

Standard-size suction cups have diameters of 2 to 3 cm and pore volumes (i.e. dead volumes) in the mL range. The rather large volumes limit their application where high spatial resolution is required, e.g. to distinguish between bulk and rhizosphere soil solution, or when the influence of the sampling on water fluxes or soil structure needs to be minimal. In order to allow for a higher spatial resolution and to reduce disturbances, micro suction cups with diameters between 1 and 2.5 mm have been developed during the last decade. The most common types are polymerous cups (Eijkelkamp Agrisearch Equipment, Netherlands), ceramic capillaries (PI Ceramic, Germany) and hollow fibers made of polyvinyl alcohol, polysulfone or polyacrylonitrile (Yanai et al., 1993; Jones and Edwards, 1993; Menzies and Guppy, 2000).

The influence of micro suction cup materials on soil solution samples has been studied by Jones and Edwards (1993), Yanai et al. (1993), Göttlein et al. (1996), Spangenberg et al. (1997), Cabrera (1998). Only Menzies and Guppy (2000) studied the influence of a micro cup sampler on TM in the soil solution. They found that no inorganic analytes including TM were sorbed in detectable quantities using a polyacrylonitrile hollow fiber.

The aim of our work was to fill a number of knowledge gaps identified above. In particular, data are missing for alkaline conditions and for the influence of DOC. Little data is available for micro suction cups. For this purpose, we performed laboratory tests in which we compared different commercially available standard-size and micro suction-cups. The tests were performed at weakly acidic and alkaline pH, at an ionic strength typical for soil solutions in forest soils, at TM concentrations found in polluted and non-polluted soils and with different additions of DOC.
2.3 Material and methods

2.3.1 Suction cups characteristics

a) Standard size suction cups

Four types of commercially available standard size suction cups were tested:
1) A ceramic type from Soilmoisture Equipment Corp. (Santa Barbara, U.S.A). 2) A borosilicate glass type (EcoTech GmbH, Bonn, Germany). 3) A Teflon (PTFE) type (Prenart ApS, Copenhagen, Denmark). The Teflon material, which is in contact with the soil solution, is intimately mixed with a silicate to reduce its hydrophobicity. 4) A nylon membrane type (EcoTech GmbH, Bonn, Germany). The nylon membrane is supported by a polyethylene substructure and covered by a polyethylene shield.

b) Micro suction cups

Three different micro suction cup materials were tested:
1) A ceramic capillary (PI Ceramic, Lederhose, Germany). The ceramic capillary is a new type of ceramic made from pure aluminium oxide (very few contamination products). 2) A hollow fibre made of polyvinyl alcohol (PVA; Yanai et al., 1993). 3) A tube made of an unknown polymer (Rhizon MOM; Eijkelkamp Equipment, Giesbeek, Netherlands).

Physical dimensions and properties of the different standard size suction cups are given in Table 2.1 and of the different micro suction cups in Table 2.2.

Table 2.1: Properties of the standard size suction cups as given by the manufacturer, except pore volume that was determined in this study. Average values are listed except for pore size.

<table>
<thead>
<tr>
<th>Cup material</th>
<th>Ceramic</th>
<th>Borosilicate glass</th>
<th>Teflon</th>
<th>Nylon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cup dimension L x OD&lt;sup&gt;a&lt;/sup&gt; [cm]</td>
<td>5.5 x 2.1</td>
<td>6 x 3.2</td>
<td>9.5 x 2.1</td>
<td>9.5 x 3.2</td>
</tr>
<tr>
<td>Porous area [cm&lt;sup&gt;2&lt;/sup&gt; (cm length)&lt;sup&gt;-1&lt;/sup&gt;]</td>
<td>6.60</td>
<td>10.05</td>
<td>6.60</td>
<td>10.05</td>
</tr>
<tr>
<td>Pore volume [mL (cm length)&lt;sup&gt;-1&lt;/sup&gt;]</td>
<td>0.4</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Max. pore size [μm]</td>
<td>2.5</td>
<td>1</td>
<td>2</td>
<td>0.45</td>
</tr>
<tr>
<td>Bubble point [hPa]</td>
<td>1500</td>
<td>1500</td>
<td>450</td>
<td>2000</td>
</tr>
</tbody>
</table>

<sup>a</sup> outer diameter
Table 2.2: Properties of the micro suction cups materials as given by the manufacturer or in the literature (see Material and Methods section), except pore volume that was determined in this study. Average values are listed except for pore size.

<table>
<thead>
<tr>
<th>Cup material</th>
<th>Ceramic</th>
<th>Polyvinyl alcohol</th>
<th>Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD [cm]</td>
<td>0.1</td>
<td>0.09</td>
<td>0.25</td>
</tr>
<tr>
<td>Porous area [cm² (cm length)^{-1}]</td>
<td>0.31</td>
<td>0.28</td>
<td>0.79</td>
</tr>
<tr>
<td>Pore volume [µL (cm length)^{-1}]</td>
<td>2.8</td>
<td>1.8</td>
<td>10</td>
</tr>
<tr>
<td>Max. pore size [µm]</td>
<td>1</td>
<td>0.04</td>
<td>0.1 (average)</td>
</tr>
<tr>
<td>Bubble point [hPa]</td>
<td>n.s.¹</td>
<td>n.s.</td>
<td>2000</td>
</tr>
</tbody>
</table>

¹ outer diameter
² not specified

2.3.2 Laboratory tests

a) Standard size suction cups

Prior to the experiments, the standard suction cups were cleaned, first with 100 mL 1 M HCl, then with 100 mL 1 M NaOH, at a flow rate of 40 mL h^{-1}, by means of a peristaltic pump. Before and after the NaOH washing, the cups were rinsed with doubly-deionised water (Millipore Milli-Q). The final rinsing was stopped when pH and conductivity had reached the values of doubly-deionised water. Then synthetic soil solutions were percolated through the cups. The composition of these solutions is given in Table 2.3. They mimic the composition of soil solutions from an acidic (pH 4.5) and a calcareous (pH 7.5) sandy soil. All chemicals were reagent grade or higher. The pH was adjusted to the desired value by means of HCl (Merck suprapur) or NaOH (Merck pro analysis). Trace metals were added to the synthetic soil solutions at 3 different concentrations (level 1-3, Table 2.3). A stepwise protocol was adapted from Grossmann et al. (1990) as follows:

step 0: pre-conditioning phase with percolation of 2500 mL solution with TM concentrations at level 1.
step 1: percolation of 250 mL solution with TM concentrations at level 2.
step 2: percolation of 250 mL solution with TM concentrations at level 3.
step 3: percolation of 250 mL solution with TM concentrations at level 1.
Sorption of trace metals by standard and micro suction cups

Step 4: percolation of 250 mL solution with TM concentrations at level 3 plus 9 mg L\(^{-1}\) DOC.

A flow rate of 20 mL h\(^{-1}\) was chosen, which is comparable to field conditions.

**Table 2.3:** Composition of the solutions used in the laboratory tests of suction cup materials. For the standard size suction cups, TM concentrations refer to the concentrations in step 3, 1, and 2, respectively, and DOC to the concentrations in step 1/2/3 and 4, respectively. For the micro suction cups, TM concentrations refer to the concentrations in step 1/3/5, 2 and 4, and pH, DOC and other ions refer to the concentrations in step 1/3/5/2 and 4, respectively.

<table>
<thead>
<tr>
<th>Ion [(\mu\text{M})]</th>
<th>Standard size suction cups</th>
<th>Micro suction cups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>acidic solution</td>
<td>alkaline solution</td>
</tr>
<tr>
<td>pH</td>
<td>4.5</td>
<td>7.5</td>
</tr>
<tr>
<td>DOC [mg L(^{-1})]</td>
<td>0/9(^a)</td>
<td>0/9(^a)</td>
</tr>
<tr>
<td>Cu</td>
<td>0.083/0.153/1.408</td>
<td>0.018/0.061/1.046</td>
</tr>
<tr>
<td>Zn</td>
<td>0.426/0.820/7.005</td>
<td>0.356/0.688/6.08</td>
</tr>
<tr>
<td>Cd</td>
<td>0.011/0.027/0.262</td>
<td>0.013/0.028/0.245</td>
</tr>
<tr>
<td>Pb</td>
<td>0.003/0.006/0.045</td>
<td>0.001/0.004/0.024</td>
</tr>
<tr>
<td>Na</td>
<td>113</td>
<td>114</td>
</tr>
<tr>
<td>Mg</td>
<td>217</td>
<td>216</td>
</tr>
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<td>K</td>
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</tr>
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<td>Ca</td>
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<td>1933</td>
</tr>
<tr>
<td>Cl</td>
<td>418</td>
<td>1031</td>
</tr>
<tr>
<td>NO(_3)</td>
<td>161</td>
<td>1175</td>
</tr>
<tr>
<td>SO(_4)</td>
<td>519</td>
<td>522</td>
</tr>
<tr>
<td>CO(_3)</td>
<td>0</td>
<td>835</td>
</tr>
</tbody>
</table>

\(^a\) leaf litter extract  
\(^b\) free soil percolation water

The DOC added to the solution in the last step was obtained from an extract of litter from spruce (Picea abies L.), poplar (Populus tremula L.), birch (Betula pendula L.) and willow (Salix viminalis L.). Extraction was performed according to Luster et al. (1996).
Sorption of trace metals by standard and micro suction cups

At different time steps, metal concentrations in the percolates were compared with the initial concentrations.

In an additional experiment, we investigated the DOC sorption by nylon cups. Cups were first washed with 1 M NaOH, rinsed with 1 L of doubly-deionised water and then percolated with one of the following two solutions:
- synthetic soil solution of pH 4.5 or pH 7.5 to which TM and 9 mg L$^{-1}$ DOC from the same leaf and needle litter extract were added, as in step 4 of the previous experiment.
- percolation water collected at the bottom of a lysimeter stocked with young forest vegetation (mixed stand of spruce, poplar, willow and birch). The lysimeter soil consisted of 15 cm top soil composed of a homogenised weakly acid loam, 80 cm subsoil composed of a calcareous loamy sand from a river bank and a 50 cm quartz sand and gravel drainage packing (Table 2.3, alkaline solution of micro suction cups, step 4).

b) Micro suction cups

The ceramic and PVA micro suction cups were cleaned with 6.5 mL 1 mM HNO$_3$ at a flow rate of 2 mL h$^{-1}$, the polymer cups with 13 mL of a 1 mM HNO$_3$ solution at a flow rate of 4 mL h$^{-1}$. Then, the ceramic and PVA cups were rinsed with about 25 mL doubly-deionised water, the polymer cups with about 50 mL, until pH and conductivity of the rinsing water remained almost unchanged after percolation through the cups.

The sequence of test solutions percolated through the micro suction cups was slightly different from the one used for the standard size suction cups:
- step 1: percolation of 12 mL (25 mL for polymer cups) synthetic soil solution with TM concentrations at level 1.
- step 2: percolation of 12 mL (25 mL for polymer cups) solution with TM concentrations at level 3.
- step 3: percolation of 12 mL (25 mL for polymer cups) solution with TM concentrations at level 1.
- step 4: percolation of 12 mL (25 mL for polymer cups) free percolation water from either a lysimeter with a calcareous subsoil as described above or with an acidic subsoil and spiked with TM at level 3.
step 5: percolation of 12 mL (25 mL for polymer cups) synthetic soil solution with TM concentrations at level 1.
The flow rate was similar as for the cleaning procedure. The composition of the various solutions is given in Table 2.3.

2.3.3 Analytical methods
Concentrations of Cu, Zn, Cd, Pb, Na, Mg, K, Ca and Mn in solution were determined by inductively-coupled plasma mass spectrometry (Elan 6000; Perkin-Elmer, Wellesley, MA), concentrations of Cl\(^-\), NO\(_3\)\(^-\), PO\(_4\)\(^{3-}\) and SO\(_4\)\(^{2-}\) by ion chromatography (DX-120; Dionex, Sunnyvale, CA), DOC with a TOC-Analyser (Shimadzu TOC-V, Tokyo, Japan) and pH by means of a glass electrode (C 4000-8; Radiometer Analytical SAS, Villeurbanne, France). Prior to analysis, the samples were filtered through membrane filters of 0.45 μm pore size (ME 25; Schleicher and Schuell, Dassel, Germany). The light absorption by DOC was measured by spectrometry at 254 nm (Varian Cary 50, Malgrave, Australia). Accuracy of all analytical data was checked by analysing certified standard reference materials with each series of analysis. With these measures, a laboratory precision of 0.15 units for pH, and less than 10 % for all other parameters, was achieved. Metal speciation in soil solution was calculated by means of the WHAM 6.0 model (Tipping, 1998).

2.4 Results

2.4.1 Cleaning of standard size suction cups
Acid washing led to an initial release of Pb from nylon and ceramic suction cups. At the end of the washing phase, Pb levels in the percolate were below the detection limit. Large amounts of Si and also some Na were released from the borosilicate glass cups, whereas Mg, Al, Ca and Cl\(^-\) were released from the ceramic cups. Nylon cups released about 4 mg L\(^{-1}\) DOC during their first use. After washing with NaOH and rinsing with 4 L distilled water, the DOC release decreased to about 0.5 mg L\(^{-1}\) and remained constant as long as the cups were in use. The release of DOC was larger at alkaline (0.8 mg L\(^{-1}\)) than at acidic conditions (0.4 mg L\(^{-1}\)). Dissolved organic carbon release from Teflon and ceramic cups was only about half of that of nylon cups.
2.4.2 Cleaning of micro suction cups

The trace metals Cu, Zn, Cd and Pb were detected in the outflow of the cups during the acid washing procedure. Zinc release from all 3 cups ranged between 0.4 and 0.5 μM and Cu release between 0.04 and 0.08 μM. Ceramic cups leached almost 4 μM Pb and 1.2 nM Cd, polymer cups less than 0.6 nM Cd and no Pb. The PVA cups did not release any Cd and about 0.09 μM Pb. Significant amounts of Al, K and Mn were released only by the ceramic cups. Also Cl⁻, NO₃⁻ and SO₄²⁻ were detected in the percolate of the cups, reaching concentrations of about 2 μM, 1000 μM and 1 μM, respectively. At the end of the rinsing phase, the cation concentrations did no longer exceed the detection limit while the anion concentrations remained above their detection limits.

a) Sorption of trace metals, major cations and anions by standard size suction cups at acidic pH

The cadmium concentrations in the outflow of all cups closely followed the initial concentrations under all tested conditions (Figure 2.1). Also Zn exhibited weak sorption towards the end of step 3 (highest TM level), more at ceramic than at Teflon and nylon cups (Table 2.4, Table 2.5). At lower concentrations neither Cd nor Zn retention occurred. Lead was completely sorbed by Teflon and ceramic cups at the highest concentrations, somewhat less at lower ones, while almost no Pb was sorbed by nylon cups. Recovery of all TM in the percolating solution is shown in Table 2.4 and sorption characteristics are summarized in Table 2.5.

Figure 2.2 shows the Cu concentrations of the acidic synthetic soil solution before and after passing through the cups. Copper concentrations in the percolate of the nylon cup closely followed the initial concentrations at the beginning of each percolation step. However, some Cu was sorbed after leaching 35 pore volumes of the solution with the highest concentration (step 2) in the absence of DOC. Sorption reached 16% at the end of the step. At the lower concentration (step 1), no sorption was observed. The ceramic cup showed strong retardation of the Cu breakthrough. Even after 114 pore volumes, concentrations in the percolate had not yet reached the initial concentrations, in particular in presence of DOC. For the PTFE cup, Cu retardation was even stronger.
Sorption of trace metals by standard and micro suction cups

Figure 2.1: Cd concentrations in the percolate collected from different standard size suction cups at pH 4.5. Volumes are given in multiples of the pore volume of the respective cup. The reference lines show the expected metal concentrations if no interaction with the cup materials occurred, i.e. the concentration measured in the initial solution.

Figure 2.2: Cu concentrations in the percolate collected from different standard size suction cups at pH 4.5. Volumes are given in multiples of the pore volume of the respective cup. The reference lines show the expected metal concentrations if no interaction with the cup materials occurred, i.e. the concentration measured in the initial solution.
Table 2.4: Percentage of metal, anion and DOC recovery with standard suction cups in absence/presence of DOC from a leaf litter extract (step 3 and 5, respectively, both with the highest TM concentrations) after 88 pore volumes for nylon, 66 for Teflon, 83 for glass and 68 for ceramic (corresponding approximately to a percolate of 250 mL for all cups except ceramic (150 mL), n.d.: not determined).

<table>
<thead>
<tr>
<th></th>
<th>Nylon</th>
<th>Teflon</th>
<th>Glass</th>
<th>Ceramic</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.5</td>
<td>7.5</td>
<td>4.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Cu</td>
<td>84/95</td>
<td>41/80</td>
<td>11/25</td>
<td>n.d./n.d.</td>
</tr>
<tr>
<td>Zn</td>
<td>81/92</td>
<td>88/98</td>
<td>84/90</td>
<td>4/206</td>
</tr>
<tr>
<td>Cd</td>
<td>91/99</td>
<td>84/88</td>
<td>93/89</td>
<td>22/77</td>
</tr>
<tr>
<td>Pb</td>
<td>91/87</td>
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<td>3/7</td>
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<td>103/101</td>
<td>98/102</td>
<td>102/98</td>
</tr>
<tr>
<td>NO₃</td>
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<td>101/96</td>
<td>96/95</td>
<td>103/97</td>
</tr>
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<tr>
<td>DOC</td>
<td>56</td>
<td>66</td>
<td>70</td>
<td>62</td>
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Breakthrough extended into the subsequent steps when the next solution was already applied.

The major nutrient anions were only very weakly sorbed, except that nylon retained about 10% of the applied nitrate. All cups showed some Na, Mg and Ca and strong K retention (Table 2.4).

b) Sorption of trace metals, major cations and anions by standard size suction cups at alkaline pH

The retention of Cd increased in the order nylon < Teflon << glass = ceramic (Figure 2.3). Glass and ceramic cups sorbed Cd almost completely. At lower metal concentrations, the sequence of retention on the different cups was similar. Addition of DOC resulted in the desorption of previously sorbed Cd from the ceramic cup after about 75 pore volumes. Zinc sorption looked like Cd but was found slightly lower for
Table 2.5: Qualitative evaluation of the general sorption characteristics of the different standard size suction cup materials. This evaluation is based on the laboratory tests with synthetic soil solution of pH 4.5 and 7.5 in the absence/presence of DOC (step 3/5, see Material and Methods) (0 negligible sorption (<5%); +: low sorption (5-20%), ++: considerable sorption (20-40%), +++: high sorption (>40%), d: desorption, n.d.: not determined).

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<td>+++/d+++</td>
<td>+++</td>
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<td>+++/d+++</td>
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</tbody>
</table>

Figure 2.3: Cd concentrations in the percolate collected from different standard size suction cups at pH 7.5. Volumes are given in multiples of the pore volume of the respective cup. The reference lines show the expected metal concentrations if no interaction with the cup materials occured, i.e. the concentration measured in the initial solution.
nylon and higher for the other cup materials (Table 2.4 and Table 2.5). Zinc was retained on Teflon cups in absence of DOC but was desorbed as soon as DOC was added (Table 2.4 and Table 2.5).

There was much less Cu sorption in the presence than in the absence of DOC (Figure 2.4). All cup materials except nylon showed complete sorption of Cu in the absence of DOC at pH 7.5. Lead was almost completely sorbed under all conditions (Table 2.4 and Table 2.5). With regard to major nutrient ions, initial concentrations were generally reached except for Ca, Mg and Na which were partly retained by all cups (Table 2.4). Furthermore, glass cup sorbed K completely.

![Figure 2.4: Cu concentrations in the percolate collected from different standard size suction cups at pH 7.5. Volumes are given in multiples of the pore volume of the respective cup. The reference lines show the expected metal concentrations if no interaction with the cup materials occurred, i.e. the concentration measured in the initial solution.](image)

c) **Sorption of trace metals, major cations and anions by micro suction cups at acidic pH**

Cadmium concentrations in the percolate of 3 different micro suction cups are plotted together with the initial concentrations in Figure 2.5. Both Cd and Zn concentrations were not much affected by any cup material (Figure 2.5; Table 2.6). In average, Pb was least sorbed on polymer cups (Table 2.6). As some Pb was released from the ceramic
Sorption of trace metals by standard and micro suction cups material during the entire test, these data were not further evaluated (see question marks in Table 2.6).

Figure 2.5: Cd concentrations in the percolate collected from different micro suction cups at pH 4.5. Volumes are given in multiples of the pore volume of the respective cup. The reference lines show the expected metal concentrations if no interaction with the cup materials occurred, i.e. the concentration measured in the initial solution.

Table 2.6: Qualitative evaluation of the general sorption characteristics of the different micro suction cups. This evaluation is based on the laboratory tests, with synthetic soil solution of pH 4.5 and 8.0 in the absence/presence of DOC (step 2/4, see Material and Methods) (0 negligible sorption (<5%); +: low sorption (5-20%); ++: considerable sorption (20-40%), +++: high sorption (>40%), d: desorption, n.d.: not determined).

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<td>0/+++</td>
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</tr>
</tbody>
</table>
Sorption of trace metals by standard and micro suction cups

Initial Cu concentrations were reached after 71 and 278 pore volumes with both PVA and ceramic cups, respectively (Figure 2.6). The polymer cup retained less Cu in presence of DOC (Table 2.6). Recoveries could not been reported in a table since the percolation volumes used divided by the dead volume of the different micro suction cups gave very different pore volumes.

No sorption of major cations (Na, Mg, K, Ca) and anions (Cl\(^-\), NO\(_3^-\), SO\(_4^{2-}\)) was observed.

![Figure 2.6: Cu concentrations in the percolate collected from different micro suction cups at pH 4.5. Volumes are given in multiples of the pore volume of the respective cup. The reference lines show the expected metal concentrations if no interaction with the cup materials occurred, i.e. the concentration measured in the initial solution.](image)

Cups made of PVA retarded Cd and Zn breakthrough (Figure 2.7). In absence of DOC, initial Cd concentrations were reached with delay with ceramic cups. Cadmium sorption by polymer cups was reduced by DOC (Figure 2.7). Sorption of Zn by PVA and polymer cups was quite similar to Cd (Table 2.6). Additional Cu and Pb in step 2 (no DOC present) was almost completely sorbed by all cup materials (Figure 2.8 and Table 2.6). In the presence of DOC, however, Cu was less sorbed or was even desorbed from the
Figure 2.7: Cd concentrations in the percolate collected from different micro suction cups at pH 8.0. Volumes are given in multiples of the pore volume of the respective cup. The reference lines show the expected metal concentrations if no interaction with the cup materials occurred, i.e. the concentration measured in the initial solution.

Figure 2.8: Cu concentrations in the percolate collected from different micro suction cups at pH 8.0. Volumes are given in multiples of the pore volume of the respective cup. The reference lines show the expected metal concentrations if no interaction with the cup materials occurred, i.e. the concentration measured in the initial solution.
cup material. The addition of DOC resulted in the desorption of previously sorbed Pb from the cups, except for complete sorption of Pb by the ceramic material.

No retention was observed for major cations (Na, Mg, K, Ca) and anions (Cl\(^-\), NO\(_3^-\), SO\(_4^{2-}\)) (data not shown).

e) Sorption of DOC by standard size suction cups

In step 5 of the laboratory test, somewhat less DOC from a leaf litter extract was retained by nylon and ceramic cups from the alkaline synthetic soil solution than from the acidic one (Table 2.4). It was the other way around for cups made from teflon. For the alkaline solution, the lowest retention was observed for the glass cup (Table 2.4). In the separate test of the alkaline percolation water from the lysimeter, UV absorption matched the initial values almost immediately and remained unchanged for more than 420 dead volumes (data not shown).

f) Sorption of DOC by micro suction cups

Light absorption at 256 nm of the TM spiked acid drainage water from the lysimeters (step 4 of the tests for metal sorption) was reduced by about 10% in the outflow of all cups compared to the light absorption of the initial solution (Figure 2.9). Breakthrough happened somewhat later for ceramic and PVA than for polymer cups. In the case of the alkaline solutions, the light absorption was not reduced by any of the cup materials (Figure 2.9). As for the acidic conditions, breakthrough happened earlier for the polymerous cup.
Sorption of trace metals by standard and micro suction cups

Figure 2.9: UV absorptions measured at 254 nm in the percolate collected from different micro suction cups at pH 4.5 and 8.0. All UV absorptions are expressed against the absorption of the reference in the initial solution. Volumes are given in multiples of the pore volume of the respective cup.

2.5 Discussion

2.5.1 Trace metal sorption in absence of dissolved organic carbon

Trace metal sorption by cup materials was weaker for acidic than alkaline solutions as also found in previous studies (Grossmann et al., 1990; Wenzel and Wieshammer, 1995; Wenzel et al., 1997; Andersen et al., 2002). The higher concentration of protons at low pH as an additional competitor of the TM for the binding sites at the cup material results in an earlier breakthrough of TM in the effluent of the suction cups. In general, metal sorption by the cups increased in the order: Cd < Zn << Cu < Pb. This confirms the findings of Wenzel and Wieshammer (1995), that Zn and Cd are generally less strongly sorbed than Pb and Cu. This sorption sequence of TM at cup materials corresponds to metal sorption onto clay, silicate or oxide minerals (McBride, 1989).

Our data generally confirmed results of previous studies that reported virtually no effect of nylon on sampled TM concentrations (Grossmann et al., 1990; Grossmann and Udluft, 1991; Wenzel and Wieshammer, 1995; Wenzel et al., 1997), except for Pb,
Sorption of trace metals by standard and micro suction cups

as previously noted by Grossmann et al. (1990) and Grossmann and Udluft (1991). The response of the metal concentrations in the outflow from nylon cups to changes in the initial concentration was very fast.

Teflon cups have not yet been evaluated systematically with respect to their influence on TM concentrations under realistic conditions. In our tests, Teflon cups showed similar effects as ceramic cups under both acidic and alkaline conditions, i.e. strong sorption except for Zn and Cd at pH 4.5. This strong metal retention capacity which is not expected from plastic materials because of their lower CEC, can be explained by the silicate material that is mixed into the Teflon in order to decrease hydrophobicity (Prenart ApS) (Maitre et al., 1991).

Ceramic cups proved to be unsuitable for monitoring TM in solution, as was expected because of their high CEC and pH-dependent specific metal binding sites (Grossmann et al., 1990). The borosilicate glass material showed by far the worst behaviour, as metals were almost completely sorbed under all conditions.

Our investigation of micro suction cups showed that the pure aluminium oxide ceramic used for the construction of the micro capillaries sorbed TM much less than the ceramic material used for the construction of the standard suction cups, especially at acidic pH. The polymerous cups proved to be suited for Zn and Cd sampling but performed worst with Cu. The PVA cups showed very little metal sorption except for Cu and Pb under alkaline conditions.

In order to reduce metal sorption, a pre-conditioning phase, as previously proposed by other authors (Grossmann et al., 1990; Guggenberger and Zech, 1992; Koch and Grupe, 1993; Wenzel and Wieshammer, 1995), is recommended to get reliable data, as it allows the cup materials to equilibrate more rapidly with the soil solution.

2.5.2 Sorption of dissolved organic carbon

The "new" DOC originating from a leaf litter extract was much more sorbed on cup materials than the "old" DOC from the lysimeter drainage water. The higher sorption can be related to the higher hydrophobicity. Dissolved organic carbon from a leaf litter extract corresponds to DOC from fresh litter before leaching into the soil. Guggenberger et al. (1998) found that functional groups were reduced as DOC leached through the soil profile. O-alkyl C increased while carboxyl C and aromatic C decreased with depth due to selective sorption of the latter two more hydrophobic DOC fractions. The DOC fraction in soil leachate, which corresponds to the lysimeter drainage water
used in our study, as "old" DOC source, is chemically similar to the hydrophilic fraction of the litter extract, with weaker sorption properties, higher degree of oxidation, increased saccharides concentration and lower molecular weight than the hydrophobic fraction (Guggenberger et al., 1998; Huang et al., 1998). The assumption that the DOC sorbed on the cup materials was primarily the more reactive hydrophobic fraction is also supported by the finding of Guggenberger and Zech (1992) that the hydrophobic fractions of DOC had decreased after flushing soil solution through ceramic P80 suction cups. The higher the content of hydrophobic DOC, the more sorption occurred at the cup material, in particular when also the latter was hydrophobic. Of the different cup materials investigated in our study, borosilicate glass, which had been specifically developed for the monitoring of DOC, sorbed the smallest amount of "new" DOC. Ceramics, because of their hydrophilic properties, sorbed less DOC than the more hydrophobic materials Teflon and nylon. "Old" DOC, i.e. DOC from the lysimeter drainage water sorbed much less onto the nylon cups.

Comparing DOC from the same source, our tests showed generally more DOC sorption at lower than at higher pH. Such increase of DOC sorption on mineral surfaces with decreasing pH is well-known (Tipping and Hurley, 1988).

The release of some DOC from the nylon cups themselves is not expected to affect metal concentration by complexation according Wenzel et al. (1997).

2.5.3 Trace metal sorption in presence of dissolved organic carbon

Dissolved organic carbon can influence TM retention by cup materials in two ways. Firstly, DOC can complex the metals. If the complexes are not sorbed, this can prevent metal retention by the cups. Speciation calculations performed with the WHAM 6.0 model (Tipping, 1998) for both acidic and alkaline synthetic solutions in presence of DOC predicted a complexation rate with DOC at pH 4.5 of 67% for Cu, 4% for Zn, 4% for Cd and 52% for Pb. At pH 7.5, the calculated complexation rate with DOC was higher with 85% for Cu, 7% for Zn, 10% for Cd and 94% for Pb. Secondly, DOC sorption by the suction cups can increase their exchange capacity, and, thus, the sorption of some metals (Grossmann et al., 1990). In most cases we noted less metal sorption with than without DOC, at given pH and TM concentrations. Thus, DOC prevented metal sorption rather than enhancing it. This was particularly obvious at alkaline pH where, in some cases, the addition of DOC led to strong release of previously sorbed metals. At pH 4.5, this DOC effect was less pronounced, which can
be explained by the weaker complexation of TM by DOC (Christensen and Christensen, 2000) as well as by stronger sorption of DOC to the cup materials at low pH. In some cases we found that DOC addition increased TM sorption to the cups, e.g. in the case of Cu sorption by ceramic standard suction cups at pH 4.5. Since "new" DOC is more sorbed by cups than "old" DOC, our results suggest that more metals are sorbed in the presence of "new" DOC than in the presence of "old" DOC.

Borosilicate glass was included in this study because it was supposed to exhibit low DOC sorption. Our results confirmed this expectation. The hypothesis that this would also lead to lower metal retention in presence of DOC due to the formation of stable soluble organo-metal complexes, however, was not confirmed. All TM were sorbed completely at the glass cup also in presence of DOC. Either, the adsorptive forces between TM and binding sites on the glass were stronger than the bonds between TM and DOC, or the metal-DOC complexes were sorbed to the binding sites of the glass, e.g. by the formation of ternary complexes. Nylon and PVA proved to be the most suitable materials for monitoring TM in the presence of DOC.

The studies of Guggenberger and Zech (1992) and of Grossmann et al. (1990) were the only investigations we found in the literature on the influence of DOC on metal sorption by suction cups. Guggenberger and Zech (1992) compared Al, Cu, Pb, SO$_4^{2-}$ sorption in presence of DOC by field-equilibrated and new acid-washed ceramic suction cups. Their results with respect to Cu, Pb and SO$_4^{2-}$ sorption agree with ours. Grossmann et al. (1990) studied TM sorption onto nylon, aluminium oxide and ceramic cups in the presence and absence of DOC extracted from a Rendzina. Ceramic cups were not tested for metal sorption in presence of DOC because they were assumed to behave like aluminium oxide. The results obtained with nylon cups generally agree with ours.

2.6 Conclusions

Systematic evaluation of a given suction cup material under realistic conditions is the basis for a correct interpretation of soil solution data and for avoiding mistakes in calculating metal fluxes and element budgets. Our tests highlight the importance of taking the effects of DOC on the sorption of trace elements into account. Metal retention was generally reduced in the presence of DOC, especially at alkaline pH. "New" DOC obtained from leaf litter sorbed more strongly onto the different cup
materials than the "old" DOC. This explains why the "old" DOC is expected to mobilize TM and reduce metal sorption by the cups more effectively than the "new" DOC. Thus, the accuracy in TM monitoring of soil solution can be assumed to be higher in deeper mineral soil horizons than just below the litter layer. At acidic pH the mobilizing effect of DOC was reduced due to the stronger sorption of DOC onto the cups. Nylon membranes proved to be the most suited material available for the construction of suction cups if metals are going to be analysed. Only Cu in absence of DOC and Pb in absence and presence of DOC were sorbed strongly onto nylon at alkaline pH. Hollow fibers made of polyvinyl alcohol (PVA) were found to be the best suited material for the construction of micro suction cups. They showed very little sorption of metals, except for Cu at alkaline pH in the absence of DOC and for Pb in both absence and presence of DOC.

2.7 Acknowledgements

We thank the staff of the Central Laboratory of the Swiss Federal Research Institute WSL for performing most of the chemical analyses. We are also grateful to the technical staff of the department soil ecology at WSL, Roberto Bucheli, Daniel Christen, Noureddine Hajjar, Beat Peter and Alois Zürcher for their help with analyses. We thank Axel Göttlein for providing the ceramic capillaries. This research was funded partly by the Swiss National Science Foundation and the Swiss Federal Research Institute WSL.
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Chapter 3

Heavy metal concentrations and speciation in the soil solution of a forest model ecosystem: Seasonal variability and plant influence

D. RAIS, B. NOWACK, R. SCHULIN, L. Y.L. ZHAO, J. LUSTER

Submitted to Environmental Science & Technology
3.1 Abstract

In a lysimeter study aiming at evaluating the potential of trees to phytostabilise heavy metal contaminated soils, the heavy metal concentrations and speciation in soil solution of young forest model ecosystems were investigated. The following factors were varied: topsoil with or without heavy metals, subsoil acidic or calcareous, and presence or absence of plants. Copper, Zn, and Cd concentrations were significantly larger in the contaminated topsoils than in the control, both in the absence and presence of plants, but varied relatively little over time. While Cu was governed by complexation with DOC, Zn and Cd variations in the contaminated topsoils were negatively correlated with phosphate. Based on this, plant uptake of phosphate was assumed to be responsible for about two times larger Zn and Cd concentrations in the presence than in the absence of plants. On the other hand, Cu concentrations did not differ between planted and unplanted systems. However, smaller fractions of free Cu ($Cu^{2+}$) in the absence than in the presence of plants indicated a difference in the metal binding properties of DOC. In the acidic subsoil, Zn concentrations were somewhat larger below contaminated topsoil than below uncontaminated topsoil in the presence of plants, with maximum concentrations in winter and spring. In the calcareous subsoil, none of the heavy metals was increased below the contaminated topsoil.

3.2 Introduction

Large contents of heavy metals (HM) in soils can have adverse effects on soil biota, plant growth and the quality of crops. In addition, groundwater and surface water quality can be adversely affected if potentially toxic metals are leached from contaminated sites. Total HM contents alone are considered poor predictors of mobility and bioavailability of metals in soils (Tack and Verloo, 1995). The chemical reactivity of a metal depends on its concentration and speciation in the soil solution (Stumm and Morgan, 1996). According to the free-ion activity model, bioavailability and toxicity of metals to aquatic organisms are determined by the concentrations of the free-metal ions (Brezonik et al., 1991). The validity of the model is also generally supported in plant nutrition (Parker et al., 1995). Recently adopted approaches to define critical loads of HM are based on dissolved concentrations of HM in the soil solution (Rieuwerts et al., 1998).
Heavy metal concentrations and speciation in the soil solution

The presence of plants can strongly influence the composition of the soil solution, and, thus, also the concentration of metal species. This can occur e.g. through direct metal uptake by roots, excretion of $\text{H}^+$ or $\text{HCO}_3^-$ in exchange to the uptake of nutrient ions (Marschner, 1995), consumption of $\text{O}_2$ in the rhizosphere (McLaughlin et al., 1998), and exudation of low-molecular weight organic substances that can form metal complexes (Jones and Darrah, 1994; McLaughlin et al., 1998; Dousset et al., 2001). In addition, the production of dissolved organic carbon (DOC) from decomposing soil organic matter and residues is controlled by biological activity (Dai et al., 1996) that in turn is closely related to the activity of plants (Kandeler et al., 2002). Therefore, the influence of plants on metal concentrations and speciation in soil solution can be expected to show seasonal fluctuations corresponding to the annual cycle of plant activities.

Tyler (1981), Bergkvist (1987) and Keller and Domergue (1996) reported seasonal variations in metal concentrations of soil solutions collected with zero-tension lysimeters from unpolluted soils below forest vegetation and attributed these to changes in acidity, DOC and soil moisture. On the other hand, Denaix et al. (2001) found no seasonal variation in dissolved Zn, Cd and Pb concentrations of a metal-contaminated acidic soil under birch and grass vegetation. Also Federer and Sticher (1994) found no significant seasonal variations of Cu, Zn, and Cd concentrations for a calcareous fluvisol covered with grass and polluted by dust emissions from a metal smelter. Using soil lysimeters spiked with sewage sludge, McLaren et al. (2004) found slightly larger concentrations of Cd and Zn in the leachate from bare forest soils than from grass covered pasture soils, ascribed this to larger metal uptake by the grass, but gave no information on seasonal fluctuations in the composition of the leachates. Derome and Nieminen (1998) sampled the soil solution of contaminated podsols in dependence of the distance to a metal smelter using zero-tension lysimeters. They observed large fluxes of Cu and Zn in the vicinity of the smelter and explained this with the damaged tree stands and ground vegetation. This would argue in favour of reduced metal mobility in the presence of plants.

The limited number of investigations dedicated to the effect of vegetation on the concentration and speciation of heavy metals in the soil solution of contaminated and uncontaminated soils justifies further systematic in-situ studies in order to better assess the influence of plants on the mobility and bioavailability of heavy metals in soils. One
particular question is whether there is a seasonal variation in the risk involved for organisms and groundwater.

Therefore, we wanted to examine the effects of vegetation and their seasonally varying activity on Cu, Zn and Cd concentrations and speciation in the soil solution of metal contaminated soils. This study was part of a large model ecosystem experiment during which numerous plant and soil parameters were investigated in order to evaluate the potential of trees to phytostabilise HM contaminated sites.

3.3 Material and Methods

3.3.1 Experimental set-up and treatments

The model ecosystems were established in May 2000 in the Open Top Chamber (OTC) facility of the Swiss Federal Institute for Forest, Snow and Landscape Research in Birmensdorf (Switzerland), which consists of hexagonal OTC of 3 m height and 3 m width. Below ground, each OTC contains two lysimeters with 3 m² surface area each. Each lysimeter was planted with the same collection of trees, including 6 Norway spruce (*Picea abies* L.), 2 birch (*Betula pendula* L.), 2 willow (*Salix viminalis* L.), and 4 poplar (*Populus tremula* L.) as well as 6 herbaceous plant species. The soil in the lysimeters consisted of a topsoil (0 to 15 cm depth), a subsoil (15 to 95 cm depth), and a drainage packing of quartz sand layers with increasing grain size towards the bottom (95 to 145 cm depth). The topsoil was a loam from an agricultural field at Birr (Switzerland). In one lysimeter of each OTC, the subsoil was an acidic loamy sand from an Alisol at Eiken (Switzerland), in the other a calcareous sandy loam from a Calcaric Fluvisol at Brugg (Switzerland). Selected properties of the homogenized soils before they were filled into the lysimeters are given in Table 3.1. In half of the OTC, a mixture of a filter dust from a non-ferrous metal smelter and CdO was incorporated into the topsoil manually using a small harrow. The amount of this contamination was chosen to give average total metal contents of 410 mg kg⁻¹ Cu, 2800 mg kg⁻¹ Zn, 10 mg kg⁻¹ Cd, and 100 mg kg⁻¹ Pb. The manual procedure was chosen to avoid the handling of large amounts of contaminated soil, and because soil solution samplers and soil physical probes had to be installed prior to the contamination in order to avoid subsoil contamination along these instruments. The drawback was a large short-range variability. Total metal contents in 3 selected contaminated lysimeters shortly after addition and before trees were planted span a range from 1000 to 7000 mg kg⁻¹ Zn,
250 to 1700 mg kg\(^{-1}\) Cu, and 50 to 220 mg kg\(^{-1}\) Pb. However, similar high degrees of small-scale variability in metal contents were found previously in metal-contaminated soils under field conditions (Geiger and Schulin, 1995). There were four replicate lysimeters for each treatment combining topsoil contamination or control with each of the two subsoils. As plant-free references, four soil columns, one per treatment, were installed in the immediate vicinity of the OTC at a shaded place. The columns were 78 cm in diameter and packed with topsoil, subsoil and quartz sand in the same way as the OTC lysimeters. At the inside, the column walls made of stainless steel were lined with a polyethylene foil to avoid direct contact with the soil.

<table>
<thead>
<tr>
<th>Table 3.1: Selected physical and chemical properties of the soils used in the model ecosystems (with plants) and plant-free soil columns.</th>
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<tbody>
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<tr>
<td>Texture [% sand; silt; clay]</td>
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<tr>
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<td>C(_{\text{inorg}}) (g kg(^{-1}))</td>
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<tr>
<td>C(_{\text{org}}) (g kg(^{-1}))</td>
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<tr>
<td>P(_{\text{org}}) (mg kg(^{-1}))</td>
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<tr>
<td>CEC (mmol(_{\text{c}}) kg(^{-1}))</td>
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<tr>
<td>Base saturation [%]</td>
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<tr>
<td>Cu(_{\text{tot}}) (mg kg(^{-1}))</td>
</tr>
<tr>
<td>Zn(_{\text{tot}}) (mg kg(^{-1}))</td>
</tr>
<tr>
<td>Cd(_{\text{tot}}) (mg kg(^{-1}))</td>
</tr>
<tr>
<td>Pb(_{\text{tot}}) (mg kg(^{-1}))</td>
</tr>
</tbody>
</table>

Each year, during the growing season from May to September, the model ecosystems and plant-free references were irrigated artificially by sprinkling during the night. The roofs of the OTC closed automatically during rainfall, and the soil columns were shielded with a permanent roof. The irrigation water mimicked the composition of the average local rainfall (70 NH\(_4^+\), 70 NO\(_3^-\), 3.2 PO\(_4^{3-}\), 17 Cl\(^-\), 3.1 SO\(_4^{2-}\), 4.3 Na\(^+\), 7.7 K\(^+\), 5 Ca\(^{2+}\), 1.3 Mg\(^{2+}\), 0.15 Zn\(^{2+}\) (μmol L\(^{-1}\)); pH 5.5) and the amount was adjusted to maintain sufficient soil moisture in the planted systems to allow for optimal plant
growth. From October to April, planted lysimeters and plant-free soil columns received natural precipitation.

In the following, the abbreviation HM is also used to denote treatments with contaminated topsoils. For treatments with uncontaminated topsoil, CO ("control") is used.

3.3.2 Sampling and analysis of soil samples

In each of three selected lysimeters, 35 samples of 40 ml each were taken randomly within a 15 x 15 cm area using a cylindrical auger with an inner diameter of 2 cm and a sampling depth of 12 cm. The samples were dried at 40 °C and sieved to 2 mm. Exchangeable cations were extracted with 1 M NH₄Cl (soil to extractant ratio 1:10; 1 hour at 20 °C on an end-over-end shaker). Total HM contents were determined by means of extraction with 2 M HNO₃ (soil:extractant ratio 1:10; 2 hours at 96 °C on a horizontal shaker). Metal concentrations in both extracts were analysed using inductively-coupled plasma atomic emission spectrometry (Optima 3000, Perkin-Elmer, Wellesley, MA). Inorganic C was expelled from the soil samples as CO₂ by addition of H₂SO₄. The CO₂ was trapped on a resin carrying NaOH and determined gravimetrically. Phosphorus fractions were determined according to Kuo (1996), i.e. total extractable P (P_{extr.}) by extraction of the organic matter free soil (dry digestion at 550 °C) with 0.5 M H₂SO₄, and organically bound P (P_{org.}) by calculation as difference between P_{extr.} and inorganic P (extraction of the untreated soil with 0.5 M H₂SO₄). Phosphorus in all extracts was analysed colorimetrically (Murphy and Riley, 1962). Other soil parameters were determined as described by Blaser et al. (1997). The base saturation was calculated as the ratio between the sum of exchangeable Ca²⁺, Mg²⁺, K⁺, and Na⁺ and the cation exchange capacity.

3.3.3 Soil solution sampling

Soil solutions were sampled at 10, 40 and 70 cm depth from July 2002 until December 2003, i.e. beginning 2 years after installation of the model ecosystems. They were collected using 10 cm long porous suction cups made of a nylon membrane in a supporting structure of polyethylene (EcoTech GmbH, Bonn, Germany). In previous laboratory experiments, this type of suction cups was found to cause the smallest bias (Rais, 2005). The samplers were installed vertically with the center of the cup at a given depth. There were two samplers at each depth in each lysimeter and soil column.
connected with Teflon tubings to the same sampling bottle. Thus, each sample represents a composite sample from two different positions. The vacuum was kept at 500 hPa, except during freezing conditions in winter, when the vacuum pumps had to be switched off. The sampling bottles were made of polypropylene (EcoTech GmbH, Bonn, Germany). They were kept in boxes buried in soil pits in order to keep them cool and, thus, to minimise changes in soil solution concentrations due to microbial or algal activity. One week before each sampling event, collection bottles were emptied. Thus, a sample represents the solution collected during 1 week. Precipitates that had formed in the bottles since the last sampling were dissolved in 50 ml 1.5 % HNO₃. Prior to installation, tubings and sampling bottles were soaked with 6.5% HNO₃ for at least 12 h, then rinsed with doubly-deionised water (Millipore, Bedford, MA). Suction cups were percolated with 100 ml 1 M HCl followed by rinsing with doubly-deionised water.

3.3.4 Chemical analysis of solution samples

For chemical analysis, soil solution samples from planted lysimeters with the same treatment were pooled except for 3 sampling dates when all 4 replicates were analysed (October 2002, December 2002 and June 2003). As 3 months were required for the samplers to reach steady state conditions, only samples collected after September 2002 were considered for data analysis.

Concentrations of Na, Mg, Al, K, Ca, Mn, Cu, Zn, and Cd in solution were determined by inductively-coupled plasma mass spectrometry (Elan 6000, Perkin-Elmer, Wellesley, MA), of Cl⁻, NO₃⁻, PO₄³⁻, and SO₄²⁻ by ion chromatography (DX-120, Dionex, Sunnyvale, CA), NH₄⁺ by flow injection analysis (FIAS 300, Perkin-Elmer, Wellesley, MA), DOC as non-purgeable organic carbon with a TOC-Analyser (Shimadzu TOC-V, Tokyo, Japan) and pH potentiometrically (Electrode C 4000-8; Radiometer Analytical SAS, Villeurbanne, France). Total metal concentrations were calculated by summing the concentrations of the collected soil solution with the volume-corrected concentrations in the dissolved precipitates formed in the bottles. Prior to analysis, the samples were filtered (0.45 μm; ME 25, Schleicher and Schuell, Dassel, Germany). Certified standard reference materials were analysed with each series of analysis. Analytical errors were less than 0.15 units for pH and 10 % for all other parameters.
Heavy metal concentrations and speciation in the soil solution

Labile Zn was measured by differential pulse anodic stripping voltammetry with a hanging mercury drop electrode (Processor 693 VA and stand 694 VA, Metrohm, Switzerland). First, samples were purged for 2.5 minutes with nitrogen. Then 3 measurement cycles were performed as follows. Deposition for 20 s at -1100 mV; sweeping during 16.2 s from -1100 mV to -800 mV. The mercury drop had a size of 0.35 mm². Between cycles, the samples were stirred for 4 s at 2000 r.p.m. Calibration buffers ranging from 0 to 10 µM Zn were prepared in 0.2 M 2-morpholinoethanesulfonic acid at pH 6.5 to 6.8. The detection limit was 0.2 µM.

Free Cu (Cu²⁺) activity was measured by means of a copper selective electrode (Model 9429, Thermo Orion, MA) coupled with a Ag/AgCl reference electrode (in 3 M KCl) (Model 6.0733.100, Metrohm, Switzerland). Solution pH was measured potentiometrically (Electrode 6.0234.100, Metrohm, Switzerland). The Cu potential was recorded when the drift was less than 0.5 mV in 30 s. This took about 10 min at low pCu²⁺ and 5 min at high pCu²⁺. The method of Sauvé et al. (1995) was used for calibration. The calibration solutions were prepared using 1 mM iminodiacetic acid, 0.1 mM Cu(NO₃)₂, 6 mM NaOH and 2.5 mM potassium hydrogen-phthalate. The pH of the calibration solutions ranged from 2.5 to 11.0 adjusted by addition of HNO₃. The pCu²⁺ values of the calibration solutions were calculated using ChemEQL (Müller, 1996). Electrode potential and pCu²⁺ were linearly correlated from pCu²⁺ 11.5 to 4.5. The detection limit was 10⁻¹² M Cu²⁺.

3.3.5 Statistical analysis and speciation modelling

Standard errors of the treatment means were calculated from the replicate samples taken at each of the 3 following dates: 10 October 2002, 12 December 2002 and 5 June 2003. To evaluate differences between treatments, multivariate analysis of variance (MANOVA) was performed on log₁₀-transformed data based on the 3 dates mentioned above using SAS version 8.2 (SAS, Inc.). The significance level was chosen as P < 0.05. Multiple regression analysis with STATISTICA version 5.1 (StatSoft, Inc.) was used to identify the parameters explaining most of the variance of HM solubility. In a first step, a correlation matrix was calculated including the HM as well as all major cations, major inorganic anions, DOC and pH. In a second step, each parameter with a significant correlation was omitted once and the calculation repeated in order to determine the parameter with the least contribution to the coefficient of determination (R²). This parameter was then dropped from the regression model and the procedure.
repeated, until finally a model equation was obtained in which all parameters made a large contribution to $R^2$.

Metal speciation in soil solution for comparison with measured free and labile concentrations was calculated using WHAM 6.0 (Tipping, 1998). As suggested by Bryan, Tipping et al. (2002) for fresh waters but also used by Tipping (2005) for soil solutions, fulvic acid concentrations were calculated by multiplying DOC concentrations with a factor 1.3. For the prediction of labile Zn with WHAM, the sum of free Zn and inorganic Zn complexes was considered, although, according to Batley and Florence (1976) also weak organic complexes should be included.

3.4 Results

3.4.1 Heavy metal concentrations in topsoil and subsoil

As the type of subsoil had no significant effect on the dissolved HM concentrations in the planted topsoils ($P > 0.05$; $N = 4$), topsoil concentrations were averaged over the two subsoils. Dissolved Cu, Zn and Cd concentrations were significantly larger in the planted HM topsoils than in the CO topsoils ($P < 0.0001$; $N = 8$; Figure 3.1 to Figure 3.3). Zinc and Cd concentrations were in average about 2 times larger in the HM topsoils with plants than without plants, whereas Cu concentrations did not differ between the two treatments. In the HM topsoils with plants, Cu concentrations varied between 1.0 and 1.6, Zn between 14 and 25, and Cd between 0.02 and 0.04 μmol L$^{-1}$. In the HM topsoils without plants, Cu concentrations varied between 1.0 and 2.0, Zn between 7 and 14, and Cd between 0.01 and 0.016 μmol L$^{-1}$. In the CO topsoils, Cu concentrations varied between 0.2 and 0.6 μmol L$^{-1}$ with plants, and between 0.1 and 0.3 μmol L$^{-1}$ without plants, while Zn and Cd were below detection limit (0.05 μmol L$^{-1}$, and 0.2 nmol L$^{-1}$, respectively) most of the time in both cases. In general, Cd varied in a similar way as Zn in the HM topsoils, as demonstrated by the close correlation between the concentrations of the two metals (Figure 3.4).

The contamination of the topsoils did not significantly increase HM concentrations in the subsoil solution, neither in the presence ($P > 0.05$; $N = 4$ for both the acidic and calcareous subsoil, at 40 and at 70 cm depth) nor in the absence of plants (Figure 3.1 to Figure 3.3, Table 3.2). However, in the presence of plants, there was a tendency that Zn concentrations in the acidic subsoils were larger below HM than below CO topsoils,
Heavy metal concentrations and speciation in the soil solution

Figure 3.1: Dissolved Cu concentrations in the metal contaminated (HM) and uncontaminated topsoil (CO) and the subsoils at 40 cm with (left) and without plants (right). Standard errors are given for the 3 dates (indicated by arrows) for which replicate solution samples were available (in the topsoil N = 8; in the subsoil N = 4).

Figure 3.2: Dissolved Zn concentrations in the metal contaminated (HM) and uncontaminated topsoil (CO) and the subsoils at 40 cm with (left) and without plants (right). Zero means lower than detection limit. Standard errors are given for the 3 dates (indicated by arrows) for which replicate solution samples were available (in the topsoil N = 8; in the subsoil N = 4).
Figure 3.3: Dissolved Cd concentrations in the metal contaminated (HM) and uncontaminated topsoil (CO) and the subsoils at 40 cm with (left) and without plants (right). Zero means lower than detection limit. Standard errors are given for the 3 dates (indicated by arrows) for which replicate solution samples were available (in the topsoil N = 8; in the subsoil N = 4).

Figure 3.4: Measured Cd concentrations in the soil solution of the metal-contaminated (HM) topsoil vs. predicted values obtained using Equation (1c) (Table 3.4). Full symbols are for model ecosystems with plants and empty symbols for plant-free references.
in particular in winter and spring (Figure 3.2, Table 3.2). Without plants, there were larger Cu, Zn, and Cd concentrations in the acidic subsoil below HM topsoil at one to two sampling dates in winter and spring (Figure 3.1 to Figure 3.3). By contrast, in the presence of plants, dissolved Cu concentrations in the calcareous subsoils tended to be larger in the CO than in the HM treatments (Figure 3.1, Table 3.2). In all treatments, Cu concentrations were larger in the calcareous than in the acidic subsoil (Figure 3.1, Table 3.2). In the calcareous subsoil, Zn and Cd concentrations were most of the time below or close to detection limits (0.05 μmol L⁻¹ and 0.2 nmol L⁻¹, respectively). The pH-values and DOC concentrations for the CO and HM treatments in the acidic subsoils were larger in the absence than in the presence of plants (Table 3.2). This was more pronounced at 40 than at 70 cm depth.

3.4.2 Correlation between HM concentrations and other soil solution parameters

The correlation matrix for all data from HM topsoils with and without plants revealed a significant positive correlation between Cu and DOC, Cl⁻, and SO₄²⁻ (Table 3.3). Multiple regression analysis revealed that Cu concentrations could be modelled sufficiently with DOC only (Table 3.4, Equation 1a). Zinc and Cd were correlated significantly with pH, Cl⁻, NO₃⁻, PO₄³⁻, SO₄²⁻, K, and Na (Table 3.3), but the variations in Zn and Cd concentrations were explained sufficiently by a model based on the negative correlation with both PO₄³⁻ and pH (Table 3.4, Equation 1b and 1c). Figure 3.5 shows the seasonal variations of the governing parameters DOC, pH, and PO₄³⁻ in the HM topsoils. In the presence of plants, DOC concentrations exhibited major maxima in autumn and a minor maximum in May. The pH value increased in late spring and summer. This seasonal variation was overlaid by a decrease over the whole observation period. Phosphate did not vary much except for smaller concentrations towards the end of the observation period. In the absence of plants, there were no peak concentrations of DOC in autumn. While pH, as in the presence of plants, exhibited an overall decrease with time, there was no increase in spring and summer. Phosphate concentrations increased during the observation period and were about two times larger than in the presence of plants.

In the CO topsoil and in the calcareous subsoil (CO and HM), variations in Cu concentrations were explained also sufficiently by variations in DOC (Table 3.4, Equations 2 and 3). Equation 2 (Table 3.4) predicts the measured Cu concentrations
Table 3.2: Average heavy metal concentrations as well as pH and DOC concentrations in the subsoil solutions at 40 and 70 cm depth below the control (CO) or metal-contaminated (HM) topsoil; mean and standard deviation for the individual sampling dates are given.

<table>
<thead>
<tr>
<th>Type of subsoil</th>
<th>Soil depth (cm)</th>
<th>Plants</th>
<th>Treatments</th>
<th>Cu (µmol L⁻¹)</th>
<th>Zn (µmol L⁻¹)</th>
<th>Cd (nmol L⁻¹)</th>
<th>pH</th>
<th>DOC (µmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcareous</td>
<td>40</td>
<td>with</td>
<td>CO</td>
<td>0.184±0.028</td>
<td>&lt;0.05</td>
<td>&lt;0.2</td>
<td>8.53±0.10</td>
<td>1069±208</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HM</td>
<td>0.139±0.013</td>
<td>&lt;0.05</td>
<td>&lt;0.2</td>
<td>8.54±0.11</td>
<td>638±110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>without</td>
<td>CO</td>
<td>0.155±0.042</td>
<td>&lt;0.05</td>
<td>&lt;0.2</td>
<td>8.57±0.06</td>
<td>797±203</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HM</td>
<td>0.155±0.025</td>
<td>&lt;0.05</td>
<td>&lt;0.2</td>
<td>8.57±0.05</td>
<td>706±107</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>with</td>
<td>CO</td>
<td>0.146±0.022</td>
<td>&lt;0.05</td>
<td>&lt;0.2</td>
<td>8.55±0.10</td>
<td>867±248</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HM</td>
<td>0.104±0.017</td>
<td>&lt;0.05</td>
<td>&lt;0.2</td>
<td>8.54±0.11</td>
<td>481±107</td>
</tr>
<tr>
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<td></td>
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<td>CO</td>
<td>0.168±0.038</td>
<td>&lt;0.05</td>
<td>&lt;0.2</td>
<td>8.57±0.05</td>
<td>1001±214</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>HM</td>
<td>0.172±0.033</td>
<td>&lt;0.05</td>
<td>&lt;0.2</td>
<td>8.57±0.07</td>
<td>917±281</td>
</tr>
<tr>
<td>Acidic</td>
<td>40</td>
<td>with</td>
<td>CO</td>
<td>0.028±0.007</td>
<td>&lt;0.05</td>
<td>0.8±0.3</td>
<td>6.33±0.30</td>
<td>280±33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HM</td>
<td>0.027±0.008</td>
<td>0.13±0.08</td>
<td>0.6±0.2</td>
<td>6.44±0.29</td>
<td>287±41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>without</td>
<td>CO</td>
<td>0.037±0.019</td>
<td>&lt;0.05</td>
<td>&lt;0.2</td>
<td>7.12±0.39</td>
<td>614±99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HM</td>
<td>0.068±0.049</td>
<td>0.14±0.25</td>
<td>0.3±0.5</td>
<td>7.47±0.31</td>
<td>777±149</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>with</td>
<td>CO</td>
<td>0.015±0.006</td>
<td>&lt;0.05</td>
<td>0.9±0.4</td>
<td>5.81±0.13</td>
<td>194±26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HM</td>
<td>0.018±0.005</td>
<td>0.07±0.05</td>
<td>0.8±0.6</td>
<td>6.04±0.28</td>
<td>207±27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>without</td>
<td>CO</td>
<td>0.028±0.005</td>
<td>&lt;0.05</td>
<td>1.2±0.5</td>
<td>5.96±0.40</td>
<td>704±100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HM</td>
<td>0.058±0.029</td>
<td>0.07±0.12</td>
<td>1.0±0.2</td>
<td>6.71±0.28</td>
<td>1341±108</td>
</tr>
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</table>
Table 3.3: Correlation matrix for heavy metals, pH, DOC as well as major cations and anions in the contaminated topsoil solutions. Marked correlations with a star are significant at \( p < 0.05 \) (N=36).

<table>
<thead>
<tr>
<th></th>
<th>Cu</th>
<th>Zn</th>
<th>Cd</th>
<th>pH</th>
<th>DOC</th>
<th>Cl</th>
<th>NO$_3^-$</th>
<th>PO$_4^{3-}$</th>
<th>SO$_4^{2-}$</th>
<th>K</th>
<th>Mg</th>
<th>Ca</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>1</td>
<td>-0.32</td>
<td>-0.08</td>
<td>0.24</td>
<td>0.61*</td>
<td>0.34*</td>
<td>0.3</td>
<td>0.35*</td>
<td>0.05</td>
<td>0.22</td>
<td>0.25</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>-0.32</td>
<td>1</td>
<td>-0.9*</td>
<td>-0.33*</td>
<td>0.22</td>
<td>0.41*</td>
<td>-0.66*</td>
<td>-0.79*</td>
<td>0.36*</td>
<td>-0.53*</td>
<td>-0.14</td>
<td>0.01</td>
<td>0.65*</td>
</tr>
<tr>
<td>Cd</td>
<td>-0.08</td>
<td>0.9*</td>
<td>1</td>
<td>-0.33</td>
<td>0.44*</td>
<td>0.49*</td>
<td>-0.76*</td>
<td>-0.86*</td>
<td>0.48*</td>
<td>-0.72*</td>
<td>-0.11</td>
<td>0</td>
<td>0.67*</td>
</tr>
<tr>
<td>pH</td>
<td>0.24</td>
<td>-0.33*</td>
<td>-0.33</td>
<td>1</td>
<td>-0.14</td>
<td>-0.02</td>
<td>0.34*</td>
<td>0.37*</td>
<td>-0.39*</td>
<td>0.61*</td>
<td>0.5</td>
<td>0.48*</td>
<td>-0.11</td>
</tr>
<tr>
<td>DOC</td>
<td>0.61*</td>
<td>0.22</td>
<td>0.44*</td>
<td>-0.14</td>
<td>1</td>
<td>0.71*</td>
<td>-0.29</td>
<td>-0.54*</td>
<td>0.85*</td>
<td>-0.43*</td>
<td>0.38*</td>
<td>0.41*</td>
<td>0.39*</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>0.34*</td>
<td>0.41*</td>
<td>0.49*</td>
<td>-0.02</td>
<td>0.71*</td>
<td>1</td>
<td>-0.23</td>
<td>-0.45*</td>
<td>0.77*</td>
<td>-0.28</td>
<td>0.38*</td>
<td>0.47*</td>
<td>0.47*</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>0.3</td>
<td>-0.66*</td>
<td>-0.76*</td>
<td>0.34*</td>
<td>-0.29</td>
<td>-0.23</td>
<td>1</td>
<td>0.77*</td>
<td>-0.29</td>
<td>0.7*</td>
<td>0.16</td>
<td>0.21</td>
<td>-0.58*</td>
</tr>
<tr>
<td>PO$_4^{3-}$</td>
<td>0.15</td>
<td>-0.79*</td>
<td>-0.86*</td>
<td>0.37*</td>
<td>-0.54*</td>
<td>-0.45*</td>
<td>0.77*</td>
<td>1</td>
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<td>0.63*</td>
<td>-0.12</td>
<td>-0.13</td>
<td>-0.58*</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
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<td>0.36*</td>
<td>0.48*</td>
<td>-0.39*</td>
<td>0.85*</td>
<td>0.77*</td>
<td>-0.29</td>
<td>-0.59*</td>
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<td>-0.46*</td>
<td>0.29</td>
<td>0.36*</td>
<td>0.43*</td>
</tr>
<tr>
<td>K</td>
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<td>-0.53*</td>
<td>-0.72*</td>
<td>0.61*</td>
<td>-0.43*</td>
<td>-0.28</td>
<td>0.7*</td>
<td>0.63*</td>
<td>-0.46*</td>
<td>1</td>
<td>0.36*</td>
<td>0.3</td>
<td>-0.34*</td>
</tr>
<tr>
<td>Mg</td>
<td>0.22</td>
<td>-0.14</td>
<td>-0.11</td>
<td>0.5</td>
<td>0.38*</td>
<td>0.38*</td>
<td>0.16</td>
<td>-0.12</td>
<td>0.29</td>
<td>0.36*</td>
<td>1</td>
<td>0.9*</td>
<td>0.07</td>
</tr>
<tr>
<td>Ca</td>
<td>0.25</td>
<td>0.01</td>
<td>0</td>
<td>0.48*</td>
<td>0.41*</td>
<td>0.47*</td>
<td>0.21</td>
<td>-0.13</td>
<td>0.36*</td>
<td>0.3</td>
<td>0.9*</td>
<td>1</td>
<td>0.13</td>
</tr>
<tr>
<td>Na</td>
<td>0.01</td>
<td>0.65*</td>
<td>0.67*</td>
<td>-0.11</td>
<td>0.39*</td>
<td>0.47*</td>
<td>-0.58*</td>
<td>-0.58*</td>
<td>0.43*</td>
<td>-0.34*</td>
<td>0.07</td>
<td>0.13</td>
<td>1</td>
</tr>
</tbody>
</table>
in the CO topsoil well (Figure 3.6). The predictions of the measured Cu, Zn, and Cd concentrations in the HM topsoils are generally good but there are several "outliers" (Figure 3.6 to Figure 3.8).

In the cases, where HM concentrations were below or close to the detection limit most of the time (Cu in the acidic subsoil; Zn and Cd in the uncontaminated topsoil and in both subsoils), either the basis for model calculations was too small, or the scatter in the data was too large to perform meaningful regression analysis. The data presented in Table 3.2 suggest for the acidic subsoil a weak positive correlation between Cu and DOC and a negative correlation between Cd and pH. A negative correlation in the acidic subsoil was also observed between Zn and Cd below the HM topsoil, while below the CO topsoil, average Zn concentrations remained below detection limit (Table 3.2).

<table>
<thead>
<tr>
<th>Table 3.4: Model equations to predict Cu, Zn and Cd concentrations (mol L⁻¹) in the soil solution of the planted and unplanted soil systems; coefficients are given together with their standard errors (SE); statistical significance: *** for p &lt; 0.001, and NS for p &gt; 0.1.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
</tr>
<tr>
<td>Metal-contaminated topsoil (HM)</td>
</tr>
<tr>
<td>1a) log [Cu]</td>
</tr>
<tr>
<td>1b) log [Zn]</td>
</tr>
<tr>
<td>1c) log [Cd]</td>
</tr>
<tr>
<td>Control topsoil (CO)</td>
</tr>
<tr>
<td>2) log [Cu]</td>
</tr>
<tr>
<td>Calcareous subsoil (40 cm)</td>
</tr>
<tr>
<td>3) log [Cu]</td>
</tr>
</tbody>
</table>

47
Heavy metal concentrations and speciation in the soil solution

Figure 3.5: DOC, pH and PO$_4^{3-}$ concentrations in the soil solution of the planted (left) and unplanted (right) contaminated (HM) topsoils. Concentrations are in µmol L$^{-1}$. Standard errors are given for the 3 dates (indicated by arrows) for which replicate solution samples were available (N = 4).

Figure 3.6: Measured Cu concentrations in the soil solution of the metal-contaminated (HM) and uncontaminated (CO) topsoil vs. predicted values obtained using Equation (1a) and (2) (Table 3.4), respectively. Triangles: HM treatment; circles: CO treatment. Full symbols are for model ecosystems with plants and empty symbols for plant-free references.
Heavy metal concentrations and speciation in the soil solution

Figure 3.7: Measured Zn concentrations in the soil solution of the metal-contaminated (HM) topsoil vs. predicted values obtained using Equation (1b) (Table 3.4). Full symbols are for model ecosystems with plants and empty symbols for plant-free references.

Figure 3.8: Measured Cd concentrations in the soil solution of the metal-contaminated (HM) topsoil vs. predicted values obtained using Equation (1b) (Table 3.4). Full symbols are for model ecosystems with plants and empty symbols for plant-free references.
3.4.3 Metal speciation

Table 3.5 lists measured and calculated concentrations of Cu and Zn species for solution samples obtained from the HM topsoil at different sampling dates. In presence of plants, the measured fraction of free Cu (Cu$^{2+}$) was smaller in May than in July and December. In absence of plants, this fraction was low throughout the year and smaller than in the planted topsoil in July and December. For the treatments with plants, the Cu$^{2+}$ concentrations calculated with WHAM were in good agreement with the measured Cu$^{2+}$ concentrations in July and December. In May, Cu$^{2+}$ was overestimated by the model by a factor of about 3. For the treatments without plants, WHAM overestimated Cu$^{2+}$ at all times. The deviation was particularly large in July. According to WHAM, between 85 and 95% of the Cu in solution was bound to dissolved fulvic acids (data not shown). Major inorganic Cu species were calculated to be hydrogen carbonate and carbonate complexes.

**Table 3.5**: Total soluble, free and labile metal concentrations as measured in the metal-contaminated topsoil in the presence and absence of plants. Standard errors of 2 samples are reported. The free Cu (Cu$^{2+}$) concentrations as well as the labile Zn predicted were calculated with the WHAM 6.0 model. The labile Zn represents the sum of all inorganic species given by the model.

<table>
<thead>
<tr>
<th></th>
<th>May</th>
<th>July</th>
<th>December</th>
</tr>
</thead>
<tbody>
<tr>
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<td>no plants</td>
<td>plants</td>
</tr>
<tr>
<td>Cu total (μmol L$^{-1}$)</td>
<td>1.38±0.13</td>
<td>1.50±0.01</td>
<td>1.30±0.04</td>
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<tr>
<td>Cu$^{2+}$ measured (nmol L$^{-1}$)</td>
<td>2.3±0.6</td>
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<tr>
<td>Cu$^{2+}$ predicted (nmol L$^{-1}$)</td>
<td>6.1</td>
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<tr>
<td>Cu$^{2+}$ measured vs. Cu tot.</td>
<td>0.0017</td>
<td>0.0023</td>
<td>0.0063</td>
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<tr>
<td>Zn total (μmol L$^{-1}$)</td>
<td>15.2±3.75</td>
<td>7.49±1.73</td>
<td>20.51±5.32</td>
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<tr>
<td>Zn labile measured (μmol L$^{-1}$)</td>
<td>8.36±1.0</td>
<td>5.58±1.1</td>
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<tr>
<td>Zn labile predicted (μmol L$^{-1}$)</td>
<td>12.32</td>
<td>6.67</td>
<td>18.10</td>
</tr>
<tr>
<td>Zn labile measured vs. Zn tot.</td>
<td>0.55</td>
<td>0.75</td>
<td>0.52</td>
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</table>

There were no seasonal variations in the fraction of labile Zn in presence of plants and only relatively small variations in the absence of plants. The fraction of labile Zn
tended to be larger in absence than in presence of plants. Labile Zn was systematically overpredicted by the model, especially in the presence of plants, i.e. the WHAM calculations predicted a smaller proportion of Zn complexed with fulvic acids (10 to 19 %) than measured (26 to 54 %).

3.5 Discussion

3.5.1 Metal solubility control in the topsoil

For both the HM and CO topsoils, the strong correlation with DOC indicates that Cu solubility was governed mainly by the formation of complexes with dissolved organic matter (DOM).

The strong negative correlation of Zn and Cd in the HM topsoils with phosphate and the weak negative correlation with pH suggest that the soil solution concentrations of these two metals were governed mainly by the equilibrium with their phosphates. Indeed, speciation calculations using SOILCHEM (Sposito and Coves, 1995) show that solutions with a similar composition as those found in the HM topsoil are oversaturated with respect to hopeite \( \text{Zn}_3(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O} \) except for small Zn and phosphate concentrations (Table 3.6). According to Lindsay (1979), hopeite can control the solubility of available, i.e. freshly added Zn, and according to Kabata-Pendias and Pendias (2001), Zn solubility is often negatively correlated with P compounds in soils. Examining the topsoil of our experiment with EXAFS, Voegelin et al. (2005) found that the ZnO, which was the major component of the HM filter dust added at the beginning of the experiment, had transformed after 9 months into new phases. Large fractions could be assigned to Zn phytate, the Zn salt of phytic acid. The latter is considered the most important organic phosphorus compound in soils from which phosphate is produced by phytase (Svenson, 1986). Zinc phytate is virtually insoluble between pH 5 and 8 and exhibits a Zn to phytate ratio of 5 (Persson et al., 1998; Habermann et al., 2002). Based on the organic phosphorus content (Table 3.1; corresponding to 4.6 mmol kg\(^{-1}\) phytate) and the average Zn content of the HM topsoil (42.8 mmol kg\(^{-1}\)) it is possible that most of the phytate is present as Zn phytate. It can now be assumed that the degradation of Zn phytate by phytase leads to the release of both phosphate and Zn, the solution concentrations of which are then controlled by Zn phosphate.

In contrast to Zn, the HM topsoil solutions are largely undersaturated with respect to \( \text{Cd}_3(\text{PO}_4)_2 \), and, at large Cd, high pH, and large partial pressure of CO\(_2\), \( \text{CdCO}_3 \)
Table 3.6: Results of solubility calculations performed with SOILCHEM (Sposito and Coves, 1995) for solutions mimicking the soil solutions in the topsoil for different p(CO$_2$) and pH as well as PO$_4^{3-}$, Zn, and Cd concentrations; concentrations of Ca (500 µmol L$^{-1}$), Mg (50 µmol L$^{-1}$), K (100 µmol L$^{-1}$), Na (25 µmol L$^{-1}$), SO$_4^{2-}$ (50 µmol L$^{-1}$), Cl$^-$ (30 µmol L$^{-1}$), NO$_3^-$ (100 µmol L$^{-1}$), fulvic acid 1 (50 µmol L$^{-1}$), fulvic acid 2 (500 mmol L$^{-1}$) were the same in all cases; as Ca phosphate allowed to precipitate either as octacalcium phosphate (OCP, Ca$_4$H(PO$_4$)$_3$ + 2.5 H$_2$O) or β-tricalcium phosphate (TCP; β-Ca$_3$(PO$_4$)$_2$), both were considered.

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<th>p(CO$_2$) [Pa]</th>
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<th>PO$_4$ (µmol L$^{-1}$)</th>
<th>OCP % Ca as OCP</th>
<th>TCP % Ca as TCP</th>
<th>Zn (µmol L$^{-1}$)</th>
<th>TCP % Zn as TCP</th>
<th>OCP % Zn as hopeite</th>
<th>TCP % Zn as ZnCO$_3$</th>
<th>OCP % Zn as ZnCO$_3$</th>
<th>Cd (µmol L$^{-1}$)</th>
<th>OCP or TCP % Cd as Cd$_2$(PO$_4$)$_2$</th>
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</tr>
</tbody>
</table>
Heavy metal concentrations and speciation in the soil solution

(Octavite) can precipitate (Table 3.6), which is in accordance with the calculations by Lindsay (1979) and Soon (1981). However, for sewage sludge amended topsoils with a total content of freshly added Cd of 7.5 mg kg⁻¹, Soon (1981) measured soluble Cd concentrations of about 50 nmol L⁻¹ at a pH value of about 7.5. This reflects very closely the conditions in our HM topsoils. Soon (1981) concluded that adsorption to exchange and surface complexation sites controlled soluble Cd concentrations below pH 8 rather than precipitation. In addition, he found that solubility decreased linearly with pH. Based on this, a strong dependence of Cd solubility on phosphate in our soils can only be explained by indirect effects. The excellent correlation between Cd and Zn concentrations suggests that the latter metal is involved. Cadmium may be sorbed to Zn phosphate precipitates, or increasing Zn concentrations may lead to an increasing release of Cd from exchange sites.

3.5.2 Heavy metal concentrations in the contaminated topsoils: seasonal variations and plant influence

Based on the considerations on solubility control, the almost constant Zn and Cd concentrations in the plant free reference can be explained by the opposite temporal trend in phosphate concentrations and pH. The decreasing trend in pH which, as an overall trend, is also recognisable in the planted systems, indicates ongoing irreversible equilibration processes. The larger Zn and Cd concentrations in the presence than in the absence of plants can be attributed to the smaller phosphate concentrations. The latter may be explained by plant uptake of phosphate. Smaller phosphate concentrations in the rhizosphere than in bulk soil are described by Jungk (2002). The small seasonal variations of the Zn and Cd concentrations in the presence of plants with somewhat lower values in winter and spring reflect mainly the opposite behaviour of phosphate which, again, may be caused by the seasonally different plant activity. In conclusion, in neutral to slightly alkaline soils with sufficiently large P contents, plants may indirectly increase Zn and Cd concentrations in the soil solution via nutrient uptake.

The Cu concentrations in the HM topsoil solution of the plant free reference exhibit the same seasonal pattern as DOC with a maximum in spring (Figure 3.1 and Figure 3.5). The latter is in accordance with findings by Kaiser et al. (2002). In the planted topsoil, however, the response of Cu concentrations to fluctuations in DOC is damped. In particular, the large peak concentrations of DOC in autumn were only partly reflected.
by an increase in Cu concentrations. Based on the findings by Kaiser et al. (2002) and Kalbitz et al. (2004), these DOC peaks very likely are not a "normal" seasonal variation, but can be attributed to enhanced DOC mobilisation caused by the yearly pruning of the deciduous trees, a "clear-cutting like measure".

3.5.3 Heavy metal concentrations in the subsoil

Since the metal concentrations in the subsoil solutions below both planted and unplanted HM topsoils were not or only little larger than in the control, retention by precipitation or sorption of HM must have occurred upon soil solution entering the uppermost layers of the subsoils. For the calcareous subsoil, precipitation of all metals can be explained by the large increase in pH. In the acidic subsoil, DOC solubility is likely to be smaller and the proportion of positive charges on hydroxide surfaces larger than in the topsoil which may have led to the precipitation of Cu DOC complexes (Kalbitz et al., 2000). Furthermore, the drop of HM concentrations from top- to sub-soil indicates that in general preferential flow was of little importance in our lysimeters. This is supported by observations of Camobreco et al. (1996) that homogenised soil columns adsorbed all applied metals, while undisturbed soil columns exhibited some metal breakthrough.

As in the topsoil, the solubility of Cu in both subsoils seemed to be controlled mainly by DOC. This explains, for instance, the larger Cu concentrations in the calcareous subsoil below the CO than below the HM topsoils with plants. The smaller DOC concentrations below the HM topsoil may be caused by an indirect effect of the HM contamination in the topsoil on microbial activity.

In the acidic subsoil below HM topsoil, Zn concentrations depended mainly on pH as is expected for lithogenic Zn (Lindsay, 1979). The concentrations, however, were smaller than typical lithogenic Zn. Furthermore, in the presence of plants, Zn concentrations below the CO topsoil were smaller than below HM topsoil, despite similar pH. This indicates that some Zn from the contaminated topsoil may have been translocated and formed a better soluble Zn phase than the background lithogenic Zn. Also Cd seemed to depend mainly on pH in the acidic subsoil. Lithogenic Cd in the pH range 6 to 7 typically shows constant values at about 0.1 µmol L⁻¹ (Lindsay, 1979), which is about 2 orders of magnitude larger than the concentrations found in the acidic subsoil.
The tendency to larger Zn and Cd concentrations in the acidic subsoil in the presence of plants in winter and spring can be explained by lower pH values during this time of the year (data not shown), that may have been the result of reduced nitrate uptake by plants and microorganisms. Tyler (1981) also reported maximum Zn and Cd concentrations in soil solution during winter. The large metal peaks in winter below HM topsoil in the absence of plants, however, cannot be explained with a corresponding drop in pH.

3.5.4 Metal speciation in the contaminated topsoil

The excellent agreement between measured and calculated Cu$^{2+}$ concentrations in the HM treatment with plants in July and December indicates that the assumptions made in WHAM for estimating the cation-binding properties of average isolated fulvic acid from DOC (Bryan et al., 2002) is valid for our system. Also the measured and calculated Cu$^{2+}$ concentrations in May can be considered similar within a confidence range of 95% (Bryan et al., 2002).

The mostly smaller Cu$^{2+}$ fraction in the absence than in the presence of plants as well as the large overestimation of Cu$^{2+}$ by WHAM for the plant free system, indicate a difference in DOC quality in terms of metal binding properties. The DOC in the soils without plants seemed to form more stable complexes with Cu than the DOC in the presence of plants. Considering this and that DOC concentrations were similar with and without plants, larger total Cu concentrations should have been measured in the absence of plants. The fact that total Cu concentrations in soil solution did not depend on the presence of plants suggests that in the absence of plants Cu was also more strongly bound to the solid soil organic matter.

If the assumptions on the chemical identity of labile Zn (Batley and Florence, 1976) are correct, the systematic overestimation of measured labile Zn by the WHAM speciation model both in presence and absence of plants indicates that the DOC in our systems exhibits either a larger binding capacity for Zn, or forms more stable complexes with Zn than the model DOC.
3.6 Acknowledgements

We are grateful to the staff of the Central Laboratory of the Swiss Federal Research Institute for Forest, Snow and Landscape Research (WSL) who performed most of the chemical analyses. We also thank the technical staff of the Soil Ecology group at WSL for their help with the chemical analysis and installation of the soil solution monitoring system. The Bioindication group led by Madeleine Goerg-Günthard as well as Peter Bleuler are acknowledged for maintaining the experimental facility. This research was supported by funds from the Swiss National Science Foundation, the Swiss Federal Research Institute WSL, and the Swiss Federal Institute of Technology Zürich.
Chapter 4

Effects of heavy metals on dissolved organic matter, sulphate, phosphate, and nitrate in the soil solution of a model forest ecosystem

D. Rais, B. Frey, B. Nowack, R. Schulin, J. Luster

Submitted to Soil Biology and Biochemistry
4.1 Abstract

The effects of heavy metal (HM) pollution on microbial activity and soil solution concentrations of dissolved organic carbon (DOC), sulphate, phosphate, and nitrate were investigated in forest model ecosystems. In a lysimeter study, the factors topsoil contamination (with/without HM), subsoil type (acidic/calcareous) and plant cover (with/without plants) were considered. Biological parameters of topsoil samples from the planted lysimeters revealed a significant reduction of the microbial activity by HM contamination. In good agreement with these results, DOC and $SO_4^{2-}$ concentrations were smaller in the contaminated planted topsoil than in the uncontaminated planted topsoil. No HM effects were observed in absence of plants with DOC and $SO_4^{2-}$ concentrations at a similar level as in the planted contaminated topsoil. These results suggest on one hand a base level of biodegradation in the absence of plants that was not affected by HM contamination. On the other hand the stimulation of the microbial activity by root exudates was reduced under HM stress. In contrast to DOC and $SO_4^{2-}$, $PO_4^{3-}$ concentrations were smaller in the contaminated than in the control topsoil not only in presence but also in absence of plants. This was attributed to $PO_4^{3-}$ solubility in the contaminated topsoil being controlled by $Zn_3(PO_4)_2$. Larger $NO_3^{-}$ concentrations in the HM treatment than in the control in the planted topsoil were inconsistent with the HM effects on nitrification potential. The much larger concentrations in absence than in presence of plants suggest that $NO_3^{-}$ was controlled mainly by plant uptake.

Keywords: biodecomposition, heavy metal contamination; mineralisation; soil microbial assays; soil solution

4.2 Introduction

The fertility of soil ecosystems depends on the turnover of soil organic matter (SOM) which is decomposed by the soil fauna and microorganisms. Any contaminant that poisons soil organisms can therefore affect the sustainability and productivity of the ecosystem. The toxicity of heavy metals (HM) to organisms depends on their concentrations and speciation in the soil solution (Rieuwerts et al., 1998; Dumestre et al., 1999; Kiikkila et al., 2002). Heavy metals have been observed to be toxic to all kinds of soil organisms when present in excessive concentrations, including the macro- (Nahmani et al., 2003; Lukkari et al., 2004) and the micro-fauna (Bruce et al., 1997;
Effects of heavy metals on soil solution parameters

Boyd and Williams, 2003) as well as bacteria and fungi (Baath, 1989; McGrath, 1994; Giller et al., 1998 for reviews).

The biodegradation of the major elements C, N, P and S depend almost entirely on microbial processes. Microbial assays have been used widely to evaluate the toxicity of HM to microorganisms and microbial processes (Giller et al., 1998 for review). At high concentrations, HM generally decrease the size of the microbial community in terms of biomass (Chander et al., 2002), change the community structure (Gremion et al., 2004; Hartmann et al., 2005) and reduce activities such as SOM decomposition (Kandeler et al., 2000). More specifically, Kandeler et al. (1996) stated that the enzymes involved in the C-cycling such as the beta-glucosidase are generally less affected than other enzymes, but results on the sensitivity of the beta-glucosidase to HM are conflicting (Hinojosa et al., 2004; Kuperman and Carreiro, 1997; Effron et al., 2004; Geiger et al., 1998). The supply of P and S to soil organisms depends largely on the decomposition of SOM and on the cleavage of phosphate and sulphate esters catalysed by microbial phosphatase and arylsulphatase, respectively (Killham et al., 1983; Killham, 1994). Phosphatase and arylsulphatase are generally found to be highly sensitive to HM contamination of soils (Kandeler et al., 2000; Stuczynski et al., 2003; Effron et al., 2004; Roy et al., 2004). Regarding the N-cycle, most studies reported a reduction of the nitrification in the presence of HM, assessed either by the nitrification potential (Sauvé et al., 1999; Smolders et al., 2001) or by the release of nitrate to the soil solution (Cela and Sumner, 2003).

The use of microbial assays to assess soil quality in terms of nutrient turnover has been questioned (Brookes, 1995; Kapustka, 1999; Gil-Sotres et al., 2005). The lack of standardised methods in sample collection, storage and treatments make the comparison of data obtained from different laboratories virtually impossible (Gil-Sotres et al., 2005). Moreover, such laboratory procedures often artificially optimise conditions compared to effective soil conditions, especially when measuring enzyme activities (Verchot and Borelli, 2005). In particular, the influence of plant activity on microbial activities is not taken into account. Furthermore, in the field, the high degree of spatial and temporal variations in microbial populations, community structures, and functions are often larger than the effects due to pollution. This can make the results from microbial assays difficult to interpret and can explain part of the conflicting results (Gil-Sotres et al., 2005).
A complementary approach to investigate the effects of HM on nutrient turnover in a soil is to monitor products of biodegradation processes such as dissolved organic carbon (DOC), dissolved organic nitrogen (DON), \( \text{SO}_4^{2-} \), \( \text{PO}_4^{3-} \) and \( \text{NO}_3^- \) in the soil solution. Advantages of this method compared to microbial assays are, firstly, to look \textit{in situ} at the undisturbed soil system under natural climatic conditions and in the presence of plants, secondly, to integrate over a larger soil compartment thereby reducing the influence of spatial heterogeneity, and, thirdly, the possibility to assess seasonal variations.

Little is known about the effects of HM on different soil solution constituents such as DOC, DON, \( \text{SO}_4^{2-} \), and \( \text{PO}_4^{3-} \). To knowledge, only Merckx et al. (2001) addressed DOC production under HM contamination.

Plants stimulate the microbial activity in the rhizosphere by root exudation (Brimecombe et al., 2001; Kandeler et al., 2002). The plants themselves benefit from the accelerated release of nutrients. In addition, plants can also produce some enzymes that are involved in mineralisation processes such as acid phosphatase (Speir and Cowling, 1991; Tadano and Sakai, 1991). Therefore, it is important to investigate the effects of HM stress on nutrient turnover in the presence of plants, although soil solution concentrations of nutrient ions can also be greatly influenced by the sink of nutrient uptake.

The objective of the present study was to investigate the effects of HM on dissolved organic matter (DOM), sulphate, phosphate, and nitrate in the soil solution of a young forest model ecosystem, including seasonal variations, and to interpret the results in terms of HM influence on the mineralisation potential of a soil and nutrient uptake by plants. Microbial assays were used to help in differentiating between microbial and plant effects.

4.3 Material and methods

4.3.1 Experimental set-up and treatments

Forest model ecosystems were established in May 2000 in the Open Top Chamber (OTC) facility of the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) in Birmensdorf, Switzerland. Eight OTC of 3 m height and 3 m width were used, that were equipped with roofs that can be automatically closed during rainfall. Below ground, each OTC contains two lysimeters with 3 m\(^2\) surface each. Each lysimeter was
planted with the same collection of tree species, including 6 individuals of Norway spruce (Picea abies L.), 2 birch (Betula pendula L.), 2 willow (Salix viminalis L.) and 4 poplar (Populus tremula L.) as well as 6 herbaceous plant species. The soil in the lysimeters consisted of a loamy topsoil (0 to 15 cm depth), a sandy to loamy-sandy subsoil (15 to 95 cm depth), and a drainage packing of quartz sand (95 to 145 cm depth). The topsoil was obtained from an agricultural field at Birr (Aargau, Switzerland). In half of the OTC it was manually mixed by means of a small harrow with filter dust obtained from a non-ferrous metal smelter and CdO. The amount of this artificial contamination was chosen to give average total metal contents in the topsoil of 410 mg kg\(^{-1}\) Cu, 2800 mg kg\(^{-1}\) Zn, 10 mg kg\(^{-1}\) Cd, and 100 mg kg\(^{-1}\) Pb.

In one lysimeter of each OTC, the subsoil was an acidic loamy sand (pH 4.2) taken from a Haplic Alisol at Eiken (Aargau, Switzerland), in the other a calcareous sandy loam (pH 7.4) originating from a Calcaric Fluvisol at Brugg (Aargau, Switzerland). Selected soil properties are given in Table 4.1. From the beginning of May to the end of September, the model ecosystems were irrigated artificially by sprinkling during nights. The irrigation water mimicked the composition of the ambient rainfall (pH of 5.5). During the other months, the roofs of the OTC remained always open so that the lysimeters received natural precipitation.

Table 4.1: Selected physical and chemical properties of the soils used in the model ecosystems and plant-free soil columns. No C/N ratio could be calculated in the acidic subsoil since \(N_{\text{tot}}\) was below detection limit. (n.d.: not determined)

<table>
<thead>
<tr>
<th></th>
<th>Topsoil</th>
<th>Acidic subsoil</th>
<th>Calcareous subsoil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture [% sand; silt; clay]</td>
<td>36 ; 49 ; 15</td>
<td>87 ; 8 ; 5</td>
<td>74 ; 16 ; 10</td>
</tr>
<tr>
<td>pH (0.01 M CaCl(_2))</td>
<td>6.55±0.12</td>
<td>4.2</td>
<td>7.4</td>
</tr>
<tr>
<td>C(_{\text{org}}) (g kg(^{-1}))</td>
<td>15.1</td>
<td>3.2</td>
<td>11.2</td>
</tr>
<tr>
<td>C(<em>{\text{org}}/) N(</em>{\text{tot}})</td>
<td>10</td>
<td>n.d.</td>
<td>18</td>
</tr>
<tr>
<td>P(_{\text{avail}}) (mg kg(^{-1}))</td>
<td>49±5</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>P(_{\text{org}}) (mg kg(^{-1}))</td>
<td>862</td>
<td>84</td>
<td>54</td>
</tr>
</tbody>
</table>
The study comprised four replicates of each of the four combinations of topsoil contamination (with/without heavy metal dust) and subsoil type (acidic/calcareous subsoil). The experiment lasted from May 2000 to December 2003.

As plant-free references, four soil columns made of stainless steel were installed in the immediate vicinity of the OTC at a shaded place. The columns were 78 cm in diameter and were packed with topsoil, subsoil and quartz sand in the same way as the OTC lysimeters. Prior to filling the columns with soil, the inside part was lined with a polyethylene foil to avoid direct contact of the soil with the steel wall. As in the OTC, a roof excluded natural precipitation from the beginning of May to the end of September. The irrigation scheme was the same as in the OTC.

In total, 8 combinations of topsoil contamination (with/without heavy metal dust), subsoil type (acidic/calcareous) and vegetation cover (with/without plants) were investigated. The following abbreviations are used to denote treatments: HM, heavy metal contaminated topsoil; CO, control.

4.3.2 Sampling and analysis of the soil samples

Soil chemical and physical properties were determined from samples taken from the homogenized top- and sub-soil batches before they were filled into the lysimeters. The samples were dried at 40°C and sieved to 2 mm.

Phosphorus fractions were determined according to (Kuo, 1996), i.e. total extractable P ($P_{extr}$) by extraction of the organic matter free soil (dry digestion at 550 °C) with 0.5 M H$_2$SO$_4$, organically bound P ($P_{org}$) by calculation as difference between $P_{extr}$ and inorganic P (extraction of the untreated soil with 0.5 M H$_2$SO$_4$), available P ($P_{avail}$) by short extraction of the untreated soil with 0.5 M NaHCO$_3$. Phosphorus in all extracts was analysed colorimetrically (Murphy and Riley, 1962). All other soil parameters were determined as described by Blaser et al. (1997).

4.3.3 Soil Solution Sampling

Soil solutions were sampled at 10, 40 and 70 cm depth (centre of the cup) every 3 to 4 weeks from July 2002 until December 2003, i.e. after the soil had equilibrated for more than 2 years. Soil solution was collected using 10 cm long porous suction cups made of a nylon membrane (EcoTech GmbH, Bonn, Germany). In previous laboratory experiments, this type of suction cups had been found to cause the smallest bias (Rais, 2005). Two soil water samplers were installed at each depth in each lysimeter and
Effects of heavy metals on soil solution parameters

connected with Teflon tubings to the same sampling bottle. Thus, each of these samples represents a composition sample from two different positions at the respective depth. The vacuum was kept at 500 hPa at all time, except during freezing conditions in winter-time, when the vacuum pumps had to be switched off. The sampling bottles were made of polypropylene (EcoTech GmbH, Bonn, Germany). The bottles were kept in plastic boxes which were buried in soil pits in order to keep them cool and thus to minimise changes in soil solution concentrations due to microbial or algae activity. Each time samples were taken for analysis, collection bottles were emptied one week before. Thus, a sample represents the solution collected during 1 week. Precipitates that had formed in the bottles since the last sampling event were dissolved in 50 ml 1.5 % HNO₃. Prior to use, all plastic- and glass-ware, including collection bottles used in the field, were cleaned and soaked in 6.5% HNO₃ for at least 12 h, then rinsed with doubly-deionised water (Millipore, Bedford, MA).

4.3.4 Chemical analyses of solution samples

For chemical analysis, soil solution samples from lysimeters with the same treatments and from the same depth were pooled except for 3 sampling dates when all 4 replicates were analysed (October 2002, December 2002 and June 2003).

Concentrations of Cu, Zn, and Cd in solution were determined by inductively-coupled plasma mass spectrometry (Elan 6000, Perkin-Elmer, Wellesley, MA), concentrations of NO₃⁻, PO₄³⁻, and SO₄²⁻ by ion chromatography (DX-120, Dionex, Sunnyvale, CA), DOC and DON with a TOC/TN-Analyser (Shimadzu TOC-V, Tokyo, Japan), and pH potentiometrically (Electrode C 4000-8; Radiometer Analytical SAS, Villeurbanne, France). Total metal concentrations were calculated by summing the concentrations of the collected soil solution with the volume-corrected concentrations in the dissolved precipitates that had formed in the bottles. Prior to analysis, the samples were filtered (0.45 μm; ME 25, Schleicher and Schuell, Dassel, Germany). Accuracy was continuously checked by analysing certified standard reference materials. Deviations from reference values were less than 0.15 units for pH and 10 % for all other parameters.
As 3 months were required for the soil solution samplers to reach steady state conditions, only samples collected after September 2002 were considered for data analysis.

4.3.5 Soil sampling in the planted topsoil for determination of soil biological parameters

Nine soil cores were taken in August 2003 (approximately 200 g dry weight) from each replicate of the contaminated and uncontaminated planted topsoils above each of the two subsoil types. The samples from each treatment were pooled and sieved to 2 mm. A sample was immediately used for basal respiration, microbial C biomass determination and nitrification potential, while the rest was kept frozen at −20°C before enzymatic analyses. The determination of the water content of the soil by drying (105°C) was used to relate the data on a g dry weight basis.

4.3.6 Soil biological analyses

a) Microbial biomass C

Microbial biomass C ($C_{\text{mic}}$) of field-moist samples was determined by the chloroform fumigation-extraction method of Vance et al. (1987). The filtered extracts of both fumigated and unfumigated soil samples were analysed for soluble organic C using a total organic C analyser (Shimadzu TOC-5000, Tokyo, Japan). Microbial biomass ($C_{\text{mic}}$) was estimated on the basis of the difference between the organic C extracted from the fumigated soil and the one from the unfumigated soil ($E_C$) using the equation: $C_{\text{mic}} = E_C \times 2.22$ (Wu et al., 1990).

b) Soil basal respiration

Basal respiration was determined according Zimmermann and Frey (2002). Water contents of the soil samples were adjusted to 2/3 of their water holding capacity. The moist soil samples were placed in 100 ml tubes and incubated in 250 ml air-tight glass vessels at 22°C for 4 days. The CO$_2$ produced from the soil was absorbed in NaOH and determined titrimetrically. The CO$_2$ evolution rates were determined after the soils had reached a relatively constant CO$_2$ production rate at 22°C. This was generally the case after 24 h.
Effects of heavy metals on soil solution parameters


c) Acid phosphatase

The phosphomonoesterase activity (phosphatase) was determined using para-nitrophenyl phosphate (pNPP) as a substrate using the method described by Tabatabai and Bremner (1969). Shortly, 1 g soil samples were incubated in a THAM buffer containing citric, maleic, and boric acids (pH 6.5) as well as pNPP at 37 °C for 1 h. The p-nitrophenol (pNP), the degradation product of the pNPP by phosphatase, was determined colorimetrically. The phosphatase activity was expressed as µg (pNP) per gram dry matter and hour.

d) Beta-glucosidase

The beta-glucosidase activity was measured as described by Alef and Nannipieri (1995). Briefly, 5 g of fresh soil were incubated in an acetate buffer (pH 6.2) containing a salicin substrate for 3 h at 37 °C. The concentration of the metabolised product (saligenin) was measured photometrically at 578 nm using phenol standards.

e) Nitrification potential

Nitrification potentials were determined using the shaken slurry method (Hart et al., 1994). Triplicate 15 g soil samples were weighed into 250 ml Erlenmeyer flasks. To each sample, 100 ml of a sterile solution (pH 7.2) containing 1.5 mM NH₄⁺ and 1.0 mM potassium phosphate buffer (K₂HPO₄: KH₂PO₄) was added, and slurries were incubated on an end-over-end shaker (180 rpm) at 25°C for 24 h. After 3, 5, 22, and 24 h, 10 ml aliquots of slurry were withdrawn and centrifuged. The supernatants were frozen and stored until they were analysed colorimetrically for NO₃⁻. Nitrification potential was calculated by linear regression of accumulated NO₃⁻ over time.

4.3.7 Statistical analysis

Standard errors of the treatment means were calculated from the replicate samples taken at each of the 3 following dates: 10 October 2002, 12 December 2002 and 5 June 2003. To evaluate differences between treatments, multivariate analysis of variance (MANOVA) was performed on log₁₀-transformed data based on the 3 dates mentioned above using SAS version 8.2 (SAS, Inc.). The significance level was chosen as P < 0.1.
Analyses of variance (ANOVA) were performed with SYSTAT 10 version 10 (SPSS, Inc.) to evaluate differences between treatments for the biological data.

4.4 Results

4.4.1 Heavy metals

The dissolved concentrations of Cu, Zn and Cd are reported in Table 4.2 for the different soils and treatments. Heavy metal concentrations were much higher in the contaminated topsoil than in the uncontaminated topsoil, but no significantly increased heavy metal concentrations in the subsoils below the contaminated topsoils were found (Rais, 2005).

Table 4.2: Concentration range of protons and heavy metals in the soil solution of planted and unplanted soil systems for CO/HM treatments.

<table>
<thead>
<tr>
<th></th>
<th>Topsoil with plants</th>
<th>Acidic subsoil with plants</th>
<th>Calcareous subsoil with plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.4-8.0/7.5-8.0</td>
<td>5.8-6.8/6.0-7.0</td>
<td>8.4-8.7/8.4-8.8</td>
</tr>
<tr>
<td>Cu (µmol L⁻¹)</td>
<td>0.19-0.62/1.0-1.6</td>
<td>0.02-0.04/0.02-0.05</td>
<td>0.15-0.24/0.12-0.16</td>
</tr>
<tr>
<td>Zn (µmol L⁻¹)</td>
<td>0.02-1.0/13.1-24.9</td>
<td>0.02-0.09/0.02-0.24</td>
<td>0.03-0.04/0.02-0.03</td>
</tr>
<tr>
<td>Cd (nmol L⁻¹)</td>
<td>0.10-0.51/20.0-44.3</td>
<td>0.28-1.1/0.37-0.90</td>
<td>0.11-0.51/0.10-0.32</td>
</tr>
<tr>
<td>pH</td>
<td>7.4-8.1/7.6-8.3</td>
<td>7.2-7.8/7.5-8.0</td>
<td>8.5-8.6/8.5-8.6</td>
</tr>
<tr>
<td>Cu (µmol L⁻¹)</td>
<td>0.09-0.27/1.0-2.0</td>
<td>0.03-0.08/0.03-0.15</td>
<td>0.11-0.23/0.13-0.19</td>
</tr>
<tr>
<td>Zn (µmol L⁻¹)</td>
<td>0.03-0.14/6.9-14.0</td>
<td>0.02-0.05/0.02-0.41</td>
<td>0.02-0.08/0.03-0.13</td>
</tr>
<tr>
<td>Cd (nmol L⁻¹)</td>
<td>0.10-0.78/10.5-16.0</td>
<td>0.09-0.23/0.10-1.2</td>
<td>0.09-0.21/0.10-0.83</td>
</tr>
</tbody>
</table>

4.4.2 Dissolved organic matter

Dissolved organic carbon and DON did not differ significantly between topsoils over different subsoils, neither for the metal-contaminated topsoil, nor for the control. In the presence of plants, DOC concentrations were significantly larger in the uncontaminated topsoil than in the metal-contaminated topsoil (P = 0.020, N = 8) (Figure 4.1 and Figure 4.2). Peak concentrations were observed between September and November and to a lesser extent in spring. In the absence of plants, DOC
concentrations were similar in contaminated and control topsoils (Figure 4.1 and Figure 4.2). Both were of similar magnitude as the concentrations in the contaminated topsoils with plants but exhibited a smoother time course than in presence of plants.

In the calcareous subsoils with plants, larger DOC concentrations were found for the CO than for the HM treatment (Figure 4.1). The difference was statistically significant in October 2002 (P = 0.043, N = 3). Without plants, DOC was the same in treatments with and without contamination (Figure 4.1). In the acidic subsoils there was no difference between the CO and HM treatment, neither in the presence nor in the absence of plants (Figure 4.2). Without plants, the DOC concentrations in both treatments were larger than with plants (Figure 4.2).

In the presence of plants, DON behaved similar as DOC in the topsoil and both subsoils in terms of differences between treatments and seasonal variations. The coefficient of correlation between DOC and DON was $R^2 = 0.94$ for the control and $R^2 = 0.92$ for the contaminated topsoils. With plants, the average DOC/DON ratio was about 12 in the topsoil, 18 in the calcareous subsoil, and 15 in the acidic subsoil for the CO treatment, and 13, 19, and 15, respectively for the HM treatment. The DOC/DON ratios are thus very similar in both treatments. With plants, the DOC/DON ratio in the topsoil solution is slightly larger than the $C_{org}/N_{tot}$ ratio in the topsoil solid phase (Table 4.1), while in the calcareous subsoil the two ratios are in close agreement. Without plants, DON concentrations were not calculated from total N concentrations. Due to the very large NO$_3^-$ concentrations they would have been the result of the difference between two large numbers.

### 4.4.3 Sulphate

In contrast to the other soil solution constituents, topsoil solution concentrations of SO$_4^{2-}$ depended on the type of subsoil in the presence of plants, at least in the CO treatment where SO$_4^{2-}$ concentrations were significantly larger above the acidic than above the calcareous subsoil (P = 0.062, N = 4) (Figure 4.3 and Figure 4.4). In the HM treatment, the subsoil effect on topsoil SO$_4^{2-}$ concentrations was not significant but there was a similar tendency. In the topsoil above acidic subsoil, HM significantly decreased SO$_4^{2-}$ concentrations (P = 0.038, N = 8). Furthermore, above acidic subsoil, peak SO$_4^{2-}$ concentrations were observed in late autumn for both CO and HM
Effects of heavy metals on soil solution parameters

Figure 4.1: DOC concentrations in the calcareous subsoil (40 cm depth) and the corresponding topsoil (10 cm depth), with (left) and without plants (right). Standard errors are given for the three dates (indicated with arrows) for which replicate samples were analysed separately (N=4).

Figure 4.2: DOC concentrations in the acidic subsoil (40 cm depth) and the corresponding topsoil (10 cm depth), with (left) and without plants (right). Standard errors are given for the three dates (indicated with arrows) for which replicate samples were analysed separately (N=4).
Effects of heavy metals on soil solution parameters

treatments. Above calcareous subsoil, elevated concentrations in autumn were much less pronounced.

In the calcareous subsoil with plants, more $\text{SO}_4^{2-}$ was found in the CO than in the HM treatment (Figure 4.3). The differences were significant for sampling dates in October and December ($P = 0.080$ and $N = 4$ for both). A trend to higher $\text{SO}_4^{2-}$ concentrations in the CO treatment was also found in the acidic subsoil with plants (Figure 4.4). In the absence of plants, no metal effects were observed on $\text{SO}_4^{2-}$ concentrations (Figure 4.3 and Figure 4.4). Furthermore, the $\text{SO}_4^{2-}$ concentrations were at or below the level of the concentrations reported for the HM treatment with plants.

Figure 4.3: $\text{SO}_4^{2-}$ concentrations in the soil solution of the calcareous subsoil (40 cm depth) and the corresponding topsoil (10 cm depth), with (left) and without plants (right). Standard errors are given for the three dates (indicated with arrows) for which replicate samples were analysed separately ($N=4$).
Effects of heavy metals on soil solution parameters

**Figure 4.4:** \( \text{SO}_4^{2-} \) concentrations in the soil solution of the acidic subsoil (40 cm depth) and the corresponding topsoil (10 cm depth), with (left) and without plants (right). Standard errors are given for the three dates (indicated with arrows) for which replicate samples were analysed separately (N=4).

### 4.4.4 Phosphate

\( \text{PO}_4^{3-} \) concentrations in the topsoil did not depend on subsoil type. In presence of plants, significantly larger \( \text{PO}_4^{3-} \) concentrations were found in the CO than in the HM treatment \((P = 0.004, N = 8)\) (Figure 4.5 and Figure 4.6). In contrast to all other soil solution constituents, a similar trend was found in absence of plants. In addition, for the respective treatment, the concentrations were larger without than with plants (Figure 4.5 and Figure 4.6). No clear seasonal trend was observed for any treatment. In the subsoils, \( \text{PO}_4^{3-} \) was below detection limit (1.58 \( \mu \text{mol L}^{-1} \)).

### 4.4.5 Nitrate

Topsoil concentrations of \( \text{NO}_3^- \) were not found to depend on the type of subsoil. In the planted topsoil, the heavy metal effect was the opposite to the other soil solution constituents as \( \text{NO}_3^- \) concentrations were significantly larger in the metal-contaminated than in the control topsoil \((P = 0.016, N = 8)\) (Figure 4.7 and Figure 4.8).
Effects of heavy metals on soil solution parameters

**Figure 4.5:** $\text{PO}_4^{3-}$ concentrations in the soil solution of the topsoil (10 cm depth) above calcareous subsoil with (left) and without plants (right). Standard errors are given for the three dates (indicated with arrows) for which replicate samples were analysed separately ($N=4$).

**Figure 4.6:** $\text{PO}_4^{3-}$ concentrations in the soil solution of the topsoil (10 cm depth) above acidic subsoil with (left) and without plants (right). Standard errors are given for the three dates (indicated with arrows) for which replicate samples were analysed separately ($N=4$).

The opposite was observed in the subsoils, although due to large variability the differences were not significant. In the calcareous subsoil, $\text{NO}_3^{-}$ concentrations peaked in late spring and autumn, especially in the CO treatment. In absence of plants, there were no differences between the CO and HM treatments, neither in the topsoils nor in the subsoils. Furthermore, in absence of plants, $\text{NO}_3^{-}$ concentrations were more than one order of magnitude larger than with plants, and in both subsoils the concentrations were clearly larger than in the topsoils.
Effects of heavy metals on soil solution parameters

Figure 4.7: NO$_3^-$ concentrations in the soil solution of the calcareous subsoil (40 cm depth) and the corresponding topsoil (10 cm depth), with (left) and without plants (right). Standard errors are given for the three dates (indicated with arrows) for which replicate samples were analysed separately (N=4).

Figure 4.8: NO$_3^-$ concentrations in the soil solution of the acidic subsoil (40 cm depth) and the corresponding topsoil (10 cm depth), with (left) and without plants (right). Standard errors are given for the three dates (indicated with arrows) for which replicate samples were analysed separately (N=4).
4.4.6 Soil biological parameters

As there were no significant differences in the microbial parameters between the topsoils above acidic and calcareous subsoils, only averaged values are presented.

In presence of plants, all biological parameters were significantly reduced in the HM treatment: acid phosphatase activity by a factor of 3, microbial C biomass and nitrification potential by a factor of 2, and basal respiration and beta-glucosidase activity by a factor of 1.5 (Table 4.3).

Table 4.3: Mean values (±SD, N = 4) of microbial biomass, microbial activity expressed as basal respiration, beta-glucosidase activity, nitrification potential, and acid phosphatase activity given per g topsoil in the presence of plants. For all parameters, the two treatments CO and HM were significantly different at P < 0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Microbial C biomass (µg C g⁻¹)</th>
<th>Basal respiration (µg CO₂ g⁻¹ h⁻¹)</th>
<th>ß-glucosidase (µmol saligenin g⁻¹ h⁻¹)</th>
<th>Nitrification potential (µg N-NO₃ g⁻¹ h⁻¹)</th>
<th>Acid phosphatase (µmol PNP g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>329±67</td>
<td>3.02±0.24</td>
<td>0.238±0.011</td>
<td>0.125±0.027</td>
<td>2.36±0.21</td>
</tr>
<tr>
<td>HM</td>
<td>160±31</td>
<td>1.87±0.29</td>
<td>0.168±0.025</td>
<td>0.058±0.015</td>
<td>0.78±0.28</td>
</tr>
</tbody>
</table>

PNP = p-nitrophenyl phosphate

4.5 Discussion

4.5.1 Dissolved organic matter

Dissolved organic carbon concentrations are largely controlled by biological activity (Dai et al., 1996; Kalbitz et al., 2004) and are governed on one hand by the degradation of SOM as source, and on the other hand by degradation of DOC as sink. The HM effects on DOC concentrations, thus, reflect a combined influence on these two processes. The detrimental effects of HM on the size and activity of the microbial community and more specifically on the beta-glucosidase activity are reflected by a reduced DOC mobilisation in the planted contaminated topsoil. The enzyme beta-glucosidase is involved in the degradation of SOM and is responsible for the release of low molecular weight compounds such as glucose into the soluble C pool (Killham, 1994). Different, sometimes conflicting results on the effects of HM on the biodegradation of DOC were reported by Marschner and Kalbitz (2003): an inhibition of DOC biodegradability in presence of metals was generally attributed to the toxicity
of the free metal and metal-organic complexes; an enhancement of DOC biodegradability in presence of HM was attributed to flocculation, as larger structures will provide better attachment for microbial colonies. The lower DOC concentrations in the soil solution of the contaminated topsoil than of the uncontaminated topsoil in presence of plants are in agreement with reduced degradation of SOM by HM and with an enhancing effect of HM on DOC biodegradability. Some inhibition of DOC degradation, however, cannot be excluded if its extent is smaller than the one of the effects described before.

Mineralisation processes are known to be enhanced in the rhizosphere as a result of the stimulation of microbial activity by root exudates (Brimecombe et al., 2001; Kandeler et al., 2002). However, root exudates themselves are not expected to significantly enhance DOC concentrations in bulk soil solution since Hagedorn et al. (2002) and Hagedorn et al. (2004) reported that the major part of DOC in mineral soils originates from old organic C in the soil and not from easily degradable root exudates that are rapidly consumed by microorganisms. On the basis of these considerations, the lack of a HM effect on DOC concentrations in the absence of plants and the fact that the DOC concentrations are similar in the contaminated topsoil with and without plants, may be interpreted as follows. Without plants, there is a base level of microbial SOM degradation which is insensitive to HM contamination. On the other hand, if one assumes that plant stimulation of microbial activity is proportional to root density, it cannot be excluded that part of the HM effect on DOC in the presence of plants is an indirect effect on soil microorganisms through the impact of HM on root growth observed in our systems (Menon et al., 2005).

Without taking into account the peak concentrations in autumn, the temporal variations of DOC in the planted topsoil indicate higher concentrations in spring-summer than in winter and can be linked to microbial activity. This is in accordance with the results from Kaiser et al. (2002). The peak concentrations in autumn can be ascribed to the pruning of the deciduous trees. It is feasible that this measure leads to similar effects as clear-cutting, in particular enhanced DOC mobilisation (Piirainen et al., 2002; Kalbitz et al., 2004).

Because topsoil contamination had no significant effect on HM concentrations in the subsoils, the lower DOC concentrations in the calcareous subsoil below the contaminated topsoil than below the uncontaminated topsoil cannot be the result of a direct HM effect. Results by Menon et al. (2005) show that root density in the
calcareous subsoil tended to be smaller below the contaminated topsoil than below the uncontaminated one, which can be linked to the HM effect on root density in the topsoil. The HM effect on the DOC mobilisation in the calcareous subsoil can then be explained by assuming again that stimulation of microbial activity in the presence of plants depends on root density and thus on the amount of root exudation. Similar C decomposition processes in the top- and subsoil are also suggested by the DOC concentrations in the calcareous subsoils following mainly the ones in the topsoil solution. The slightly smaller seasonal variations in the calcareous subsoil may be explained by the generally much smaller root density compared to the topsoil (Menon et al., 2005). Leaching of DOC from the topsoil to the mineral calcareous subsoil can not be completely excluded but seems unlikely considering the similar C/N ratio between the soil matrix and the soil solution (see discussion below). In the acidic subsoil, the absence of HM effect on DOC concentrations can be related to similar root density between the CO and HM treatment (Menon et al., 2005). As DOC solubility increases with pH (Kalbitz et al., 2000), the larger DOC concentrations in the absence than in the presence of plants in this soil can be explained by the higher pH in the plant-free reference (Table 4.2).

4.5.2 Dissolved organic matter: C/N ratio

The comparison of the C/N ratio in solution with the one in the soil matrix can provide information on the way SOM is decomposed and released into solution. Major sources of DOM are humified old C (older than 4 years) and native soil C (Hagedorn et al., 2004). This old SOM is presumably made soluble after hydrolysis by exoenzymes or dissolution, which is a prerequisite for further degradation leading to plant-/soil-derived DOC (Marschner and Kalbitz, 2003). In the model ecosystems with plants, the quite similar C to N ratio in the soil solution (i.e. DOC/DON) and in the soil matrix (i.e. C\text{org}/N\text{tot}) indicates that organic N was not decomposed faster than organic C. This suggests that hydrolysis of organic N is linked to C decomposition rather than occurring selectively in response to the need for N of the microorganisms. Another explanation is that the release of DOC and DON was mainly controlled by dissolution processes from the soil organic C pool.

The lack of a HM effect on the C/N ratio in the topsoil solution suggests that the higher N requirements of the microbial community living in the uncontaminated topsoil
Effects of heavy metals on soil solution parameters

4.5.3 Sulphate

The differences in $\text{SO}_4^{2-}$ concentrations observed between the different treatments are very likely related to different enzyme activities involved in the decomposition of organic S-containing compounds. For instance, Kandeler et al. (2000) and Haanstra and Doelman (1991) reported a smaller activity of arylsulfatase in metal-contaminated soils. The very low $\text{SO}_4^{2-}$ concentrations in the absence of plants suggest that the activity of microorganisms involved in S mineralisation depends even more strongly on stimulation by root exudation than the activity of microorganisms responsible for SOM decomposition. Chowdhury et al. (2000) reported substantial mineralisation of soil organic-S with but not without plants and Hu et al. (2002) and (Hu et al. (2003) reported $\text{SO}_4^{2-}$ enrichment in the rhizosphere as a consequence of higher arylsulfatase activity. Another explanation for the larger $\text{SO}_4^{2-}$ concentrations in presence of plants may be the direct release of $\text{SO}_4^{2-}$ from dead roots as $\text{SO}_4^{2-}$ is the most abundant form of S in roots (Marschner, 1995). This plant effect on $\text{SO}_4^{2-}$ mobilisation may also explain the larger $\text{SO}_4^{2-}$ concentrations in the planted control topsoils above acidic than above calcareous subsoils because of the larger root density measured above acidic subsoils (Menon et al., 2005). We cannot explain, however, the particularly small $\text{SO}_4^{2-}$ concentrations in the planted topsoil above calcareous subsoil compared to both the calcareous subsoil and the topsoil above acidic subsoil, and the corresponding negligible HM effect.

As for DOC, the pruning of the deciduous trees is likely to be responsible for the elevated $\text{SO}_4^{2-}$ concentrations in autumn, in particular in the topsoil above acidic subsoil. The less pronounced maximum in May in all topsoils is in accordance with observations by Turrion et al. (1997).

4.5.4 Phosphate

On one hand, in the presence of plants, the smaller $\text{PO}_4^{3-}$ concentrations in the contaminated topsoil than in the control topsoil reflect the strongly reduced activity of the enzyme acid phosphatase under contamination. Similar detrimental effects of HM on the activity of this enzyme were reported by several authors (Kandeler et al., 2000; Effron et al., 2004; Roy et al., 2004). On the other hand, $\text{PO}_4^{3-}$ is known to form weakly
Effects of heavy metals on soil solution parameters

soluble solid phases with HM. In particular, Zn and Pb have been shown to form precipitates with $\text{PO}_4^{3-}$ in the range from neutral to alkaline soil conditions (Kabata-Pendias and Pendias, 2001; Sauvé et al., 1998). Therefore, a reduced $\text{PO}_4^{3-}$ solubility in the topsoil contaminated with the metal dust seems plausible. Indeed, calculations with the SOILCHEM speciation program (Sposito and Coves, 1995) showed that the soil solution in the contaminated topsoil was oversaturated with respect to hopeite ($\text{Zn}_3(\text{PO}_4)\text{I}_2$). The occurrence of such a chemical effect can also explain that $\text{PO}_4^{3-}$ is the only ion for which a negative HM effect is observed also in the absence of plants.

As discussed for $\text{SO}_4^{2-}$, P mineralisation is also expected to be enhanced by root exudation. Indeed, a larger phosphatase activity in the rhizosphere than in the bulk soil was reported by Asmar et al. (1995). In our systems, however, the larger $\text{PO}_4^{3-}$ concentrations in the absence than in the presence of plants reflect plant uptake (Jungk, 2002) and mask any opposite effect by root exudation.

4.5.5 Nitrate

Reduced nitrification potential under HM contamination as measured in the presence of plants is in accordance with the literature (Sauvé et al., 1999; Smolders et al., 2001). The opposite effect on $\text{NO}_3^{-}$ concentrations can be explained by the larger plant uptake of $\text{NO}_3^{-}$ in the control topsoil than in the contaminated topsoil as suggested by root density measurements (Menon et al., 2005). This is supported by the results obtained in the absence of plants where $\text{NO}_3^{-}$ concentrations were much larger than in the presence of plants. The large influence of plant uptake on $\text{NO}_3^{-}$ concentrations in bulk soil solution which masks or even outbalances the effects on microbial activity can be attributed to both the large demand for N and the high mobility of $\text{NO}_3^{-}$ in soils (Jungk, 2002). This is in contrast to $\text{PO}_4^{3-}$, which due to its much lower mobility in soil is only reduced by plant uptake in the rhizosphere (Jungk, 2002) and to $\text{SO}_4^{2-}$ which is taken up by plants at only a low rate (Marschner, 1995).

The small seasonal variations of the $\text{NO}_3^{-}$ concentrations in presence of plants, except for the peaks in the calcareous subsoil, suggest a general balance between nitrification and nitrate uptake while peak concentrations in the calcareous subsoil can be interpreted as the result of an imbalance. In agreement with observations by Nadelhoffer et al. (1984), such peaks occurred in summer and autumn. The pruning of the deciduous trees may have increased the effect in autumn since elevated $\text{NO}_3^{-}$
concentrations have been measured after clear-cutting (Piirainen et al., 2002; Killham, 1994).

4.6 Conclusions

In good agreement with previous studies, biological parameters demonstrated that size and activity of the microbial community in the planted topsoil were reduced by HM contamination. In accordance, DOC and $\text{SO}_4^{2-}$ concentrations were reduced in these soils. Comparison of DOC and $\text{SO}_4^{2-}$ concentrations in the soil solution in presence and absence of plants suggest that there is a base level of degradation processes in the absence of plants that is not affected by HM contamination. The stimulation of the microbial activity by root exudates may have been strongly affected under HM stress at least partly because of the indirect negative effect of HM on root growth as indicated by root density measurements. As a consequence, soil solution monitoring indicated that seasonal variations in DOC and $\text{SO}_4^{2-}$ concentrations are dampened in the presence of HM.

Concentrations of $\text{PO}_4^{3-}$ and $\text{NO}_3^{-}$ in the soil solution are governed in addition by other factors than microbial activity. Due to both the large demand by plants and its high mobility, the concentrations of $\text{NO}_3^{-}$ are mainly controlled by plant uptake. Effects of HM on nitrification potential are masked effectively. For $\text{PO}_4^{3-}$, the effect of plant uptake can be recognized by smaller concentrations in the presence than in the absence of plants. It does not mask, however, effects of HM on microbial activity and the chemical effect of reduced $\text{PO}_4^{3-}$ solubility in the presence of HM. The latter effect very likely is responsible for the fact that $\text{PO}_4^{3-}$ is the only ion for which there is a negative HM effect in the absence of plants.

Soil solution constituents, which consider the soil compartment as a whole and at natural conditions, are complementary to microbial assays and put the results of the biological tests into an ecosystem perspective.

4.7 Acknowledgments

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Chapter 5

General conclusions

The objectives of this lysimeter experiment with young forest model ecosystems were on one hand to increase our understanding of the dynamics of heavy metals in the soil solution as influenced by plants and seasonal variations in plant and microbial activity, and, on the other hand to investigate the effects of heavy metal contamination on selected soil solution parameters in order to evaluate the response of a whole soil ecosystem to heavy metal stress. In a first step, however, different suction cup materials used for the sampling of soil solution were tested for their sorption characteristics under different conditions in order to determine the best suited material with respect to its minimal influence on the soil solution chemistry, especially regarding heavy metals.

5.1 Sorption characteristics of different suction cup materials in function of the soil solution chemistry – Does DOC matter and what is the best suited suction cup material for trace metal sampling?

The sorption characteristics of a suction cup material are strongly influenced by the chemistry of the soil solution, in particular pH and dissolved organic carbon (DOC) content. As expected (Wenzel et al., 1997; Andersen et al., 2002), metal sorption generally decreased with pH as a result of increasing proton competition for the binding sites on the cup material. Our experiments underlined the importance of DOC on the sorption of heavy metals. In most cases, we noted less metal sorption with than without DOC, at given pH and heavy metal concentrations. This can be explained by the formation of metal-organic complexes that are sorbed less at cup binding sites than the inorganic metal species. Metal retention was especially reduced at alkaline pH due to the larger complex stability and to the increased DOC solubility with increasing pH (Kalbitz et al. 2000). The sorption of DOC depended also strongly on its quality. The "new" DOC originating from leaf litter was much more sorbed on the different cup materials than the "old" DOC from seepage water, primarily because of the larger
proportion of the hydrophobic fraction in the new DOC. As a consequence, our results suggest that the accuracy of soil solution monitoring with suction cups is higher in deeper mineral soil horizons than just below the litter layer.

Nylon and polyvinyl alcohol (PVA) materials used in this study for standard and micro suction cups, respectively, are the best suited materials if heavy metals are to be analysed. Teflon, ceramic and borosilicate glass materials were not found suitable for the monitoring of heavy metals in alkaline soil solution and both Teflon and ceramic sorbed Cu and Pb also strongly at low pH. None of the tested materials gave satisfactory results with respect to Pb in alkaline conditions.

5.2 Do plants and seasonal variations in plant and microbial activity change the concentration and speciation of heavy metals in soil solution?

Soil solution concentrations of Cu, Zn, and Cd were always much higher in the contaminated than in the uncontaminated topsoils. In the metal-contaminated topsoils, the concentrations of HM varied relatively little with time. For both the metal-contaminated and control topsoils, the strong correlation with DOC indicates that Cu solubility was governed mainly by the formation of complexes with dissolved organic matter (DOM). The solubility of Zn and Cd was controlled by phosphate and to a lesser extent by pH in the metal-contaminated topsoils. This suggests that the soil solution concentrations of these two metals were governed mainly by the equilibrium with their phosphates. It is assumed that the degradation of Zn phytate led to the release of both phosphate and Zn, the solution concentrations of which are then controlled by Zn phosphate. In contrast to Zn, metal-contaminated topsoil solutions are largely undersaturated with respect to Cd₃(PO₄)₂ and thus, a strong dependence of Cd solubility on phosphate can only be explained by indirect effects. The excellent correlation between Cd and Zn concentrations suggests that Cd may be sorbed to Zn phosphate precipitates, or increasing Zn concentrations may lead to an increasing release of Cd from exchange sites. Based on the considerations on solubility control, the larger Zn and Cd concentrations in the presence than in the absence of plants can be attributed to the smaller phosphate concentrations what can be explained by plant uptake of phosphate. In conclusion, in neutral to slightly alkaline soils with sufficiently
large P contents, plants may indirectly increase Zn and Cd concentrations in the soil solution through nutrient uptake.

Since the metal concentrations in the subsoil solutions below both planted and unplanted HM topsoils were not or only little larger than in the control, retention by precipitation or sorption of HM must have occurred upon soil solution entering the uppermost layers of the subsoils. As in the topsoil, the solubility of Cu in both subsoils seemed to be controlled mainly by DOC. In the acidic subsoil below HM topsoil, Zn and Cd concentrations depended mainly on pH. In the presence of plants, Zn concentrations below the CO topsoil were smaller than below HM topsoil, despite similar pH. This indicates that some Zn from the contaminated topsoil may have been translocated and formed a better soluble Zn phase than the background lithogenic Zn. The tendency to larger Zn and Cd concentrations in the acidic subsoil in the presence of plants in winter and spring can be explained by lower pH values during this time of the year, that may have been the result of reduced nitrate uptake by plants and microorganisms.

The mostly smaller Cu$^{2+}$ fraction in the absence than in the presence of plants as well as the large overestimation of Cu$^{2+}$ by WHAM for the plant free system, indicate a difference in DOC quality in terms of metal binding properties. The DOC in the soils without plants seemed to form more stable complexes with Cu than the DOC in the presence of plants. Considering this and that DOC concentrations were similar with and without plants, larger total Cu concentrations should have been measured in the absence of plants. The fact that total Cu concentrations in soil solution did not depend on the presence of plants suggests that in the absence of plants Cu was also more strongly bound to the solid soil organic matter.

5.3 Use of soil solution parameters as indicators for the mineralisation potential of a whole soil compartment. Do heavy metals show detrimental effects and what is the influence of plants?

The use of soil solution parameters to investigate the effects of heavy metals on the nutrient turnover of a soil permits to take the "pulse" of the whole soil ecosystem, including plant and microbial interactions. All the biological parameters obtained from "classical" microbial assays demonstrated that both the size and activity of the microbial community were reduced in the planted topsoil with heavy metal
contamination. In good agreement with these results, DOC and $\text{SO}_4^{2-}$ concentrations were also depleted in these soils. No such metal effect was found in absence of plants, and DOC and $\text{SO}_4^{2-}$ concentrations were at the same level as in the contaminated planted topsoil. This suggests on one hand a base level of microbial activity in the absence of plants that is not affected by heavy metal contamination. On the other hand, stimulation of microbial activity by root exudation is severely limited by HM contamination. This can be partly explained by a negative effect of HM on root density.

Phosphate and $\text{NO}_3^-$ concentrations in soil solution are governed in addition by other factors than microbial activity. Phosphate is the only soil solution parameter for which a metal effect was found in both the presence and absence of plants with lower concentrations in the heavy metal treatment than in the control. This was interpreted as a chemical effect of reduced $\text{PO}_4^{3-}$ solubility in the presence of heavy metals. A plant uptake effect was indicated by the lower concentrations in presence than in absence of plants. Due to both the large demand by plants and its high mobility, $\text{NO}_3^-$ concentrations were mainly controlled by plant uptake and the heavy metal effects on it, while the metal effects on the nitrification potential are masked.

Soil solution parameters, which take into account a whole soil compartment at natural conditions, put the results of biological tests into an ecosystem perspective. In the light of this perspective, the effect of heavy metals on nutrient concentration in the soil solution may not be as large as suggested by microbial assays since plants sometimes counterbalance the lower mobilisation of nutrients like $\text{NO}_3^-$ in contaminated soils by lower uptake. Moreover, the presence of plants does not increase the nutrient concentrations in metal-contaminated soils compared to plant-free contaminated soils by stimulation of microbial mineralisation processes.

5.4 Outlook and open questions

The results from this study that relate to the afforestation of a slightly acidic agricultural topsoil, question the efficiency of the phytoremediation of a heavy metal contaminated soil in the short term. No evidence of beneficial effects of plants in terms of reduced metal bioavailability and mobility were found in our metal-contaminated soil systems. This may change on a longer term with increasing plant uptake of the readily bioavailable metal fraction and the following possible reduction of the metal replenishment of the soil solution from the soil matrix. In order to check the validity of
such an hypothesis, long-time experiments would be needed. In a second step, a
generalisation of the knowledge gained from near-natural model ecosystems could be
investigated in field experiments even if the lack of a proper control would then be a
problem. This would contribute to improve the effectiveness of the legislative guidelines
for heavy metal contamination.

Data on total metal concentrations in the top- and sub-soil at the end of the
experiment will be available in near future. They will answer the question whether there
was translocation of heavy metals from the topsoil to the acidic subsoil.

The data and informations gained from this study on the dynamics of heavy metals
in the soil solution of a young forest model ecosystem will be compiled with data from
other research groups involved in the "Cell to Tree" framework at different ecosystem
levels in order to improve the modelling of metal pathways within a contaminated
ecosystem. This will ultimately help to improve the risk assessment of heavy metal
contamination and to prevent related hazards for human health.


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Curriculum Vitae

1974 Born on December 2 in Lausanne (Switzerland)

1981-1985 Primary school, Delémont (Switzerland)

1985-1991 Secondary school, Delémont (Switzerland)

1991-1994 High school, Porrentruy (Switzerland)

1994-2000 Study of biology, University of Neuchâtel (Switzerland)

Specialisation in chemical ecology (plant-insect interaction)

2000 Licence ès Sciences in Biology

2001-2005 PhD studies at the Swiss Federal Institute of Technology (ETH) in Zürich, and research assistant at the Swiss Federal Research Institute WSL in Birmensdorf