TRANSLOCATION OF COPPER IN AN ALPINE CHRONOSEQUENCE BY PHENOLIC-TYPE VECTORS

THÈSE N^{o} 1997 (1999)

PRÉSENTÉE AU DÉPARTEMENT DE GÉNIE RURAL

ÉCOLE POLYTECHNIQUE FÉDERALE DE LAUSANNE

POUR L'OBTENTION DU GRADE DE DOCTEUR ÈS SCIENCES

PAR

Andrea OESS
diplômée en biochimie de l'Université de Genève
de nationalité suisse et originaire de Genève (GE)

acceptée sur proposition du jury:

Prof. J.-C. Védy, directeur de thèse
Dr M. Cheshire, rapporteur
Prof. D. Genske, rapporteur
Prof. H. C. B. Hansen, rapporteur
Dr M. Robert, rapporteur
Dr S. Stoll, rapporteur

Lausanne, EPFL
1999

Pour les baleines, les étoiles et les fourmis, en passant par le Val d'Arpette.

Remerciements

Je voudrais tout d'abord remercier le Pr. Jean-Claude Vedy pour m'avoir accueillie au sein de l'unité de Pédologie, de m'avoir témoigné confiance et sympathie jusqu'au bout.

I would like to thank Dr. Martin Cheshire for his readiness to help and the good discussions we could have in Scotland, at the Arpette site and for English corrections... I would also like to thank Dr. Donald McPhail for teaching me with patience how to use the ESR machine...

Un grand merci à toutes les personnes qui m'ont aidée sur le terrain. Tout d'abord merci au Pr. Pierre Faivre et à Christianne Gallet pour m'avoir aidé dans le choix des sols. Merci à Julien Vallet qui m'a accompagné surtout pour les mesures de d'hiver, pour le pellatage des fosses dans la neige, et les mesures GPS. Merci à Rita, Francoise pour l'aide dans les déterminations floristiques, merci pour le sourire, l'énergie et la bonne humeur de Corinne (surtout le matin...car le soir on bosse !), merci pour l'accueil de Mr. Ankisi au Jardin Alpin, merci également à Anne-Laure, Jean-David, Yves et Mireille, Claudia, à Carolina, à Eric Bardou, Jens, à Fabienne et à Pascal, à Claude, à Mazem.
Merci à Philippe de Pury pour m'avoir aidée dans la détermination des sols et pour m'avoir fait découvrir ses terrains d'étude.

Merci à Serge pour l'initiation à la simulation... ainsi qu' à Nalini pour les calculs de constantes et à Hans-Rudolf Hagemann pour les mesures de constantes diélectriques.

Merci à Jean-Pascal pour son activité, son efficacité et surtout pour m'avoir aidé pour les analyses de sols. Merci aussi à David, à Jean-David, à Stéphane et à Olivier ainsi qu'à Lukas.

Un grand merci aux copines du labo, pour leur soutien, leur gentillesse, il s'agit bien évidemment de Peggy, Isabelle, Karine et Pauline ! ! !

Merci également à Gorana, Alexis Amiguet, Flavio, Jean-Daniel Bonjour, Daniel Grandjean, Cesar Pulgarin, Joanna, Denis Baize, Christophe Joerin, ainsi qu'à Dominique Grandjean.

Résumé

Les applications répétées de produits phytosanitaires contenant du Cu pour le traitement des vignes et des vergers ont contribué à considérablement augmenter les teneurs en Cu dans les sols durant les dernières décennies et par la même occasion le risque de migration et de contamination des nappes et donc des réservoirs d'approvisionnement en eau. Afin de mieux connaître les paramètres liés au sol pouvant influencer soit la migration ou la rétention de Cu dans les horizons de surface le mécanisme de translocation du Cu a été étudié sur une chronoséquence de sols peu pollués ou à faible influence anthropogène et à acidité prononcée. En particulier le rôle des phénols naturels présents dans les plantes et les fractions organiques du sol a été mis en évidence par une approche combinée faisant intervenir d'une part des mesures sur le terrain du Val d'Arpette et d'autre part une étude mécanistique sur le rôle de la dynamique de complexation et de polymérisation des phénols.

Les mesures effectuées par Résonance Paramagnétique Electronique (RPE), potentiométrie, simulation dynamique, et absorption UV ont montré que les ortho-dihydroxyphénols (ODP) sont les phénols les plus aptes à former des complexes avec le Cu et ceci même pour des pH très bas de l'ordre de 4. La quantité de phénols de type ODP libérée potentiellement par les différentes espèces de la couverture végétale a été déterminée. Le catéchol, l'acide cafféique et l'acide protocatéchique étaient les phénols complexants dominants. Les paramètres responsables de la migration du Cu ont été déterminés par le biais de la solution du sol en effectuants des prélèvements sur trois ans à l'aide de lysimètres sans tension. Les facteurs saisonniers comme les amplitudes thermiques de l'air, du sol, la pluviométrie, le degré d'ensoleillement ainsi que les paramètres physico-chimiques (pH, T°, DOC, phénols, Cu) ont été étudiés. Les paramètres responsables de la rétention du Cu ont été déterminés en effectuant des isothermes de sorption du Cu en présence des différents horizons d'un PODZOSOL OCHRIQUE. Malgré une forte affinité du Cu pour les composantes organiques insolubles des horizons A, des migrations verticales ont également pu être mises en évidence. Ces migrations étaient toujours plus élevées entre l'horizon O et l'horizon A qu'entre l'horizon A et les horizons sous-jacents et étaient proportionnels aux teneurs de Cu dans les horizons de provenance. La forte corrélation entre les phénols et les événements de migration du Cu ont indiqué que les phénols sont les vecteurs privilégiés du Cu au détriment de la phase organique dissoute totale du sol. Les études mécanistiques ont également permis de mettre en évidence 2 types de réactivités dans les groupements fonctionnels des phénols. Alors que les groupements de type ortho-dihydroxyphénols sont de bons chélatants et contrôlent la complexation du Cu, les groupes carboxyliques n'interviennent pour ainsi dire pas dans la complexation, mais jouent un rôle déterminant dans la solubilisation de ces complexes.

Abstract

Repeated applications of Cu for phytosanitary treatments of vineyards and orchards have contributed to a considerable increase of Cu levels in soils over the last decades. Therefore the risk of migration and Cu contamination of the groundwater and the drinking water supply has also increased. In order to contribute to a better understanding of these parameters, which control either the retention of Cu to soil or its mobilisation, a study has been carried out on the translocation of Cu in low polluted soils of an alpine chronosequence characterised by extremely low pH values. In particular the role of natural phenols released by plants and the organic fractions of soils was investigated by a two scale approach. On one hand field measurements were performed at the Arpette valley site and on the other hand mechanistic studies were carried out in the laboratory about the role of complexation reactions and polymerisation dynamics on these processes.

The use of Electron Spin Resonance Spectroscopy (ESR), potentiometry, dynamic simulations and UV absorption revealed that ortho-dihydroxyphenols (ODP's) were the main complexing agents of Cu(II). They were even able to form complexes at very low pH values such as pH 4. The potential of ODP's released by the different plant species from the vegetation cover was determined for the different soils. Catechol, caffeic acid and protocatechuic acid were the dominant species present. Parameters responsible for the migration of Cu were determined through sampling of the soil solution with a tension free lysimeter device. Seasonal factors such as air and soil temperature amplitude, rainfall, solar radiation as well as physico-chemical parameters of the soil solution (pH, T°, DOC, phenols, Cu) were measured. Parameters responsible for the retention of Cu were studied by performing sorption isotherms of Cu in the presence of different horizons of a PODZOSOL OCHRIQUE. Despite the strong affinity of Cu for the insoluble organic fraction of the A-horizons, vertical migration of Cu was observed. These migrations were always higher between the O- and the A-horizon than between the A- and the under-laying horizons. The quantity of migration was always proportional to the levels of Cu present in the horizons of origin. The strong correlation observed between phenol concentration and Cu migration events suggested that phenols were the favourite translocation vectors of Cu in these soils at the cost of the DOC (dissolved organic carbon) fraction. Mechanistic studies also permitted to identify two types of functional reactivities around the phenol molecules: while ortho-dihydroxyphenols had strong chelating properties and were responsible for the complexation of Cu, carboxylic groups (COOH) which were not directly involved in the complexation reaction, played a key role by maintaining these complexes in a hydrosoluble form.

Contents

PART I : FIELD APPROACH

PART II : MECANISTIC APPROACH

Introduction

Prélude....

Le cuivre, élément-trace essentiel au métabolisme des organismes vivants, peut, lorsque les concentrations mises en jeu deviennent trop élevées, devenir hautement toxique pour ces mêmes organismes. Le système sol-végétation est une importante source de réserve de Cu. Bien que sa distribution ne soit pas homogène entre les différentes couvertures pédologique de l'échelle terrestre, la quantité totale de Cu stockée dans les sols a été estimée à 3.3 x10^{13} tonnes (Nriagu, 1979).

Les sols contrôlent de par leurs propriétés physiques, chimiques et biologiques le comportement du cuivre non seulement dans l'écosystème sol-végétation proprement dit mais aussi dans tous les autres systèmes connectés, à savoir l'hydrosphère et la lithosphère. Le contrôle exercé par le sol sur le cuivre est essentiellement lié au concept de « mobilité ». Lorsque le cuivre est mobile, sa redistribution dans l'écosystème peut, par la mise en jeu de processus de transfert, contaminer les systèmes connectés. S'il est, à l'inverse, immobile, il s'accumule dans les sols qui servent alors de "source potentielle" de Cu. Les variations de propriétés du sol causées soit par des phénomènes d'anthropisation, soit par l'évolution des sols au cours du temps peuvent modifier les formes de disponibilité de Cu. A ce moment le sol peut devenir une "source active" hautement contaminante pour les milieux naturels.

Le but de ce travail est de déterminer, *in situ* et par une démarche à caractère expérimental, les facteurs majeurs du comportement du cuivre à cet interface privilégié que représente l'interface sol - solution du sol. La mobilité du cuivre dépendant, comme pour de nombreux éléments-traces métalliques, du pH de la solution du sol, nous avons choisi d'étudier plus principalement les milieux acides et, notamment, les milieux podzolisants dont on sait qu'ils représentent des milieux exemplaires pour ce type d'étude. Ils sont non seulement acides mais aussi "acidocomplexolytiques" c'est à dire producteurs de composés organiques complexants dont la présence, dans la solution du sol, contrôle largement la mobilité de substances minérales dans l'écosystème sol-végétation. Parmi les vecteurs organiques potentiels du cuivre présents dans la solution du sol nous avons concentré notre étude sur les phénols en essayant d'en préciser le rôle dans un contexte où les effets des variations saisonnières sont des paramètres déterminants de la translocation.

Un modèle-terrain a donc été sélectionné. Ce modèle se caractérise par une roche-mère acide, des altérations allant de la bisiallitisation à l'acidocomplexolyse, des pédogenèses naturelles allant de la brunification par héritage à la podzolisation. La pollution par le cuivre y est modérée voire faible. L'altitude varie entre 1'468 et 3'270 mètres. La pluviométrie moyenne interannuelle dépasse 1,500 mm, les températures moyennes variant entre –8°C degrés en hiver et 4.5 degrés en été. La couverture végétale naturelle est essentiellement représentée par des landes à Rhododendrons et à *Vaccinium myrtillus*. Ce modèle-terrain, largement étudié par ailleurs (Keller, 1991 ; Huber, 1997), a été réexaminé pour en sélectionner trois stations. Ainsi chacune des stations retenues a été analysée dans le détail de ses caractéristiques végétales et pédologiques.

Dans un premier temps différents dispositifs de mesure ont été installés permettant (1) de suivre la variation temporelle et en continu des paramètres principaux du pédoclimat (2) de prélever la fraction gravitaire de la solution du sol à l'aide de lysimètres de type "sans tension" s'inspirant de dispositifs connus et largement décrits par ailleurs. Le Cu et les phénols présents dans cette fraction de la solution du sol ont ainsi fait l'objet d'une attention particulière. La durée de ces observations a été de 3 ans.

Une deuxième étape de cette recherche a consisté à développer et mettre au point des méthodes performantes et reproductibles portant sur les constituants phénoliques de la solution du sol. La stabilité des phénols a été mesurée sous différentes conditions et différentes méthodes de pré-concentration et d'extraction des phénols ont été testées. Enfin le mécanisme d'interaction de Cu avec les phénols a été déterminé en laboratoire à l'aide de molécules synthétiques et d'échantillons de sols et de plantes.

Une troisième et dernière étape de cette recherche a permis d'analyser les solutions du sols et de proposer un mécanisme rendant compte des principales causes de rétention ou de mobilisation de Cu dans le sol.

Ce travail repose sur plusieurs hypothèses:

(1) les phénols sont présents en quantité signifiante dans la fraction gravitaire de la solution du sol des systèmes podzolisés,

(2) les phénols ou, tout au moins, certains d'entre eux sont susceptibles de former des complexes avec le cuivre,

(3) ces "complexes" Cu-phénols représentent, dans les milieux étudiés, les formes privilégiées du transfert de cet élément-trace par la fraction gravitaire de la solution du sol.

A ces trois hypothèses se rajoute une intention d'expliquer les mécanismes faisant que, sous certaines conditions le cuivre demeure mobile alors que sous d'autres, il peut s'immobiliser dans le sol et ses structures organo-minérales.

Le Cu et sa Dissemination dans l'Environnement

Le Cu a été choisi dans cette étude d'une part parce que c'est le métal le plus exploité à l'échelle du globe terrestre, mais également pour son rôle ambigu qui lui vaut d'être un élément en carence chez certains individus et en hyperaccumulation avec des effets toxiques chez d'autres. Pour cette raison les réelles implications de son exploitation intense n'ont jusqu'à présent pas été considérées et il existe actuellemnt peu d'études systématiques sur la répartition du Cu dans les sols et les produits de cultures. D'autre part cet élément a été largement étudié par les chimistes et biochimistes. Par conséquent ses propriétés de base sont bien établies.

Le Cu fait partie des éléments traces métallique (ETM). L'ensemble des ETM ne représente que 0.6 % du total des éléments de la lithosphère et la contribution du Cu ne représente que 0.0045% (Baize, 1997). Les fortes teneurs naturelles en Cu ne sont que des exceptions et sont essentiellement concentrées dans les roches sédimentaires, les dépôt de combustibles fossiles tels que le charbon, le pétrole et dans les gisements de Cu. Cependant la forte anthropisation des deux derniers siècles à contribué à modifier de manière considérable leur répartition entre les différents réservoirs terrestres. Selon les estimations d'Adriano (1986) qui sont d'ailleurs toujours actuelles 22,000 tonnes d'As, 7,000 de Cd, 400,000 de Pb, 56,000 de Cu, et 214,000 Zn sont disséminés dans l'atmosphère chaque année (Adriano, 1986). Le Cu reste actuellement le métal le plus exploité avec des valeurs de 310 millions de tonnes (Nriagu, 1984).

Les émissions de Cu proviennent de domaines aussi divers que la combustion d'énergies fossiles, de l'industrie métallurgique et électronique, des traitements aux fongicides des vignes et vergers sous forme de « bouillie bordelaise », des additions de lisiers de porcs aux cultures, des stations d'épuration, des composts d'ordure ménagères, et du traitement des piscines. Actuellement rien que les traitements phytosanitaires portent sur 4 millions d'hectares au sein de la CEE (Bourrelier & Berthelin, 1998). Les risques de contamination pour l'homme sont essentiellement liés à la contamination des eaux souterraines et autres approvisionnements en eau potables. Le risque par ingestion des produits de cultures est mal connu puisque le Cu n'est pas contrôlé dans les denrées alimentaires.

Les problèmes liés à la toxicité du Cu sont très ambigus. Le Cu est un métal essentiel à la vie, c'est à dire un oligo-élément, mais au delà d'une certaines dose cet élément devient toxique pour les organismes vivants, d'où l'attribution du terme « potentiellement toxique » (Adriano, 1986 ; Newman & McIntosh, 1991 ; Yaron *et al.*, 1996). D'autre part la toxicité des métaux est non seulement liée à la dose de Cu assimilée, mais dépend surtout de la spéciation du métal et de sa solubilité (Bell *et al*, 1991 ; Van Straalen, & Bergema, 1995, Spurgeon & Hopkins, 1996 ; Hare & Tessier, 1996 ; Lorenz *et al.*, 1997). Les métaux peuvent exister sous différentes formes en solution : en tant que ions libres, de complexes avec des ligands minéraux, organiques ou sorbés sur des surfaces minérales. A son tour la spéciation dépend des conditions de pH, des conditions redox et de la concentration des ligands en solution.

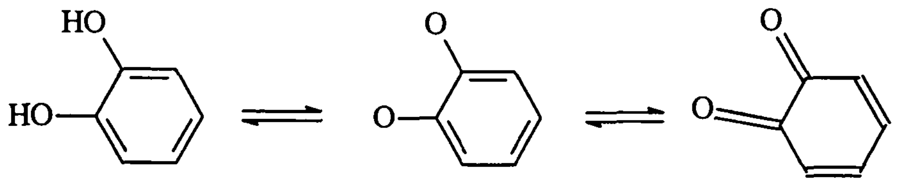
Le Cu est un métal de transition. On le trouve sous forme de sulfates, arsenates, chlorites et de carbonates. Le minéral le plus commun est la chalcopyrite $CuFeSe_2$. En fonction de son état d'oxydation sa réactivité est modifiée. Il peut exister sous forme de Cu(I), forme généralement peu stable, mais la forme la plus courante est le Cu(II). Le Cu (II) a une configuration électronique 9d et est par conséquent paramagnétique. Cette configuration lui vaut de subir une distorsion tétragonale en présence de ligands (effet Jahn Teller). La particularité de cette distorsion résulte de la formation de quatre liaisons courtes Cu-L dans le plan et de deux liaisons longues du côté opposé au plan. Le Cu ayant un caractère intermédiaire dans la classification des acides « mou » et « dur » ce qui explique en partie sa forte affinité pour les éléments O et N situés sur des macromolécules organiques. En général des complexes plus stables sont formés avec des ligands multidentés qu'avec des complexes monodentés. D'autre part son affinité pour les sites bidentés augmente dans l'ordre suivant : (O,O) < (O,N) < (N,N) < (N,S) (Buffle, 1988). Le degré de complexation diminue de façon plus importante avec la dilution pour les complexes monodentés que pour les complexes multidentés appelés aussi chelates (Stumm, 1996). La formation de complexes mono-, bi-, tri-, et tetra-dentés avec le du Cu(II) diminue la réaction de formation du complexe suivant d'un facteur 20 (Powell *et al.*, 1994).

La chimie de coordination du Cu avec des ligands organiques a été étudiée, surtout en ce qui concerne les composés souffrés ou azotés, des acides aminés par des techniques aussi diverses que la cristallographie à rayons X, l'absorption UV, la Résonance Paramagnétique Electronique (RPE) et le dichroisme circulaire à infra-rouge proche (Solomon, 1983). Les interactions Cu-acides fulviques et acides humiques ont été entre autres étudiées par les techniques de voltamétrie, des électrodes sélectives et par échange de ligands (Buffle *et al.*, 1980 ; Xue & Sunda, 1997). Bien que ces techniques permettent de mettre en évidence de nombreux éléments de la complexation comme par exemple le degré de complexation et dans certains cas le nombre de ligands autour du Cu, elles ne permettent pas d'identifier les atomes directs participant à la complexation. Par contre la RPE permet de connaître l'identité de ces atomes et son application a permis de mettre en évidence différents environnement des acides fulviques participant à la complexation du Cu (Senesi & Sposito, 1989 ; Navosha *et al.*, 1992 ; Goodman *et al.*, 1994 ; Cheshire, 1994 ; Cheshire et al., 1994). Notamment des environnements comportant 4 O, 3O, 1N, 2O et 2N sont impliqués dans ces réactions (Senesi *et al.*, 1985). L'application des techniques potentiométriques, de la RPE, de l'absorption UV et de simulation dynamiques à l'étude des interactions Cu-phénols sera discuté dans les chapitres 4 et 5.

Les Phénols

Le choix d'étudier les phénols monomères en tant que vecteurs potentiels du Cu s'est justifié par les critères suivants : leur aromaticité leur conférant une résistance plus importante par rapport aux processus de dégradation dans les milieux acides que les autres métabolites tels que les sucres et acides aminés, ils représentent potentiellement une fraction plus persistante dans l'environnement, donc capables d'interagir avec les métaux traces sur un temps plus long. Leurs fonctionnalité et en particulier les groupements COOH et catechol peuvent potentiellement former des complexes mono ou bidentés. D'autre part la simplicité de leur structure par rapport aux fractions humiques et fulviques du sol leurs attribue le pouvoir tout en servant de modèles de ces macromolécules, de clarifier certains aspects de la complexation.

Les phénols sont caractérisés par la présence d'un groupe benzène portant au moins un groupe hydroxyle (Harborne 1979). Ce sont des métabolites secondaires qu'on retrouve dans des familles de composés aussi diverses que les alcaloïdes, les flavanoides et terpenoides. L'activité des phénols provient surtout des réactions qu'ils subissent en présence d'enzymes, des minéraux et par les variations de pH et de potentiel red-ox (Andreux, 1981; Bollag, 1983; Adrian *et al.*, 1986; Leonowicz & Bollag, 1987; McBride *et al.*, 1988, McBride & Sikora, 1990; Najdia *et al.*, 1997). Les phénols sont en équilibre de pH avec l'ion phénolate. Ils peuvent également être oxydés en quinones.



phénol
forme protonée

phénolate
forme ionisée

quinone
forme oxidée

Les phénols sont des agents réducteurs tandis que les quinones sont des agents oxydants. Leurs propriétés antioxydantes sont actuellement explorées par l'industrie alimentaire et les instituts de recherche sur la prévention du cancer. Les phénols peuvent exister à l'état de monomères ou de polymères. Les formes polymérisées, plus connues sous le nom de « tannins » sont surtout reconnues pour leur attitude à former des complexes insolubles avec les protéines. L'activité des monomères est surtout connue par rapport à sa participation dans des réactions allélopathiques faisant intervenir les plantes ou les insectes (Gallet, 1992 ; Appel, 1993).

Les études qui ont portées sur les mécanismes réactionnels des macromolécules humiques en présence de cations métalliques ont montré que ce sont essentiellement les groupements COOH et phénols OH qui participent à la complexation (Stevenson 1982 ; Tan, 1982 ; Christman & Gjessing 1983). L'activité de ces groupes a souvent été attribuée au groupement COOH dû à son pK_a nettement plus bas que celui du catéchol. Bien qu'il y ait eu de nombreuses études sur le Cu et ses interactions avec la fraction humique du sol (Schnitzer & Khan, 1972 ; Sigel, 1984 ; Mc Keague *et al.*, 1986 ; Cheshire 1994), peu d'études relatent de l'interaction avec les phénols monomères. Les constantes de stabilité indiquent pourtant que la complexation faisant intervenir uniquement le groupement catechol Cu est favorable (Timberlake, 1957). Les interactions de l'acide 3-4dihydroxybenzoique en présence de Cu ont été modélisées à partir de mesures potentiométriques effectuées avec des électrodes sélectives. Deux types d'interaction ont été proposées : une interaction forte impliquant les groupements COOH et une interaction plus faible faisant intervenir le catechol (Aplincourt *et al.*, 1986, 1987,1988). Ces déductions ont également été faites sur la base du plus faible pK_a des groupement COOH. A ce jour on peut dire que l'activité des groupements catechol et COOH reste toujours incertaine et qu'il n'y a pas de preuve évidente d'une liaison plus forte impliquant l'un ou l'autre des groupements.

Paramètres de Mobilisation et d'Immobilisation du Cu dans les Sols.

Différents paramètres ont été invoqués comme influençant les processus de mobilisation et d'immobilisation: les silicates, carbonates, phosphates, oxydes, et la matière organique contribuent à la rétention des métaux lourds dans les sols (McBride *et al.*, 1997). D'autres travaux montrent que l'adsorption augmente avec le pH, la matière organique soluble et la quantité d'argiles (Stevenson, 1991 ; Harter, 1991, McBride, 1994). La matière organique soluble augmente la quantité de métal dissous (Davis, 1984 ; Temminghoff, 1997). L'importance de la matière organique solide ou soluble dans ces processus reste incontestée. Il a également été démontré que les acides fulviques peuvent empêcher la sorption des métaux lourds sur les oxydes de Fe et d'Al et sur les silicates à pH élevé. Dans certains cas ils ont une d'affinité telle pour les métaux Cu, Ni, Hg et le Pb qu'ils peuvent promouvoir leur désorption des argiles (Zacchara *et al.*, 1994 ; Xu *et al.*, 1989). Une forte corrélation entre le Cu et la DOC a été observée dans les sols cultivés (Römkens & Salomons ; 1998), mais non dans les sols forestiers à pH peu élevés. D'autre part l'utilisation d'amendements urbains organiques contribue à augmenter la quantité de métaux lourds lixiviés (Giusquiani, *et al.*, 1992).

Dans la plupart des cas les métaux traces sont accumulés en surface (McLaren & Crawford, 1973, Lagerweff *et al.*, 1976 ; Kelling *et al.*, 1977 ; Williams *et al.*, 1980 ; Emmerich *et al.*, 1982 ; Kuo *et al.*, 1983 ; Miller *et al.*, 1983 ; Mathure *et al.*, 1984 ;

Velez, 1996). Les fortes concentrations observées dans les sols de vignobles atteignant souvent 200 ppm en surface, témoignent d'un réel problème d'accumulation lié à l'utilisation continue de fongicides. L'alcalinité des sols est une matière à discussion. Bien que les teneurs de surface soient souvent plus élevées pour les sols calcaires (Quinche, 1985), des teneurs élevées ont également été mises en évidences sur sols acides (Flores Velez, 1996). Récemment des teneurs extrêmes ont été découvertes dans des luvisols au Danemark suite à l'imprégnation de poteaux électriques avec de Cr, Cu, et As. La contamination atteignait 6'000 mg/kg en surface. Dans ce cas le Cu ne s'est pas limité à la surface et entre 8'000 et 13'000 mg/kg ont été entraînés entre 1m50 et 3 m de profondeur (Lund & Fabian, 1991). Des études faites à proximité d'une région minière sur des sols bruns acides ont également montré une perte des métaux de la surface par lixiviation. L'ordre de rétention dans les profils est souvent le suivant : Pb>Cu>Zn>Cd (Merrington & Alloway, 1994). D'autres recherches effectuées dans des tourbes acides proches d'une fonderie au Canada, ont montré une accumulation préférentielle dans les 15 premiers centimètres (Dumontet, 1990). Une forte corrélation entre la MO et le Cu a également été observée dans des sols en Pologne (Dudka, 1993). Cependant les études effectuées sur les fonds pédogéochimiques (programme Aspittet) sur 45 profils forestiers répartis dans toute la France à l'abris des influences anthropogènes ont montré qu'il n'y a pas de corrélation entre l'accumulation de surface du Cu et les surfaces humifères (Baize, 1997).

Ces observations témoignent également de l'importance de la matière organique et de certaines contradictions dans les résultats qui sont le reflet de son hétérogénéité, ainsi que de la présence d'une portion seulement de substances « actives ». Le rôle de la phase fulvique comme phase complexante du Cu dans les horizons A_1-B_y d'un podzol développé sur landes humide a été souligné par Delas (1966). Plus récemment un modèle mathématique décrivant les différentes relations entre le pH, la matière organique et la teneur totale en métaux lourds appliqués à des sites contaminés a clairement indiqué qu'il y a deux fraction réactives dans le sols : une fraction organique solide qui limite l'activité du Cu, et une fraction organique soluble qui contribue à solubiliser le métal, dans des sols relativement évolués (McBride *et al.*1997).

Les paramètres saisonniers contribuent également à modifier les propriétés de migration. Des études saisonnières sur des podzols alpins ont montré des transferts préférentiels de C organique en avril et en mai (Zaboswki, & Ugolini, 1990). Tyler (1981) a montré que la lixiviation sur des podzols suédois dépendait essentiellement du groupe de métal en question. Le Fe, l'Al, le Pb, le Ni, le Cr et le V migrent surtout à la fin de l'été et en automne, tandis que le Na, le K, le Mg, le Ca, le Mn, le Zn et le Cd migrent surtout en octobre et en novembre (Tyler, 1981). Les études de Keller et Vedy (1991) qui ont portées sur des podzols sous forêt au Val d'Arpette (Suisse) ont montré que deux pics sur quatre correspondent à la période de fonte des neiges. D'autres auteurs ont également observé des lixiviations importantes durant la périodes des fontes (Jordan, 1968 ; Buurman, 1987 ; Bergkvist *et al.*, 1989).

L'eau est un paramètre essentiel en tant que phase de transport et comme milieu réducteur lorsqu'elle se trouve en grande quantité et en présence d'une forte activité biologique. Un faible potentiel redox et pH bas peuvent diminuer la stabilité de certains métaux et provoquer le transfert de la fraction échangeable vers la solution du sol en tant que ion libre ou complexé à la matière organique (Reddy & Patrick, 1977). La dégradation de la matière organique favorise souvent la mobilisation des métaux traces à l'état soluble, suite à la décomposition des ligands (Bloomfield *et al*, 1971).

Les Podzols

Les podzols ont été décrits par de nombreux auteurs (Rode, 1970; Legros & Cabidoche, 1977; Souchier, 1984; Duchaufour, 1997 ; Huber 1997). En Suisse ces sols sont surtout représentés dans les Alpes, néanmoins ils représentent une surface non négligeable à l'échelle terrestre (Figure 1).

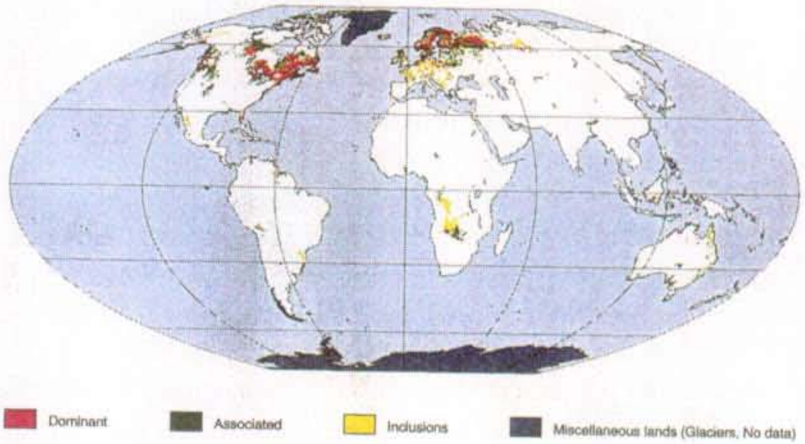


Figure 1. Répartition des Podzols dans le Monde (WRB, 1998)

Figure 2. Profil de Podzol du Val d'Arpette

La podzolisation est favorisée par une synergie de facteurs : un climat froid et humide correspondant à des zones climatiques boréales ou à un environnement de haute altitude, agissant sur une roche-mère acide et la présence d'une couverture végétale de type acidophile (landes à bruyère, rhododendrons, conifères). L'acidocomplexolyse est le processus d'altération en dominance. Il peut être divisé en deux étapes séquentielles. Une première étape de « chéluviation » ou de **translocation** l'éléments minéraux par les acides organiques provenant de l'humus, qui engendre la formation d'un horizon elluvié blanchâtre. La deuxième étape conduit à une **accumulation** de minéraux riches en fer et en l'aluminium ce qui donne lieu à une couleur rougeâtre. Il s'agit de la formation d'un horizon spodique sous-jacent (Deckers *et al.*, 1998). Toutes ces conditions contribuent à abaisser considérablement le pH du sol et favorisent la migration des cations. Les podzols constituent ainsi des terrains idéaux pour mettre en évidence les paramètres responsables de la mobilité des métaux lourds.

Approche

Le challenge de ce travail résidé dans l'intégration de différentes échelles servant de support à l'étude de processus qui ne se déroulent pas de manière linéaire dans le temps, mais qui se chevauchent et contribuent chacun à leur tour à expliciter le fonctionnement d'une autre échelle de réflexion. Contre l'attente du lecteur, le tout ne s'aligne donc pas conventionnellement sur un fil conducteur rectiligne, mais ajuste plutôt les pièces d'un puzzle autour de quelques questions essentielles : **comment le Cu migre-t-il dans les sols ? dans quelle mesure la phase humique du sol joue-t-elle le rôle de mobilisateur ou immobilisateur du Cu ? qu'elle est l'importance des phénols dans ces phénomènes ?**

Afin de réaliser cette étude une collaboration a été mise sur pied sur un plan international réunissant différents Instituts avec un *know how* spécifique aux thèmes invoqués. Des technologies de pointe ont été mises à disposition par les Instituts. L'Institut Maccaulay (Maccaulay Land Use and Research Institute) a servi d'unité d'accueil pendant plusieurs mois. Sous la direction des Dr. Martin Cheshire et Donald McPhail les interactions phénols-Cu ont été étudiés par Résonance Paramagnétique Electronique (RPE) et les méthodes d'extraction des phénols ont pu être testées. Des séjours à l'Institut de Chimie Analytique et Minérale Appliquée de l'Université de Genève ont permis de faire des simulations dynamiques de ces interactions avec les compétences du Dr. Serge Stoll. Les analyse HPLC des phénols ont été effectués au Laboratoire d'Ecotoxicologie de l'Institut de Génie Sanitaire (EPFL) avec le soutient de Lionel Spack. L'Institut de photogrammétrie a participé à la mise en place de la logistique de terrain par l'intermédiaire de Julien Vallet. Enfin, l'Institut de Pédologie et a fourni les compétences en matière d'analyses d'éléments traces ainsi que pour les analyses classiques des sols grâce à Jean-Pascal Dubois. D'autre part, la participation a des congrès internationaux a été vivement encouragée par le Pr. J.-C. Vedy. Les communications orales et présentations de posters ont permis d'échanger des idées et de discuter des résultats tout au long du travail (16ème Congrès Mondial de Science du Sol, Monpellier, 1998 ;. Colloque de l'association française pour l'étude du sol, Paris, 1998 ; Colloque de l'IHSS: analyse et diversité des substances humiques naturelles, Dijon, 1997; 5ème rencontre du GRAPE: pollution majeure en Rhône-Alpes et régions voisines, Grenoble, 1997 ; Fourth International Conference on the Biogeochemistry of Trace Elements, Berkeley CA, USA, 1997; Second International Symposium on Effect of Mineral-Organic- Micro-organism Interactions on Soil and Freshwater Environments, Nancy, 1996; Interaction of Small Solutes with Material of Environmental Relevance, Monte-Verità, 1996).

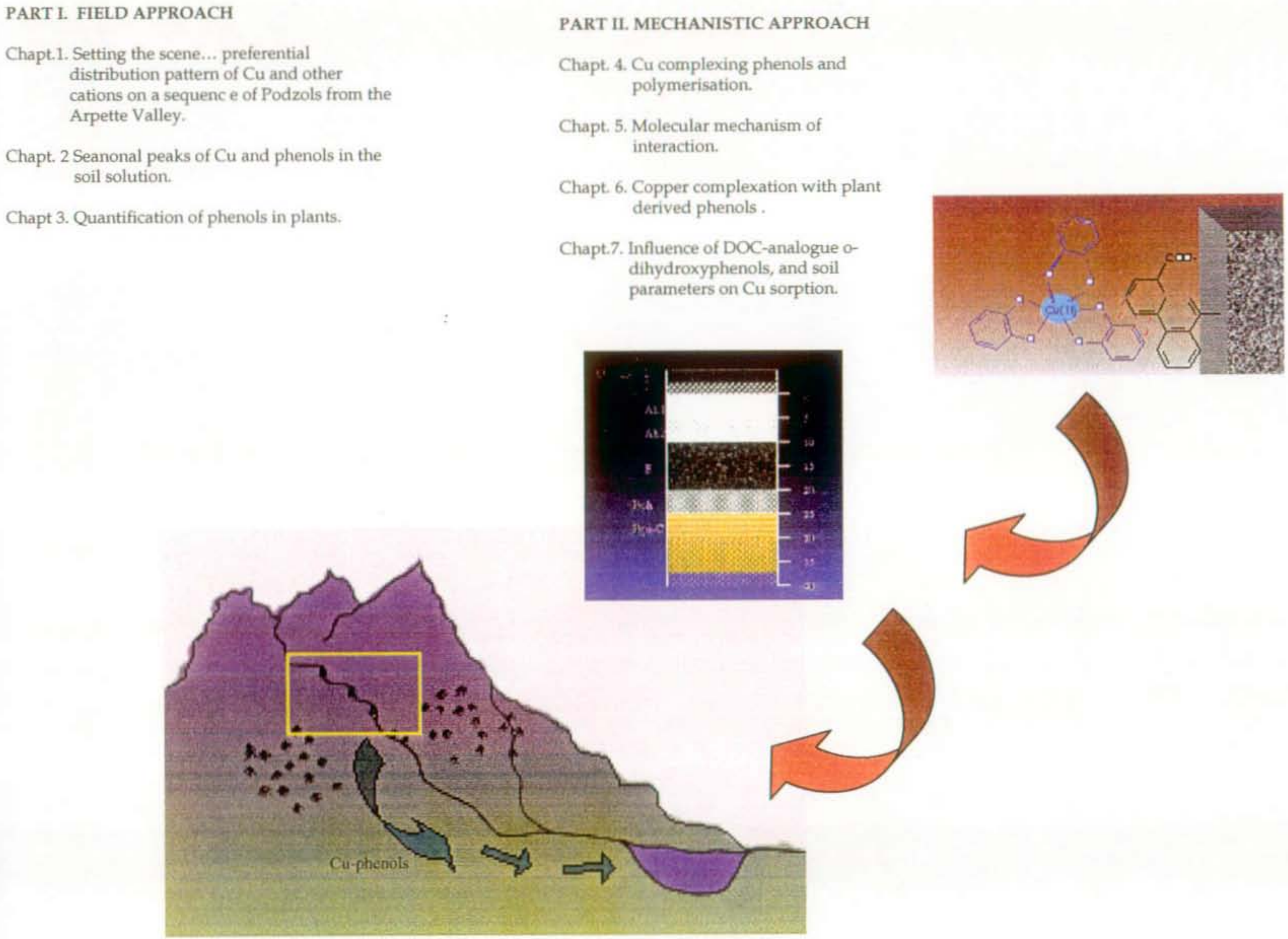
Ce travail est organisé en 2 parties : une partie TERRAIN qui rend compte de toutes les observations qui ont été faites au niveau de l'échelle du profil, de l'horizon et des réactions dans la solution du sol. La première partie est divisée en trois chapitres. Le **Chapitre 1**, présente les trois sols sélectionnés pour notre étude, quantifie leur degré de lixiviation par rapport au degré de podzolisation et les distributions du Cu au niveau des profils de sols. Le **Chapitre 2**, met en évidence l'influence des facteurs saisonniers responsables de la migration du Cu, des phénols, et de la DOC. Le **Chapitre 3** permet de quantifier les apports annuels en phénols de litière pour *Vaccinium myrtillus*, *Rhododendron ferrugineum* et les mousses.

La deuxième partie est essentiellement MECANISTIQUE et étudie les interactions Cu-phénols au laboratoire. Elle est composée de quatre chapitres. Les **Chapitres 4** et

5 s'intéressent plus spécifiquement à l'échelle moléculaire. Dans le **chapitre 4**, les principaux phénols complexants du Cu sont déterminés à l'aide de molécules de synthèse par Résonance Paramagnétique Electronique (RPE). Dans le **chapitre 5**, le mécanisme d'interaction du Cu avec les phénols en fonction du pH et le rôle de la structure des phénols est démontré à l'aide de la potentiométrie, la RPE, l'absorption UV et de simulations dynamiques. Le **chapitre 6**, est une brève communication mettant en évidence la complexation du Cu en présence de lixiviats de plantes. Le **Chapitre 7**, intègre les propriétés moléculaires à l'échelle de l'horizon par des expériences de sorption et de désorption.

La figure 3 illustre l'interdépendance de chaque chapitre traité dans ce travail.

Les chapitres 3, 4, 5 et 6 ont déjà fait l'objet de publications dans des revues internationales. Afin de garder une certaine cohésion dans la démarche, et de maintenir une qualité de travail permettant la publication, les autres chapitres ont été rédigés sur le même modèle en anglais et pourront ainsi être directement soumis aux éditeurs, sous réserve de modification du jury de thèse bien évidemment. Pour cette raison chaque chapitre contient son histoire propre et peut être lu dans l'ordre qui convient le mieux au lecteur. Au début de chaque chapitre le lecteur trouvera un résumé en français et en anglais.



PART I. FIELD APPROACH

Chapt.1. Setting the scene… preferential distribution pattern of Cu and other cations on a sequenc e of Podzols from the Arpette Valley.

Chapt. 2 Seanonal peaks of Cu and phenols in the soil solution.

Chapt 3. Quantification of phenols in plants.

PART II. MECHANISTIC APPROACH

Chapt. 4. Cu complexing phenols and polymerisation.

Chapt. 5. Molecular mechanism of interaction.

Chapt. 6. Copper complexation with plant derived phenols .

Chapt.7. Influence of DOC-analogue o-dihydroxyphenols, and soil parameters on Cu sorption.

Figure 3. Feedback between the different chapters of this study.

Introduction

Prelude...

Copper is an essential trace metal, which participates in vital functions in living organisms. However, under certain circumstances it can also become highly toxic. The soil and plant system is an important reservoir for Cu. Although Cu is unevenly distributed between land surfaces, total Cu stored in soils has been estimated to 3.3x 10^{13}tons (Nriagu, 1979).

Pressures induced by soil changes are intimately linked with mobility factors. They can alter Cu distribution between the different compartments of the environment. Depending on the physical, chemical or biological soil properties, Cu can either be mobilised or immobilised. If Cu is mobilised, its redistribution can result in a contamination of connected systems by interaction of complex processes starting at the soil - soil solution interface. If however, Cu is immobilised, it tends to accumulate in soils and the medium becomes a "potential source" of Cu. Changes in land use activity, as well as changes caused by soil evolution under the pressure of time, can sometimes modify metal behaviour in soils and cause a soil to become an active contaminating source.

The aim of this study was therefore to determine the major factors controlling the behaviour of Cu at the soil-soil solution interface by an experimental approach. Soil solution pH being a capital mobilising parameter, acidic soils from a podzol sequence were chosen. Podzols are strongly acid soils with *ochric* and *spodic* horizons resulting from the migration of organic matter, iron and aluminium. They are generally characterised by an "acido-complexolytic" activity and release large quantities of organic acids, which participate in the translocation of mineral substances. Many potential organic Cu translocation vectors exist in the soil solution. In this study we decided to concentrate on phenols, in order to specify their role in translocation processes with regard to seasonal variations.

A model site characterised by a sequence of soils developed on quartzitic parent material was selected. The dominant weathering processes varied between "bisiallitisation" and "acidocomplexolyse". The main pedogenesis ranged from inherited brunification to podzolisation. The background level of Cu pollution was low to average. The altitude of the site ranged between 1,468 to 3,270 meters. The average annual rainfall exceeded 1,500 mm with winter and summer average temperature of –8°C and 4.5 °C respectively. Dominant plant species were *Vaccinium myrtillus* and *Rhododendron ferrugineum.* The site was known from previous studies carried out on Cu and weathering processes in the Valley (Keller, 1991; Huber 1997). A careful examination of the site allowed us to select three stations. The plant and soil properties were studied in detail for each station.

In the first stage, field devices were installed in order (1) to obtain continuous field data about the pedoclimatic conditions (2) for sampling of the soil solution with tension free lysimeters. Cu and phenols were analysed from these fractions during three years.

In the second stage, the research consisted in developing state of the art techniques suitable for analysis of phenols in the solution. The stability of phenols was measured under different conditions. Several pre-concentration and phenol extraction procedures were tested. The mechanism of Cu-phenol interaction was determined in the laboratory using synthetic molecules as well as soil and plant samples.

In the third and last stage of this study, soil solutions were analysed and a mechanism, based on the different retention and mobilisation parameters was proposed.

The ongoing study was mainly based on the following three hypotheses:

(1) **Phenols are present in detectable concentrations in the soil solution of podzolised soils.**
(2) **Phenols or at least a few among them, are able to form complexes with Cu(II).**
(3) **Cu(II)-phenol complexes play a key role in Cu mobilisation in soil solutions.**

Verification of these hypothesises should also contribute piece by piece to propose a mechanism expressing the causes for mobilisation and immobilisation of Cu in soils.

Cu and its Dissemination in the Environment

Cu was chosen in this study not only because it is the most exploited metal on earth, but also for its ambiguity, with regard to its role as an oligoelement for living organisms and its toxicity under conditions of hyperaccumulation. For this reason the real implications of its extensive exploitation are not considered seriously enough and only few systematic studies on Cu have been carried out in soils and crops up todate. In addition, this element has been widely studied by chemists and biologists, and therefore the basic reactivity of Cu is known.

Cu is a metallic trace element. All the trace metals together represent only 0.6 % in the lithosphere and Cu represents 0.0045% (Baize, 1997). High levels of trace elements in soils are exceptions. Cu is mostly concentrated in sedimentary rocks, fossil fuel deposits, as coal, shale oil, crude oil and in Cu ores (Nriagu, 1979). Nevertheless, the strong pressure caused by anthropisation of the two last centuries has contributed to modify the distribution between the different compartments of the ecosystem. Estimations of 1986 revealed that 22,000 tons of As, 7,000 of Cd, 400,000 of Pb, 56,000 of Cu and 214,000 of Zn are disseminated in the atmosphere each year (Adriano, 1986) and more then 310.000 tons of Cu have been exploited (Nriagu, 1984). Cu inputs to soils originates from the combustion of fossil fuel, the metallurgic and electronic industry, the treatment of vineyards and orchards with cupric fungicides, the application of sewage sludge to cultures, water treatment stations, household and compost wastes, and the treatment of swimming pools. Within the CEE, alone the phytosanitary treatment represent more than 4 million hectares of soils treated with Cu (Bourrelier & Berthelin, 1998).

The risks of contamination are essentially linked with Cu leaching to the groundwater and the subsequent contamination of the drinking water supply. The risk by ingestion of culture production is not well known, since Cu is not controlled in food. Cu is an oligo-element, and therefore essential for life, but above a certain level it becomes toxic for organisms. Cu and other trace metals with similar properties have therefore been qualified as "potentially toxic" trace elements (Adriano, 1986; Newman & McIntosh, 1991; Yaron *et al.*, 1996). Toxicity has been mainly attributed to the substitution of other essential metals in their active site of enzymes or in structural functions of Ca and Mg (Newman & McIntosh, 1991). Although Cu toxicity can be related to some extent to total Cu content, it depends predominantly on speciation in solubility (Bell *et al.*, 1991; Van Straalen, & Bergema, 1995; Spurgeon & Hopkins, 1996; Hare & Tessier, 1996; Lorenz *et al.*, 1997). Trace metals can exist in the solution, as free ions, complexed with organic and mineral ligands or sorbed on mineral surfaces. Speciation itself depends on pH, redox-state and the concentration of ligands in the solution.

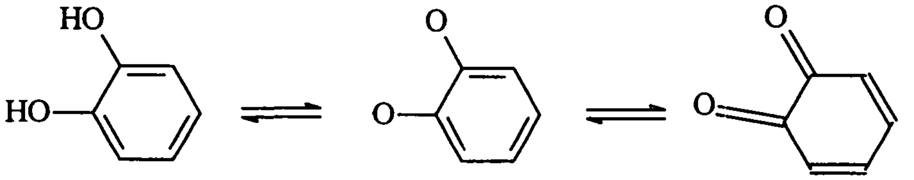
Cu is a transition metal. It can be found in sulfides, arsenides, chlorides and carbonates. The most common Cu-containing mineral is Chalcopyrite ($CuFeSe_2$). It can exist as Cu(I), but the most common form is Cu(II), which is more stable. Cu(II) has a 9d configuration and is paramagnetic. This configuration causes a tetragonal distortion in the presence of ligands (Jahn Teller effect). It results in the formation of 4 short bonds in the plane and 2 longer bonds in the adjacent plane. Cu has an intermediary character between "soft" and "hard" acids, which contributes to its high affinity for elements like O and N belonging to organic molecules. Complexes with monodentate ligands are generally less stable compared to multidentate complexes (Stumm, 1996). The affinity for bidentate sites increases in the following order: (O,O)<(O;N)<(N,N)<(N,S) (Buffle, 1988). The degree of complexation decreases with dilution and decreases more strongly for monodentate complexes than for multidentate complexes (also called chelates) (Stumm, 1996). The successive formation of mono-, bi-, tri- and tetra-dentate Cu complexes, decreases each time to the following reaction by a factor 20 (Powell *et al.*, 1994).

X-ray crystallography, UV absorption, Electron Spin Resonance spectroscopy (ESR) and near infra-red circular dichroism Cu(II) have revealed much of Cu coordination chemistry. Various S and N containing amino acids have been studied in the presence of Cu (Solomon, 1983). Ion selective electrodes and selective voltammetry techniques have been applied to complexation of Cu(II) with humic and fulvic compounds (Buffle *et al.*, 1980; Xue & Sunda, 1997). Although these approaches allow determining several important features of complexation, they do not permit us to know which atoms are directly involved in the reaction. With ESR it is possible to identify the neighbouring atoms of Cu. The application of ESR to fulvic molecules have revealed that environments bearing 4O, 3O, 1 N, 2O and 2N were involved in the bonding with Cu (Senesi *et al.*, 1985; Senesi & Sposito, 1989; Navosha *et al.*, 1992; Goodman *et al.*, 1994; Cheshire, 1994; Cheshire *et al.*, 1994).). The application of ESR, potentiometry, UV absorption and molecular simulations to study Cu-phenol interactions will be further developed in chapter 5.

Phenols

Monomeric phenols presented several advantages in our investigations. Their aromatic character makes them less prone to degradation in acidic mediums compared to other metabolites. Their functions and in particular the COOH and catechol groups offer a good opportunity to observe either mono or bisdentate complexes. Phenols are therefore potentially better vectors for Cu compared to other organic compounds. On the other hand the simplicity of their structure compared to the humic and fulvic fractions of soil makes them suitable as models for clarifying complexation properties.

Phenols are characterised by the presence of a benzene ring bearing at least one hydroxyl group (Harborne, 1979). They are secondary metabolites and are representatives of the family of compounds such as alkaloïdes, flavanoides and terpenoides. The activity of phenols has mainly been attributed to reactions occurring in the presence of enzymes, minerals and in relation with pH and redox variations (Andreux, 1981; Bollag, 1983; Adrian *et al.*, 1986; Leonowicz & Bollag, 1987; McBride *et al.*, 1988, McBride & Sikora, 1990; Naidja *et al.*, 1998). Phenols are in equilibrium with the phenolate form and can be oxidized into quinones.



phenolic protonated — phenolate ionized — quinone oxidized

Phenols are reducing agents while quinones are oxidants. Their anti-oxidant properties are currently being explored by the food industry and cancer research institutes. Phenols can exist as monomers or polymers. Their polymerised form, better known under the name of "tannin", is recognised for its ability to form insoluble complexes with proteins. The activity of monomers is more known in its participation in allelopathic reactions involving plants and insects (Gallet, 1992; Appel, 1993).

Mechanistic investigations on humic macromolecules have shown that COOH and OH groups are the most common reactive groups (Stevenson, 1982; Tan; 1982; Christman & Gjessing, 1983). The activity has predominantly been attributed to the COOH group due to its lower pK_a value. Although many studies cover Cu - humic interactions, very little is known about complexation with natural monomeric phenols. Stability constants have been determined by potentiometry for catechol (dihydroxyphenol), which indicated that the catechol group could participate in the formation of complexes with Cu (Timberlake, 1957). Other studies carried out on 3-4-dihydroxybenzoic by a combination of different approaches (pH-metric titrations, ion selective electrodes and modelling) revealed two modes of interactions with Cu. One strong interaction involving the COOH group and a second weaker interaction involving the catechol group (Aplincourt *et al.*, 1986, 1987,1988). The stronger affinity of the COOH group was again deduced from its low pK_a value. At this level the preferential activity of COOH and catechol groups remains still questionable and there is no clear evidence that one group is more active than the other one.

Cu Mobilisation and immobilisation parameters in soil

Different parameters have been described to affect Cu mobilisation and immobilisation processes in soils. Silicates, carbonates, phosphates, oxides and organic matter are known to contribute to the retention of heavy metals (McBride *et al.*, 1997). Other authors have reported that an increase in pH, organic matter and the quantity of clays promote adsorption processes (Stevenson, 1991; Harter, 1991;

McBride, 1994). Soluble organic matter increases the quantity of dissolved metal (Davis, 1984; Temminghoff *et al.*, 1997). Both the solid and soluble forms of organic matter have an importance in trace metals translocation processes. It has even been demonstrated that fulvic acids can prevent metal sorption on Fe and Al oxides and on silicious minerals when the pH is high. In certain cases they have such an affinity for metals such as Cu, Ni, Hg, and Pb that they can provoke their desorption from clays (Zacchara *et al.*, 1994; Xu *et al.*, 1989). A strong correlation between Cu and DOC has been observed in cultivated soils (Römkens & Salomon, 1998), but not in low pH forest soils (Baize, 1997). Furthermore, the application of organic matter in the form of waste amendments also increases the amount of leached metals (Giusquiani, *et al.*, 1992).

Most of the literature reports of a surface accumulation of heavy metals (McLaren & Crawford, 1973; Lagerheff *et al.*, 1976; Kelling *et al.*, 1977; Williams *et al.*, 1980; Emmerich *et al.*, 1982; Kuo *et al.*, 1983; Miller *et al.*, 1983; Mathure *et al.*, 1984; Williams *et al.*, 1984; Williams *et al.*, 1985; Yingming & Corey, 1993; *in* Flores Velez, 1996). Particularly high levels of Cu have been detected in vineyard soils. They often reach 200 ppm at the surface and confirm that repeated applications of fungicides can cause a real accumulation problem. Soil pH is an important parameter but its real effect is a matter of discussion. The highest levels of Cu are found in alkaline soils (Quinche, 1985) but high concentrations have also been detected in some acid soils (Flores Veles, 1996). Very high values have recently been detected in some Danish luvisols due to the impregnation of electric poles with Cu, Cr, and As. Contamination reached 6,000 mg/kg at the surface. In this case Cu contamination did not stay at the surface and between 8,000 and 13,000 mg/kg migrated down to 1.5 and 3 meters depth (Lund & Fabian, 1991). Studies carried out in the proximity of a mining region on acid brown soils also showed a loss in metals from the surface by lixiviation. The order of retention in the profile was the following: Pb>Cu>Zn>Cd (Merrington & Alloway, 1994). Other analysis performed on acidic virgin peat soils near a smelter in Canada, have shown a preferential accumulation in the 15 first cm of the soil (Dumontet, 1990). A strong correlation between organic matter and Cu has also been observed in sandy soils in Poland (Dudka, 1993). Nevertheless, investigations carried out on the pedogeochemical background level of Cu revealed that on a total of 45 soils all selected from low polluted areas of France, no correlation existed between Cu accumulation and the humified surfaces (Baize, 1997).

Seasonal parameters can also contribute to modify migration properties. Cu studies in alpine podzols of Washington State have shown preferential migrations in April and May (Zabowski & Ugolini, 1990). Tyler (1981) has shown that leaching in Swedish podzols mostly depended on the metal group. Fe, Al, Pb, Ni, Cr, V had a preferential leaching at the end of the summer and in autumn, while Na, K, Mg, Ca, Mn, Zn and Cd leached two times in November and one time in the autumn (Tyler, 1981). Keller & Vedy (1991) showed that two Cu peaks out of four corresponded to the snow-melting period. Other authors also report that most of the leaching occurs during the snow-melting period (Jordan, 1968; Buurman, 1987; Bergkvist *et al.*, 1989). Water is an important parameter as a transport vehicle and as a reducing medium when present in large quantities and when the biological activity is high. In some cases a low pH and redox potential has reduced metal stability causing the dissolution of the exchangeable fraction and the mobilisation of metals in a free or complexed form. (Reddy & Patrick, 1977). Organic matter degradation often enhances the mobilisation of trace metals, which occurs when the surrounding ligands are degraded (Bloomfield *et al.*, 1971).

These observations exemplify the fact that humus reactions are extremely heterogeneous and that among all only a small fraction is potentially active. The participation of fulvic acids as a complexing fraction in A1-By horizons of heath podzols has been emphasised by Delas (1966). More recently two distinctive reactive fractions in soils were proposed by modelling pH, organic matter and total metal content data's obtained from a contaminated site. One solid organic fraction, which limits Cu activity, and one soluble organic fraction, which contribute to solubilise the metal in relatively developed soils (McBride *et al.*, 1997).

Podzols

Podzols have been described by several authors (Rode, 1970; Legros & Cabidoche, 1977; Souchier, 1984; Duchaufour, 1997; Huber, 1997). In Switzerland the presence of Podzols is restricted to the alpine zone, but it still represent a fair amount of soil surface in Europe and the rest of the world (Figure 1).

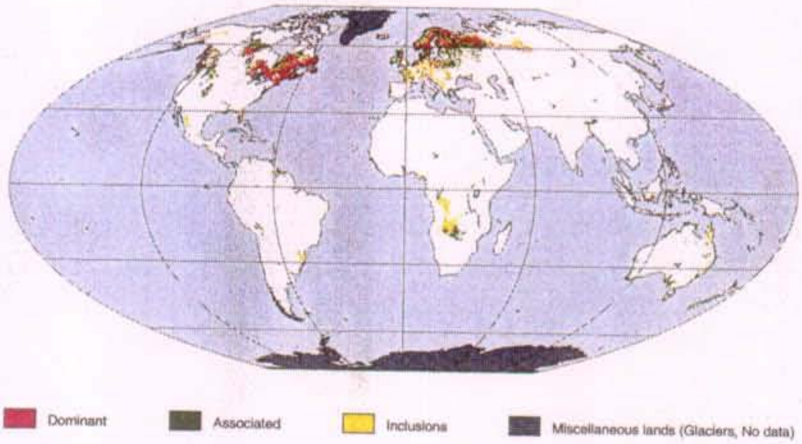


Figure 1. Podzols of the world (WRB, 1998)

Podzols are the result of synergetic conditions, which promote eluviation processes. Cool and wet climates- the boreal climatic zone, high mountain environments-, acting upon quartzitic parent materials, and by heath and /or coniferous vegetal cover. "Acidocomplexolyse" is the main process leading to podzolisation. It can be divided into three different steps. The first step consists in the **translocation** or "cheluviation" of positively charged minerals by organic acids released from the humus. In a second stage the whole complexes follow a vertical migration down the profile. It leads to the formation of an ash-grey coloured horizon commonly known as the E (from eluviated) horizon in the upper part of the profile. The final step consists in the **accumulation** (known. as "chilluviation") of Fe and Al minerals and organic ligands either in the mixed horizon or into two separate horizons.

Figure 2. Podzol profile from the Arpette Valley

These horizons are commonly known as the spodic horizons (*spod* from Russian under) and are characterised by a reddish-brown colour, which makes a sharp contrast with the upper horizons.

Podzolisation usually results in a decrease of soil pH and an increase in cation mobility. Podzols therefore constitute excellent model sites to observe metal migration.

Approach

The challenge in this study consisted in integrating processes, which occur at different time and size scales in order to explain non-linear and overlapping processes. Against the expectancy of the reader, this work is therefore not organised in a conventional linear way, but brings together by a successive arrangement pieces of a puzzle around three fundamental questions: **how does Cu migrate in soils? To what extent is Cu migration controlled by complexation with the humic fraction of soils? What is the importance of phenols in these processes?**

The realisation of this project was made possible thanks to an international collaboration bringing together Institutes with a specific *know how* in the fields of interest. The Institutes provided state of the art technologies. ESR spectroscopy applied to Cu-phenol interaction studies as well the testing of different techniques for phenol extraction were carried out at the Maccaulay Land Use Institute and supervised by Dr. Martin Cheshire and Dr. Donald McPhail. Molecular Simulations were performed at the "Institut de Chimie Analytique et Minérale Appliquée" under the direction of Dr. Serge Stoll. The methods for phenol analyses by HPLC were developed at the "Laboratoire d'Ecotoxycologie de l'Institut de Génie Sanitaire (EPFL)" with the assistance of Dr. Lionel Spack. Cu analyses in plants, soils and the soil solution as well as the classical pedological analyses were performed at the "Institut de Pédologie (EPFL)" with the competence of Jean-Pascal Dubois. Field set up was installed with the collaboration of the "Institut de photogrammétrie (EPFL)" and the contribution of Julien Vallet.

In addition Pr. J.-C. Vedy promoted the participation to several international congresses and excursions. Oral and poster presentations permitted me to exchange ideas and discuss results with other members of the scientific community (16ème Congrès Mondial De Science Du Sol, Monpellier, 1998 ;. Colloque De L'association Française Pour L'étude Du Sol, Paris, 1998 ; Colloque De L'ihss: Analyse Et Diversité Des Substances Humiques Naturelles, Dijon, 1997; 5ème Rencontre Du GRAPE: Pollution Majeure En Rhône-Alpes Et Régions Voisines, Grenoble, 1997 ; Fourth International Conference On The Biogeochemistry Of Trace Elements, Berkeley CA, USA, 1997; Second International Symposium On Effect Of Mineral-Organic- Micro-Organism Interactions On Soil And Freshwater Environments, Nancy, 1996; Interaction Of Small Solutes With Material Of Environmental Relevance, Monte-Verità, 1996).

This study has been organised into two parts:

The first part deals with FIELD investigations. They were carried out at the scale of the profile, horizon and soil solution. This part is divided into three chapters. In **Chapter 1**, the three soils selected for our study are presented in terms of leaching behaviour, Cu background level and vertical distribution. In **Chapter 2,** the seasonal variations of Cu, phenol and DOC are presented. In **Chapter 3**, the annual contribution of phenols released from leaf litters is estimated for three plant groups (*Vaccinium myrtillus*, *Rhododendron ferrugineum* and mosses).

The second part is essentially MECHANISTIC and deals with Cu-phenol interactions studied under laboratory controlled conditions. This part is divided into four chapters. **Chapter 4** and **5** deal with processes at the molecular level. **In Chapter 4**, the dominant Cu complexing phenols are determined by ESR. In **Chapter 5**, Cu-phenol interaction mechanism and in particular the role of catechol groups are determined by a combination of complementary analytical techniques (potentiometry, ESR, UV absorption, dynamic simulations). **Chapter 6**, is an extended abstract relating on Cu complexation with phenols originating from plant leachates. In **Chapter 7**, molecular properties observed in chapter 4 and 5 are used to explain Cu retention in soil surfaces with the support of a sorption experiment.

Figure 3, illustrates interconnection and feed back of the chapters.

Chapters 3, 4, 5, and 6 have already been accepted for publication in international journals. In order to keep cohesion in this work and to maintain a quality of presentation, which can readily be used for publication, the other chapters have been written on the same model in English. For this reason each chapter contains its own story and can be read in any preferred order by the reader. Abstracts in French and English will be found at the beginning of each chapter.

References

Adrian P., Andreux F., Metche M., Mansour M., Korte F., and Lavollay J. 1986. Autoxydation Des Ortho-Diphénols Catalysée Par Les Ions Fe^{2+} Et Mn^{2+}: Un Modèle De Formation Des Acides Humiques. C.R. Acad. Sc. Paris, T. 303:1615-1618.

Adriano D.C. 1986. Trace Elements In The Terrestrial Environment. Springer, Berlin Heidelberg New York, 533 Pp.

Aplincourt M., Bee-Debras A. and Prudhomme J.C. 1988. Complexes Du Cuivre (II) Avec Des Molécules De Synthèse Et Implications Pour l'Interaction Du Cuivre Avec La Matière Organique Des Sols. Science Du Sol 26 (3):157-168.

Aplincourt M., Bee A., Gerard C., Hugel R.P., and Prudhomme J.-C. 1987. Modelling Of The Interactions Of Metal Cations With Soil Organic Matter. Part 3: Thermodynamic Stability Of Copper(II) And Iron(III) Complexes With 3,4-Dihydroxy-Benzoic Acid. J. Chem. Research (S) 398:B3214-3241.

Aplincourt M., Bee-Debras A., Gerard C., and Hugel R.P. 1986. Modelling Of The Interactions Of Metal Cations With Soil Organic Matter. Part 1: Thermodynamic Stability Of Copper(II) Complexes With Dihydroxybenzoic Acids. J. Chem. Research (S) 134; (M)1253-1273.

Andreux F. 1981. Utilisation De Molécules Modèles De Synthèse Dans L'étude Des Processus D'insolubilisation Et De Biodégradation Des Polycondensats Humiques. Bulletin De L'AFES 4:271-292.

Appel H.M. 1993. Phenolics In Ecological Interactions: The Importance Of Oxidation. Journal Of Chemical Ecology.19 (7):1521-1552.

Baize D.1997. Un Point Sur...Teneurs Totales En Eléments Traces Métalliques Dans Les Sols (France). Ed. INRA Pp408.

Bell P.F., Chaney R.L., and Angle J.S. 1991. Free Metal Activity And Total Metal Concentrations As Indices Of Micronutrient Availability To Barley (*Hordeum Vulgare* (L.) «Klages». Plant Soil 130 :51-62.

Bergkvist B., Fokeson L. and Berggren D. 1989. Fluxes Of Cu, Pb, Cd, Cr And Nic In Temperate Forest Ecosystems. Water, Air And Soil Pollution, 47: 217-286.

Bloomfield C., Kelso W.H., and Piotrowska M.1971. Chem. Ind.1971, Pp59.

Bollag, J.M. 1983. Synhetic Reactions Of Aromatic Compounds By Fungal Enzymes. American Society For Microbiology, Washington 203-207.

Bourrelier P.H. and Berthelin J. 1998. Contamination Des Sols Par Les Eléments En Traces: Les Risques Et Leur Gestion. Rapport N°42, Académie Des Sciences, Institut De France, 480pp.

Buffle J., Deladoey P., Greter F.L., and Haerdi W. 1980. Study Of The Complex Formation Of Copper(II) By Humic And Fulvic Substances. Analytica Chimica Acta. 116: 255-274.

Buffle J. 1988. Basic Principles Of Metal Reactivity In Water. In " Complexation Reactions In Aquatic Systems", Ellis Horwood Series. PP47-89.

Buurman P. 1987. PH-Dependent Character Of Complexation In Podzol. *In* « Podzols Et Podzolisation ». Rhighi D. Et Chauvel A. Eds, INRA-AFES, Plaisir Et Paris (France), Pp 181-186.

Cheshire M.V. 1994. ESR And Mossbauer Spectroscopy Applied To Soil Matrixes ISSS, Mexico. Vol. 3a:45-64.

Cheshire M.V., McPhail D.B., Berrow M.L. 1994. Organic matter - copper complexes in soils treated with sewage sludge. Science of the Total Environment 152:63-72.

Davis J.A. 1984. Complexation Of Trace Metals By Adsorbed Natural Organic Matter. Geochim. Cosmochim. Acta 48: 679-691.

Deckers J.A., Nachtergaele F.O., and Spaargaren O.C. 1998. World Reference Base For Soil Ressources. Introduction. Acco, Leuwen/Amersfoort.Pp165.

Delas J. 1966. Etudes Des Rapports Cuivre Matière Organique Dans Un Podzol Humoferriguneux Accidentellement Enrichi En Cuivre.C.R. Acad. Sc. Paris. 262:2688-2691.

Duchaufour P. 1997. Abrégé De Pédologie. Sol, Végétation, Environnement, 5ème Édition. Masson. Enseignement Des Sciences De La Terre. Pp291.

Duchaufour P. 1977. Pédogénèse Et Classification *In* Pédologie. Masson, Paris.Pp 469.

Dudka S.1993. Baseline Concentrations Of As, Co, Cr, Cu, Ga, Mn, Ni And Se In Surface Soils, Poland.Applied Geochemistry. Suppl. Issue N°2 Pp23-28.

Dumontet S., Lévesque M.,and Mathur S.P.1990. Limited Downward Migration Of Pollutant Metals (Cu, Zn, Ni, And Pb) In Acidic Virgin Peat Soils Near A Smelter. Water, Air, And Soil Pollution 49:329-342.

Flores Velez L.M.1996. Essai De Spéciation Des Métaux Dans Les Sols: Cas Du Cu Dans Les Vignobles. Thèse De l'Université De Parisxii. Val-De-Marne.

Gallet C. 1992. Apports de la Biochimie à la Connaissance du Fonctionnement des Ecosystèmes Forestiers: Rôle des Composés Phénoliques. Dans une Pessière à Myrtille. Thèse N° 278-92, Université Claude Bernard.

Giusquiani P.L., Gigliotti G., and Businelli. 1992. Mobility Of Heavy Metals In Urban Waste-Amended Soils. J. Environ. Qual. 21 :330-335.

Goodman B.A., Cheshire M.V., Berrow M.L. and Deighton N. 1994. Identification Of Paramagnetic Species And Determination Of The Copper Coordination Environments In Organic Matter From Soils Used For The Disposal Of Distillery Wastes. Humic Substances *In* The Global Environment And Implications On Human Health. Edited by N. Senesi and T.M. Miano. Pp 1025-1030.

Harborne J.B. 1979. Plant Phenolics. In " Secondary Plant Products" Ed. Bell E.A. and Charlwood B.V. Encyclopedia Of Plant Physiology.8:329-402.

Hare L. and Tessier A. 1996. Predicting Animal Cadmium Concentrations In Lakes. Nature 380 :430 – 432.

Harter R.D. 1991. Micronutrient Adsorption-Desorption Reactions In Soils. In Micronutrients In Agriculture, 2nd Ed. SSSA Book Series N°4, Pp59-87.

Huber K. 1997. Altération Minérale Et Transferts De Matière Dans Des Sols Issus De Moraines Acides d'Âge Croissant (Chronoséquence) Des Alpes Du Sud. Thèse EPFL N°1714.

Jordan C.F. 1968. A Simple, Tension Free Lysimeter. Soil Science, Vol 105, N°2, Pp81-86.

Keller C. 1991. Etude du Cycle Biogéochimique du Cuivre et du Cadmium dans deux écosystèmes forestiers. Thèse N°916, EPFL, CH-1015 Lausanne.

Keller C. and Vedy J.C. 1991.Apport De La Lysimétrie Sans Tension Pour l'Etude Des Tansferts De Cu Et Cd Dans Les Sols Forestiers Faiblement Pollués. Science Du Sol 29(2):107-124.

Legros J.P. and Cabidoche Y.M. 1977. Les Types De Sols Dans Les Alpes Et Les Pyrénées Cristallines. Doc. De Cartographie Ecologique19:1-19.

Leonowicz A. and Bollag J.M. 1987. Laccases In Soil And The Feasibility Of Their Extraction. Soil Biology And Biochemistry 19 (3):237-242.

Lorenz S.E., Hamon R.E., Holm P.E., Domingues E.M., Sequiera E..M., Christensen T.H., And Mcgrath S.P.M. 1997. Cadmium And Zinc In Plants And Soil Solutions From Contaminated Soils. Plant Soil 189 :21-31.

Christman R.F. and Gjessing E.T. Eds., 1983. Aquatic And Terrestrial Humic Materials. Ann. Arbor Science, Michigan, 538 P.

Lund U. and Fobian A. 1991. Pollution Of Two Soils By Arsenic, Chromium And Copper, Denmark. Geoderma 49:83-103.

McBride M., Sauvé S., and Hendershot W. 1997. Solubility Control Of Cu, Zn, Cd And Pb In Contaminated Soils. European Journal Of Soil Science 48:337-346.

McBride M.B.1994. Environmental Chemistry Of Soils. Oxford University Press, New York.

McBride M.B. and Sikora F.J. 1990. Catalysed Oxidation Reactions Of 1,2-Dihydroxybenzene (Catechol) In Aerated Aqueous Solutions. Journal Of Inorganic Biochemistry 39:247-262.

McBride M.B., Sikora F.J., and Wesselink L.G. 1988. Complexation And Catalysed Oxidative Polymerisation Of Catechol By Aluminum In Acidic Solution. Soil Sci. Soc. Am. J. 52:985-993.

Mckeague J.A., Cheshire M.V., Andreux F., and J. Berthelin.1986. Organo-Mineral Complexes In Relation To Pedogenesis. Interaction Of Soil Minerals With Natural Organics And Microbes, SSSA Spec. Pub. N° 17:549-591.

Merrington G. and Alloway B.J. 1994.The Flux Of Cd, Cu, Pb And Zn In Mining Polluted Soils.Water, Air, And Soil Pollution 73:333-344.

Naidja A., Huang P.M., and J.M. Bollag. 1998. Comparison Of Reaction Products From The Transformation Of Catechol Catalysed By Birnessite Or Tyrosinase. Soil Sci. Soc. Am. J. 62:188-195.

Navosha, Y.Y., Prokhorov S.G., Strigutskiy V.P. and Thomson A.E.. 1992.Interaction Of Humic Substances With Metal Ions. Eurasian Soil Science 24 (5):1-6.

Nriagu J.O. 1978. Changing Metal Cycles In Human Health. Springer, Berlin Heiderlburg New York, 445 Pp.

Nriagu J.O. 1979. The Global Copper Cycle In Copper in the Environment. John Wiley and Sons Inc.

Newman M.C. and Mcintosh A.W. Ed. 1991. Metal Ecotoxicology: Concepts and Applications. Lewis Publishers, Pp 397.

Powell D. H., Merbach A.E., Fàbiàn I., Schindler S., and Van Eldik R. 1994.Evidence for Chelate-Induced Changeover in the Substitution Mechanism of aquated copper(II). Volume profile Analyses of Water Exchange and Complex Formation Reactions. Inorg. Chem. 33: 4468-4473.

Quinche J.P. 1985. Teneurs En Cuivre, Zinc, Plomb, Cadmium Et Mercure Des Sols De Quelques Vignes De La Suisse Et Du Tessin. Revue Suisse De Viti. Arboric. Hortic. 17 (6): 341-344.

Reddy C.N., and Patrick J.R. W.H. 1977. Soil Sci.Soc.Amer. J. 41:729.

Rode A.A. 1970. Podzol-Forming Process. Translated From Russian. Israel Program For Scientific Translators.

Römkens.F.A.M. and Salomons W. 1998. Cd, Cu and Zn Solubility In Arable And Forest Soils: Consequences Of Land Use Changes For Metal Mobility And Risk Assessment .Soil Science. 163:859-871.

Senesi N., Bocian D.F., and Sposito G. 1985. Electron Spin Resonance Investigation of Copper(II) Complexation By Soil Fulvic Acid. Soil Sci. Soc. Am. J. 49:114-119.

Senesi N., and Sposito G. 1989. Characterization And Stability Of Transition Metal Complexes Of Chestnut (Castanea Sativa L.) Leaf Litter. Journal of Soil Science 40: 461-472.

Schnitzer M., and Khan S.U., 1972. Humic Susbtances In The Environment. Marcel Dekker, Inc., New York, 327 P.

Sigel H. Ed. 1984. Metal Ions In Biological Systems, Vol.18: Circulation Of Metals In The Environment. Marcel Dekker, Inc., New York, pp 397.

Simonson R.V. 1959. Outline Of A Generalised Theory Of Soil Genesis. Soil Sci. Soc. Am. Proc. 23:152-156.

Solomon E. I. 1983. Bioorganic Perspectives In Copper Coordination Chemistry. *In* Copper Coordination Chemistry: Biochemical and Inorganic Pespectives, Ed. Kenneth D. Karlin and Subieta.

Souchier B. 1984. Les Podzols Et La Podzolisation En Climats Tempérés Et Montagnards. AFES, Livre Jubilaire Du Cinquantenaire, 1934-1984, 77-96:77-96.

Spurgeon D.J. and Hopkin S.P. 1996. Effects Of Variations Of The Organic Matter Content And Ph Of Soils On The Availability And Toxicity Of Zinc To Eathworm Eisena Fetida. Pedobiologia 40 :80-96.

Stevenson F.J. 1982. Humus Chemistry. John Wiley And Sons, Inc., New York, 443 P.

Stevenson F.J. 1991. Organic-Matter-Micronutrient Reactions In Soil. In: Micronutrients In Agriculture, 1st Ed. SSSA Book Series N°4, Pp145-186.

Stumm W. 1996. Aquatic Chemistry, Chemical Equilibria And Rates In Natural Waters. Environmental Science and Technology, Third Ed.Wiley Interscience.

Tan K.H. 1982. Principles Of Soil Chemistry. Marcel Dekker, Inc., New York, Pp 267.

Temminghoff E.J.M., Van Der Zee S.E.A. T.M., And De Haan F.A.M. 1997. Copper Mobility In A Copper-Contaminated Sandy Soil As Affected By Ph And Solid And Dissolved Organic Matter. Environ.Sci. Technol. 31:1109-1115.

Timberlake C.F.1957. A Potentiometric And Polarographic Study Of Copper-Catechol Complexes. Journal Of Chemical Society 4987-4993.

Tyler G. 1981. Leaching Of Metals From The A-Horizon Of A Spruce Forest Soil. Water, Air, And Soil Pollution, 15: 353-369.

Van Straalen N.M. and Bergema W.F. 1995. Ecological Risks Of Increased Bioavailability Of Metals Under Soil Acidification. Pedobiologia, 39 :1-9.

Bridges E.M., Batjes N.H., and Nachtergaele F.O. Ed. 1998. World Reference Base for Soil Ressources, Atlas. Acco. Pp 79.

Xue H., and Sunda W.G. 1997. Comparison Of Cu2+ Measurements In Lake Water Determined By Ligand Exchange And Cathodic Stripping Voltammetry And By Ion-Selective Electrode. Environmental Science And Technology, 31 (7): 1902-1909.

Xu H., Ephraim J., Ledin A., and Allard B. 1989. Effects Of Fulvic Acid On The Adsorption Of Cd(II) On Alumina. Science Of The Total Environment, 81/82, 653-660.

Yaron B., Calvet R., and Prost R.1996. Soil Pollution: Processes And Dynamics. Springer, Pp309.

Zabowski D. and Ugolini F.C. 1990. Lysimeter And Centrifuge Soil Solutions. Seasonal Differences Between Methods. Soil Sci. Soc. Am. J. 54:1130-1135.

Zacchara J.M., Resch C.T., and Smith S.C. 1994. Influence Of Humic Substances On Co2+ Sorption By A Subsurface Mineral Separate And Its Mineralogic Components. Geochimica And Cosmochimica Acta, 58 :555-566.

PART I

FIELD APPROACH

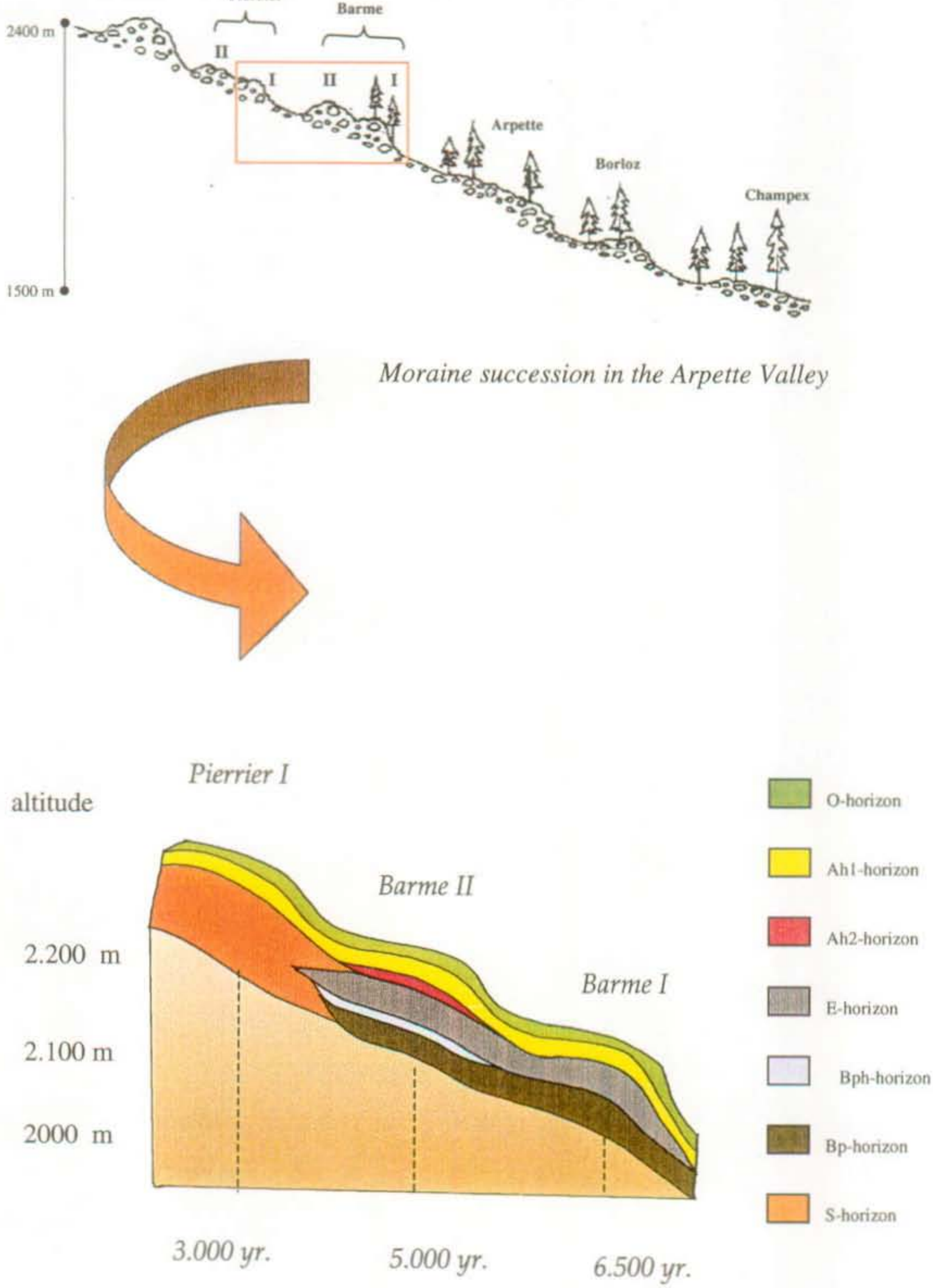
Chapter 1

Setting the scene...preferential distribution pattern of Cu and other cations on podzol profiles with increasing leaching capacities in the Arpette Valley

Abstract

Dans ce travail trois sols développés sur substrats morainiques du massif du Mont-Blanc et servant de base à l'étude de la migration du Cu et des phénols sont présentés. Il s'agit d'un RANKER ALPIN HUMIQUE, d'un PODZOSOL OCHRIQUE et d'un PODZOSOL OCHRIQUE À CARACTÈRE DURIQUE. Le degré de lessivage de ces sols a été déterminé en mesurant la quantité de Fe et d'Al extractibles à l'oxalate d'ammonium ainsi que la fraction de cations échangeables tout au long des profils. Ces mesures confirment une augmentation du lessivage avec l'augmentation de l'âge des sols qui transparaît par une morphologie bien différenciée. Les teneurs locales en ETM ont également été mesurées. Bien que les éléments Ni, Pb, Zn et Cu soient présents dans les plantes, seuls Cu et Zn subsistent dans les sols. Des teneurs élevées en Cu dépassant les normes légales suisses d'investigation ont été mesurées dans le sol le plus évolué avec une accumulation préférentielle en surface(0-15 cm).

In this study three soils from the Mont-Blanc massive developed on moraine substrates, which were used as a reference base for investigation on Cu and phenol migrations in acid soils are presented. These soils were a "RANKER ALPIN HUMIQUE", a "PODZOSOL OCHRIQUE", and a "PODZOSOL OCHRIQUE" WITH DURIPAN CHARACTER. The degree of leaching of these soils was determined by quantifying extractable Fe and Al by oxalate ammonium extraction and by measuring the total exchangeable cation fraction along the soil profiles. The background level of trace metals was also determined and indicated that while Ni, Pb, Zn and Cu were present in plants, only Cu and Zn were detectable in soils. High levels of Cu exceeding the values recommended by the Swiss legislation were found in the more evolved soil and were mostly accumulated between 0 and 15 cm.



Moraine succession in the Arpette Valley

Figure 1. Sequence of Soils selected for our study

Introduction

This study is part of a larger entity investigating Cu phenol translocation mechanisms in acid soils. It has been used as a reference document for two other studies, one on the seasonal variations of Cu and phenols in the soil solution (Oess, Chapt. 2) and another on the effect of phenols on Cu sorption to podzol horizons (Oess, Chapt. 7). This study also contributed in adjusting laboratory parameters in experiments at the molecular scale on the interaction of Cu with phenols (Chapt. 4 and 5). There was no claim in this study to bring new arguments on the mechanisms of podzolisation, which have been much discussed during the last decades (Robert *et al.*, 1980; Farmer, 1984; Souchier, 1984, Robert *et al.*, 1986; Dambrine, 1989; Huber 1997). The aim was rather to focus on specific parameters, which result from podzolisation and can help to understand the process of heavy metal translocation.

The objective of this study was to present the sequence of soils which was chosen for these studies, to determine the background level of Cu in plants and soils and finally to discuss the correlation between the vertical distribution of Cu and the increasing leaching properties of our soils.

Several criteria were considered to chose the study site. Trace metals should be present at a detectable level, but the soils should be in an area of low pollution to avoid blurring interaction with other xenobiotics. Conditions where migration processes can be observed within a reasonable amount of time are also recommended.

Previous studies carried out in the Arpette valley revealed the presence of a soil sequence with significant differences in weathering and acidification rates (Ceppi, 1993; Huber 1997). Since soil acidification and the presence of dissolved organic matter are recognised as key parameters enhancing the mobility of trace metals, we looked for soils where acidification is an active process. The background levels of Cu in a podzol studied under forest in the same valley indicated values around 16 mg/kg in the surface horizons (Keller, 1991). This indicated that there was a good chance that Cu content would be in a detectable range.

Three soils, which illustrate different stages in podzolisation were chosen from the topochronosequence. The classical soil morphology descriptions are presented in relation to increasing leaching parameters. Increasing leaching was quantified by measuring the oxalate ammonium extractable fraction of Fe and Al and the total fraction of exchangeable cations (Ca, K, Mg, Na, Al, Mn). Preferential distribution of Cd, Pb, Cr, Ni, Ti, Cu, Zn, Co in plants and in each horizon was determined for the three soils.

The site

The Arpette valley belongs to the Mont-Blanc valley and is located in a lateral valley on the Swiss side 15-km South of Martigny. The altitude in the Valley ranges from 1,468 m at the level of Champex Lake to 3,270m at the Pointe d'Orny. The valley has been shaped by the successive retreats of the Arpette Glacier and more than eight moraines are disposed perpendicular to the slope on a crystalline granitic parent material (protogine du Mont-Blanc). Glacier extension investigations have been carried out between the lowest and the highest point of the valley, which has enabled an estimation of the age of the moraines to be made (Bless, 1984; Aeschlimann, 1983, Holzhauser, 1984). The soil sequence, which has developed on top of these moraines, corresponds to rankers for the younger soils (150 to 3,000 yr.) located at the higher altitudes and to podzols for the older soils (6,500 to 10,000 yr.) at the lower altitudes (Huber, 1997). In between we are in the presence of various different stages of soil evolution tending toward podzolisation. For this study we were looking for soils strongly reflecting the transition from a ranker to a podzol. Huber (1997) showed that chemical weathering in the soil solution initiated on the rankers of the moraine named Pierrier II. We therefore selected the stage coming just after Pierrier II, where the soils are still rankers (Pierrier I), and the two following stages Barme II and Barme I where a rapid switch to well differentiated podzols was morphologically apparent. The soil profiles were dug on the upper part of the moraines and faced N-E. Figure 1, illustrates the position of the three soil stages within the topochronosequence. Lysimeters were installed in 1995 under each profile to collect the soil solution during three years, but the experimental set up and the results obtained from the study of the soil solutions are presented in another document (Chapt. 2).

Climate

The annual rainfalls exceed 1,600 mm, the average temperature is about 4.8°C (Schlussel, personal communication) and the moraines are covered with snow during 6 to 7 months per year. Since the transect is located at an altitude where climatic changes can be quite important, temperature sensors were installed between the highest and the lowest site of our transect (Pierrier I: 2,166m; Barme I: 2,003 m). The temperature was measured in the air, Soil and Soil Solution for the years of 1996, 1997 and 1998.

Despite the higher altitude the average temperature was 1.7°C higher in the Air at the Pierrier site than at the Barme site during the summer months. In the soil the temperature was also 0.9°C higher, but in the soil solution there was no significant difference. The higher temperatures observed at the Pierrier site, were attributed to an increase in solar radiation resulting from a lesser shading of the surrounding peaks. Daily amplitudes ranged between 10 and 15°C in the summer and 5° in the winter. In the soil solution weak amplitudes were observed at the Barme site (2.5 °C). At the Pierrier site the amplitudes were more pronounced and increased from 2.5°C in 1996 to 10°C in 1997 and between 10 and 20°C in the summer of 1998. This was attributed to the increase of Air temperature, which increased by 1 °C each year of study.

Measuring the snow height at several intervals during the winter indicated that Pierrier received over two times more rainfall in the form of snow. A shift of about one month in the snow-melting period was also observed between the two sites.

Vegetation cover

Although the main ecological plant groups have been studied on the different moraines (Richard, 1975), a recent study focused only on the Barme I moraine showed important discrepancies in ecological groups between the different orientations of the moraine (Bütler, 1997). Therefore we estimated that a more careful investigation was necessary for the N-E side of the moraines.

The relative abundance of each plant species was determined on 25m^2 plots by the normalised method of Braun-Blanquet (1964). The contribution in biomass for each plant was determined from the dry weight on 1m^2 plots for 1995 and 1997. The plants were harvested in August and September by cutting them off at the root and separating them by species.

On the **Barme I** moraine *Larix decidua, Sorbus aria, Rhododendron ferrugineum, Vaccinium gautherides, Vaccinium myrtillus, Homogyna alpina, Luzula sylvatica, Lycopodium annotinum, Mejampthemum bifolium* were identified.

Mosses where also present and determined with the assistance of P.Geissler (Jardin botanique, Geneva). In the order of occurrence *Pleurozium schréberi, Barbilophia lycopodioides, Hylocomium splendens, Dicranum scoparium, Hylocomium pyrenaicum* and *Sanionia uncinata* were identified. *Rhododendron ferrugineum, Vaccinium gautherides* and *myrtillus* and the total mosses had the highest recovery index.

The average dry weight between the harvests, indicated values of 242.5 g/^{m2} for the mosses, 290 g/m^2 for the *Vaccinium* species together, 143 g/m^2 for *Rhododendrons*, 39 g/m^2 for lycopodium, and 16 g/m^2 for the herbaceous strata.

On the **Barme II** moraine *Picea abies, Sorbus aucuparia, Juniperus nana, Rhododendron ferrugineum, Empetrum hermaphroditum, Vaccinium gaultherioides, Vaccinium myrtillus, Deschampsia flexuosa, Gentiana purpurea, Homogyna alpina, Luzula lutea, Melampyrum pratense* were identified. *Rhododendron ferrugineum, Empetrum hermaphroditum, Vaccinium gautherides* and *Vaccinium myrtillus* had the highest recovery index. The presence of *Empetrum hermaphroditum,* which is a primitive plant, reflects the more recent evolution of this soil compared to the soils of the Barme I site.

The average dry weight was slightly higher compared to the Barme I moraine, with values of 387 g/m^2 for mosses, 177.5 g/m^2 for *Vaccinium sp.*, 115 g/m^2 for *Rhododendron ferrugineum*, 82 g/m^2 for *Empetrum hermaphroditum*, 15 g/m^2 for the herbaceous species, 1.5 g/m^2 for lichens.

On the **Pierrier** moraine *Pinus mugo, Juniperus nana, Rhododendron ferrugineum, Salix helvetica, Empetrum hermaphroditum, Vaccinium gautherides, Vaccinium myrtillus, Anthoxanthum odoratum, Astiantia minor, Hieracium alpina, Homogyna alpina* were identified. *Rhododendron ferrugineum, Empetrum hermaphroditum, Vaccinium gautherides and Vaccinium myrtillus* were the dominant representative species.

The average dry weight was considerably lower compared to the two other moraines with values of 140.5 for *Vaccinium* sp., 71 g/m^2 for *Empetrum hermaphroditum* 40g/m^2 for *Rhododendrons*, 32 g/m^2 for the mosses, 24 g/m^2 for the herbaceous strata, 12 g/m^2 for the lichens.

When moving to the younger soils, a decrease of contribution in biomass of the shrub strata was typically joined by a decrease in mosses for which shade, acidity and humidity are the main survival conditions.

The different plant repartitions on the moraines exemplify the fact that the chosen soils belong to different stages of evolution, with different exchange cycles between the plant and soil system

Soil Sampling and Analysis

The soils were sampled from each horizon in the Barme I and Barme II profile and each 10-cm in the Pierrier profile where the horizons could not be distinguished. The soils were dried, sieved (2mm), and ground.

The amorphous forms of Fe and Al were extracted with a pH3 buffered ammonium oxalate solution following the "Tamm" method and the procedure of Schwertmann (1964).

The exchangeable forms of Al, Ca, K, Mg, Na and Mn were extracted with a 0.1 M $BaCl_2$ solution. T corresponds to the sum of the exchangeable cations. Cation concentrations were determined by an Emission Plasma Spectrometer 2000 (Perkin Elmer).

200 mg of dried and ground soils and plants were mineralised in a micro-wave oven (Milestone MLS 1200 MEGA), with 3ml HF (40%), 2 ml HCl (30%) and 5 ml HNO_3 (65%). Major elements were quantified by an Emission Plasma Spectrometer and trace elements were detected by GF-AAS (Perkin Elmer 5100). Soil sample SO-4 provided by the Canada Centre For Mineral And Energy Technology was used as a reference sample and the standard deviation for the analysis was less than 0.1% for most metals and 1 µg/g for Cu.

C was measured by combustion in a "Casumat" (Wöstoff in Bochum) and CO_2 trapped with 0.05 M NaOH solution.

Soil characteristics and cation distribution along the profile

Barme I (Figure 2)

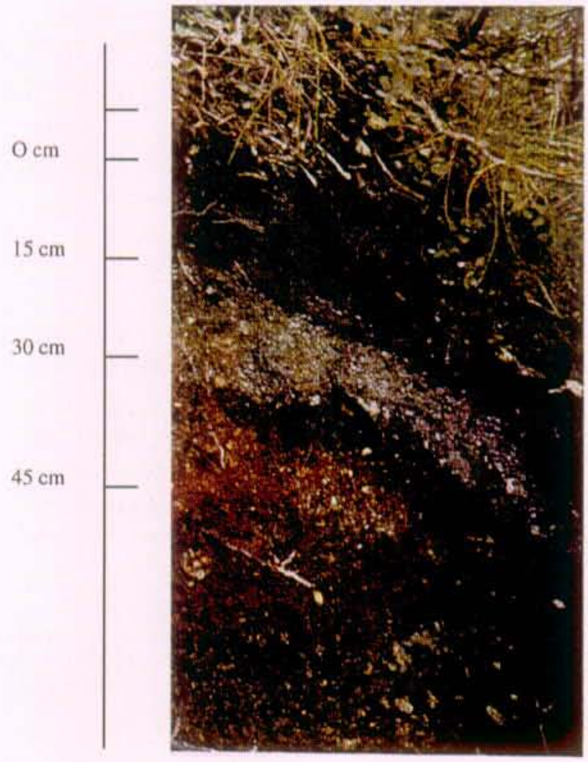
The topsoil was characterised by a very dark humic A-horizon, which underlayed a MOR humus. A decrease in Ca, K, Mg and exchangeable Al and Fe at 15 cm coincided with the appearance of a bleached horizon commonly known as the E horizon. This albic horizon is the result of the weathering of minerals by organic acids, complexation reactions and a subsequent translocation ("cheluviation") and accumulation (known as "chilluviation") (Zech *et al.*, 1993). The whole process can take several thousands of years as illustrated by this profile and leads to the formation of a spodic horizon, which is a reddish coloured Fe horizon. It usually contains illuvial amorphous material composed of organic material (Bph) and Al with or without Fe (Bps). In this case a slight Bph horizon was present, which gave place to a more developed Bps horizon. The quantity of Fe and Al was respectively 7 and 10 times higher compared to the A-horizon. In the Bps horizon a cementation was locally present and this lead to the attribution of "duripan character" connotation to this profile. The morphological characters as well as the chemical analysis suggested that this soil is an PODZOSOL OCHRIQUE (with a duric or duripan character) (Référentiel Pédologique, 1995).

BarmeII (Figure 3)

Barme I

GPS position : 571094.54 96748.58 2003.25
Slope : 48°
estimated age : 6.500 yr

PODZOSOL OCHRIQUE
(possibly with Duripan character)
REFERENTIEL PEDOLOGIQUE, 1995.



O 5-0 cm	Fresh to dry; MOR, dark brown: 7.5 YR 3/3 dry state, slightly compact and fibrous, several fine to thick roots, subhorizontal and abrupt transition (1cm).
A 0-15cm	Fresh, black: 7.5 YR 2/1, no coarse material; thick and medium size roots, ondulated and abrupt transition (1cm).
E 15-30cm	Fresh, light brownish grey 7.5 YR 7/1, sandy with numerous gravel up to 1cm in size, several thick roots; variable thickness of the horizon, abrupt transition.
Bps 30-45 cm	Fresh to wet; horizon located between rocks and blocks: bright brown 7.5 YR 5/6, silt and sandy; numerous coarse material; few and very fine roots; locally a Bph horizon in the upper part of the horizon; diffuse transition toward C.
Bps-C > 45 cm	Fresh to wet, progressive change of colour, brown10YR 4/6, sandy, numerous coarse material of all sizes, structure with particles, stop at 50 cm due to moraine blocks.

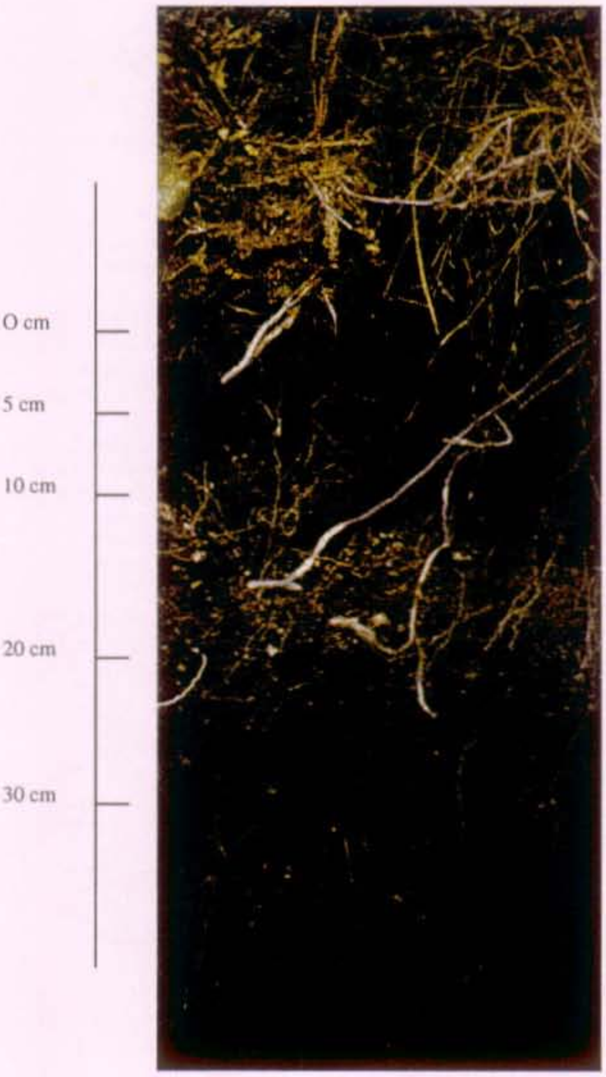
			TAMM (mg/g)		exchangeable cations (m.e./100g) +- 0.1%							T.M. (mg/kg)	
horizon	pH (H_2O)	C (%)	Fe	Al	Ca	K	Mg	Na	Al	Mn	T	Zn	Cu
O	3.7	44.0	1.2	1.3	16.78	1.79	3.85	0.20	3.40	0.31	31.6	67.9	173.4
A	3.8	34.2	1.8	1.6	4.67	1.07	1.29	0.18	5.71	0.02	16.6	25.4	139.7
E	3.9	2.7	0.5	0.9	0.29	0.10	0.09	0.14	2.43	0.00	4.3	6.4	6.8
Bps	4.5	6.0	14.5	26.0	0.40	0.05	0.08	0.14	6.44	0.00	7.5	18.2	3.3
Bps-C	4.6	6.3	17.0	25.0	0.33	0.04	0.06	0.13	5.91	0.00	6.9	27.3	0.7

Figure 2. Soil characteristics and cation distribution along the Barme I profile.

Barme II

GPS position: 571026.13 96625.98 2070.60 32
slope: 40°
orientation: N
estimated age: 5.000 yr

PODZOSOL OCHRIQUE
REFERENTIEL PEDOLOGIQUE, 1995.



O 4-Ocm	Fresh to dry; MOR, brown: 7.5 YR 4/3, large roots, fragmental Organic material, wavy and abrupt transition.
Ah_1 0-5cm	Fresh, black humic horizon, very dark brown: 7.6 YR 213, few small roots, no coarse material, wavy and abrupt transition.
Ah_2 5-10cm	Fresh, fresh black horizon but lighter, greyish brown 7.5 YR 4/2 with washed quartz grains (coarse sands), a few fine and medium sized roots.
E 10-20cm	Humid and fresh, brownish grey 7.5 YR 5/1 and light grey 7.5 YR 8/1. Sandy with numerous gravel of size up to 1 cm, many small fine roots. Abrupt transition. A complete leaching of the organic matter was noticed (5.4%).
Bph20-25 cm	Fresh, brownish black 7.5 YR 3/2, sandy loamy, numerous coarse material (gravel and rocks), many fine small roots, wavy and abrupt transition.
Bps25-36 cm	Fresh, horizon located between rocks and blocks, brown 7.5 YR 4/4 and bright brown 7.5 YR 5/6, sandy loamy, numerous coarse material, less roots, diffuse transition toward C.
Bps-C >36 cm	Progressive change of colour, sandy, numerous coarse material, stop on blocks at 45 cm (C horizon not reached).

			TAMM (mg/g)		exchangeable cations (m.e./100g) +- 0.1%							T.M. (mg/kg)	
horizon	pH (H_2O)	C (%)	Fe	Al	Ca	K	Mg	Na	Al	Mn	T	Zn	Cu
O	3.8	43.2	0.9	1.4	19.71	1.96	4.39	0.21	2.24	0.21	34.5	50.5	12.3
Ah1	4.0	34.6	1.7	1.3	5.03	0.37	1.45	0.14	4.41	0.02	15.1	23.1	3.1
Ah2	3.8	18.7	1.2	0.9	1.15	0.33	0.46	0.14	4.27	0.00	9.0	10.6	4.0
E	3.9	2.9	0.9	0.6	0.36	0.08	0.11	0.14	4.03	0.00	6.4	6.7	1.0
Bph	4.1	7.9	5.5	5.0	0.53	0.07	0.11	0.14	13.77	0.00	15.2	9.2	0.3
Bps	4.3	5.6	9.7	13.9	0.28	0.02	0.05	0.14	7.54	0.00	8.8	22.2	0.3

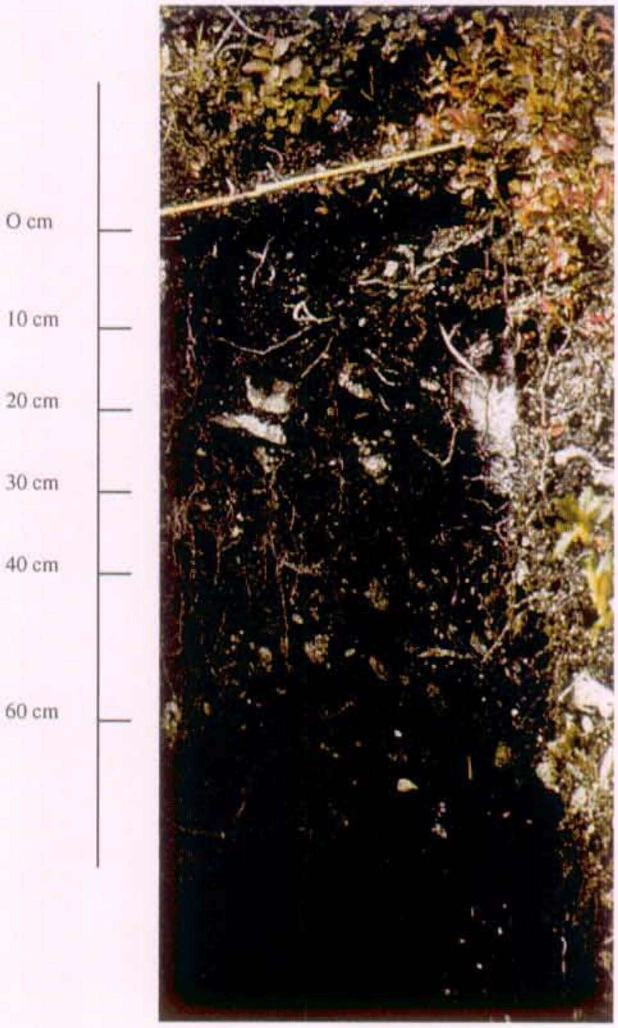
Figure 3. Soil Characteristics and cation distribution along the Barme II profile.

Pierrier I

GPS position: 570456.23 96299.13 2165.98
slope: 40°
orientation: NE
estimated age: 3.000 yr

RANKER ALPIN HUMIQUE

REFERENTIEL PEDOLOGIQUE, 1995.



O 5-Ocm	Dry, MOR, brownish black: 7.5 YR 2/2 ;. Many very fine to medium size roots, wavy and abrupt transition.
Ah 0-10cm	Fresh and very organic; reddish black: 2.5 YR 2/1, many fine to medium size roots. Numerous leached quartz grains , abundant coarse material (mostly gravels), several pebbles, wavy and diffuse transition. Remark: no lumpy structure.
S1 10-20 cm	Fresh, dark brown 7.5 YR 3/3, brown 7.5R4/4, sandy, numerous fine roots, abundant coarse material (more than in Ah). Particular but not cemented structure, diffuse transition.
S2 20-30 cm	Brownish black 7.5 YR 3/2, same as for S1.
S3 30-40 cm	Dull yellowish brown 10 YR 4/3, same as for S1.
S4 40-50 cm	Brown 10 YR 4/4, same as for S1.
S-C 50-60 cm	Fresh, brown 10 YR 4/4and dull yellow orange 10 YR 6/3, sandy, few but very fine roots, numerous coarse material, structure with particles. Stop at 60 cm.

			TAMM (mg/g)		exchangeable cations (m.e./100g) +- 0.1%							T.M. (mg/kg)	
horizon	pH (H_2O)	C (%)	Fe	Al	Ca	K	Mg	Na	Al	Mn	T	Zn	Cu
O	3.8	24.7	2.7	3.1	4.42	0.93	1.11	0.18	8.90	0.10	19.2	37.6	4.4
Ah	3.9	18.4	2.0	4.0	1.45	0.45	0.54	0.15	8.00	0.02	12.1	25.5	2.2
S1	4.1	8.1	2.9	11.8	0.71	0.19	0.31	0.15	8.08	0.02	12.0	30.0	1.0
S2	4.2	7.0	5.7	18.1	0.49	0.14	0.19	0.13	7.48	0.01	9.6	43.9	1.0
S3	4.4	4.1	3.3	7.4	0.33	0.09	0.12	0.14	4.05	0.01	5.6	53.5	0.1
S4	4.6	2.0	2.9	2.4	0.55	0.06	0.07	0.12	1.92	0.00	3.7	49.1	0.9

Figure 4. Soil characteristics and cation distribution along the Pierrier profile.

The morphological and chemical analysis indicated that the development stage of this soil is anterior to the Barme I stage. The A-horizon was subdivided into two horizons (Ah1 and Ah2). Although the black humic character was dominant in this horizon, a slight change of colour due to the presence of quartz grains and a decrease in exchangeable cations indicated that the bleaching was in the middle of it's process here. The exchangeable cation concentration in the E horizon was in the same range as for the Barme I profile but slightly lower. A pronounced Bph horizon was present with an important Al accumulation and a higher organic matter content compared to the E horizon. In the Bps horizon the quantity of Al and Fe extractable by ammonium oxalate was about 5 to 7 times higher than in A- and largely satisfy the RP (Référentiel Pédologique, 1995) requirements of the formation of a spodic horizon.

Pierrier (Figure 4)
Although similar topsoil characteristics were observed compared to the Barme I and Barme II profiles, no E-horizon was discernible in this profile. Nevertheless, the analysis at different soil depths of the S horizon revealed a first accumulation of Al between 10 and 20 cm (S1), followed by a stronger Al accumulation between 20 and 30 cm (S2) and with the beginning of an Fe accumulation. These features indicate that we are getting close to the formation of a Bph horizon. No organic matter accumulation was observed in the deeper horizons. The processes leading to podzolisation had definitely started, but the podzolic stage has not been reached yet. Therefore we can speak about a RANKER ALPIN HUMIQUE (Référentiel Pédologique, 1995).

Trace metal distribution trends

Analysis was carried out for eight elements (Cd, Pb, Cr, Ni, Ti, Cu, Zn, Co) but only Zn and Cu were present in detectable levels by AAS (>10 ppm. Both cations showed a surface accumulation (mostly in the O and A-horizon), but different concentrations were observed between the profiles and different accumulation patterns occurred with depth. Figure 5, illustrates the different accumulation patterns of Zn, Cu and organic carbon on the different profiles.

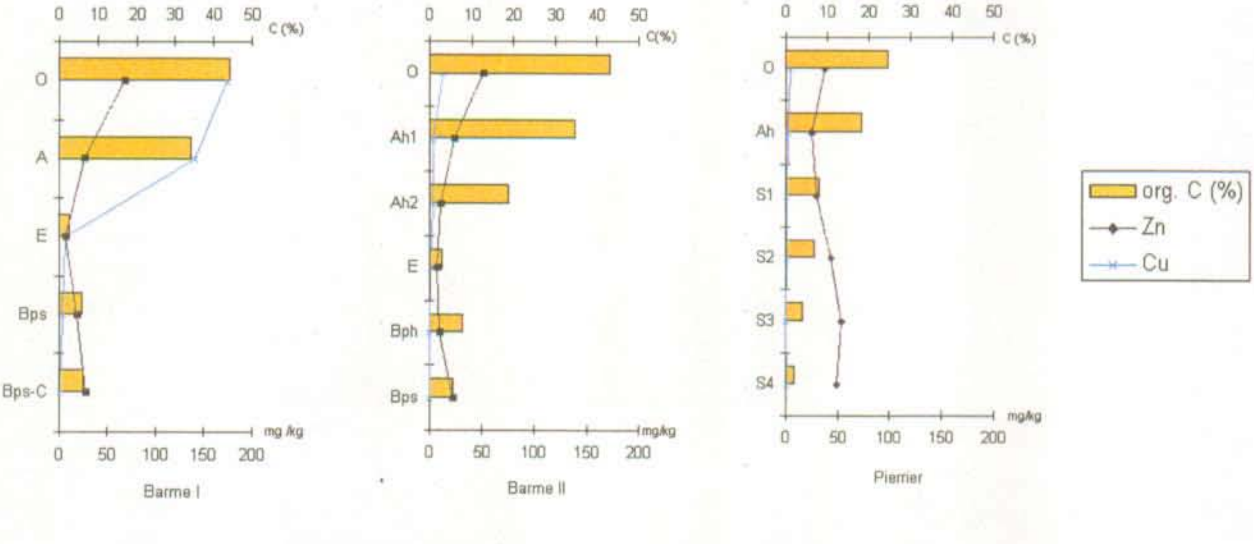


Figure 5. Distribution of Zn, Cu and % of organic matter on the three profiles

Cu concentration was highest on the Barme I profile. The highest accumulation was observed in the O and A-horizon (173.4 and 139 mg/kg). A significant decrease in Cu was observed in the E, Bps and Bps-C horizons more gradually. Cu concentration exceeded by three times Zn levels in the surface horizons. Zn decreased much and increased again in the Bps and Bps-C horizons. Cu was correlated with the percentage of surface organic carbon and Zn with surface and deeper horizon organic carbon percentage.

On the Barme II profile, Cu concentration was more than two fold lower than Zn. Cu decreased rapidly with depth. Zn distribution was similar as in Barme I profile. Organic carbon decreased linearly down to the E horizon and increased again in the Bph horizon. The subdivision of the A-horizon into two distinct Ah1 and Ah2 horizons was confirmed and reflected the different levels of organic matter accumulated between the two horizons. Cu and Zn decreased proportionally to the percentage of organic carbon, but the effect was more pronounced for Zn which was present in higher concentrations in this profile.

On the Pierrier profile Cu concentration was even lower and mostly distributed in the topsoil fraction. Zn showed a particular accumulation in the S2 and S3 horizons.

The soil pH did not vary much between the profiles. pH was about 3.7 in the surface horizons and reached about 4.6 at the C-horizon. No particular correlation between pH and Zn or Cu distribution was observed.

Trace metal content in plants

Al, Fe, Mn, Cu, Pb, Zn, Cr, Co and Cd were analysed in the plants with the highest biomass contribution. Cr, Co and Cd concentrations were below 1 µg/kg and were disregarded for our discussion. Fe, Al, and Mn range between 0.1 and 0.5 mg/kg.

Zn was the most abundant cation in all plant species, followed by Cu, Pb and Ni. Zn particularly accumulated in *Vaccinium sp.* and in *mosses. Pb* preferentially accumulated in mosses. Cu accumulated in *Vaccinium sp.* and *Rhododendron ferrugineum,* but there was no correlation between Cu accumulation in the top-layer of the soil and the concentration in plants. At the opposite Cu levels plants were higher in plants from the less contaminated soils.

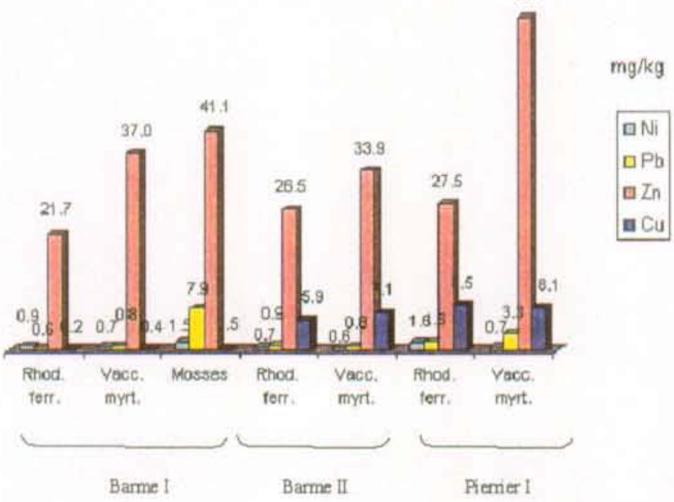


Figure 6. Distribution of trace metals in plants

Discussion, Conclusion

The morphological and chemical parameters confirmed that the chosen soil sequence represented determining stages in podzol formation. The soil named as a RANKER ALPIN HUMIQUE represented the initial stage of podzolisation. The PODZOSOL OCHRIQUE of the Barme II station was particularly interesting as it reflected the "active" podzolisation process, where "cheluviation" and "chilluviation" are still continuing processes and have not yet reached equilibrium. It was exemplified by an increasing soil stratification into distinct "organo leached", "leached", "organo spodic" and "spodic" horizons, which occurred between 3,000 and 5,000 years. In the oldest profile (Barme I), a regression in stratification was observed but an increase in sharpness of transition between horizons, indicated that some kind of equilibrium in the podzolisation process had been reached. The disappearance of the Bh-horizon was attributed to a further migration down and mixing of organic matter with the Bs-horizon.

Comparing these soils with the podzols described by Legros (1977) on a N-W transect in the area of Chamonix with similar stational conditions (altitude, parent material and vegetation), revealed that the soils of Barme I and Barme II had reached a more advanced stage of evolution with more marked elluviated and spodic horizons.

The soil and plant system is a particularly interesting phase to observe, as it reflects the atmospheric inputs at the surface and the migration tendencies at the bottom of the soil profile. Cu and Zn are the most exploited heavy metals on the planet and over 56,000 tons for Cu and 214,000 tons for Zn are disseminated in the atmosphere each year (Adriano, 1986). This intense activity was also reflected in our soils since only Zn and Cu were above the detectable limits. Both metals had different distributions in soils. Cu was preferentially accumulated in the surface horizon. Up to 173 mg/kg of Cu was detected in the surface soils of the Barme 1 moraine. It exceeded both the indicative values (40 mg/kg) and the limits determined for investigations (150 mg/kg) recommended by the Swiss legislation (Osol, 1998). The low Cu concentrations observed in plants indicated that plants were not responsible for this accumulation. In order to establish the rate and the origin of this contamination in the valley further sampling and analysis are needed. An increase of surface accumulation with soil age was also observed.

Zn was also accumulated in the surface horizons but some accumulation also occurred in the deeper horizons. The deeper accumulation increased in the less elluviated soils and indicated that leaching was favoured on less developed soil. Therefore, we can say that the stratification is a good indicator of leaching activity in the past but it does not always promote leaching in the present. Leaching could actually be more intense in the earlier stages of podzolisation.

The different accumulation trends of Cu and Zn indicate that different translocation mechanisms are involved in these soils. In order to get a better understanding of these processes we suggest the reader to proceed with the next chapters.

References:

Adriano D.C. 1986. Trace Elements in the Terrestrial Environment. Springer, Berlin Heidelberg New York, 533 pp.

Aeschlimann H. 1983. Zur Gletschergeschichte des Italienischen Mont Blanc Gebietes: Val Veni- Val Ferret-ruitor, Doktor Arbeit, Universität Zürich, 106 pp.

Référentiel Pédologique 1995. Baize D. and Girard M.C. Ed. INRA. Paris. pp 332.

Bless R.1984. Beiträge zur spät- und postglazialen Geschichte der Gletscher im nordöstlichen Mont-Blanc Gebiet, Doktor Arbeit, Physische Geographie, N°15, pp116.

Braun-Blanquet J. 1964. Pflanzensoziologie: Grudnzüge der Vegetationskunde. Springer, Wien.

Bütler R. and Domergue F.-L.1997. Valeurs Indicatrices de la Végétation et des Sols d'une Moraine Granitique de l'Etage Subalpin. Rev. Ecol. Alp. Grenoble, 4:1-12.

Ceppi M. 1993. Evolution des Sols Dans le Val d'Arpette. Travail de Diplôme de 3ème cycle (IATE-Pédologie, EPFL, CH-1015 Lausanne). pp 107.

Dambrine E., Robert M. and Ranger J. 1989. La Podzolisation à l'Etage Subalpin: un Processus Typiquement Hivernal. C.R. Acad. Sci.Paris. 308:1797-1989.

Farmer V.C. 1984. Distribution of allophane and Organic Matter in Podzol B Horizons: Reply to Buurman and Van Reeuwijk. Journal of Soil Science. 35: 453-458.

Holzhauser H. 1984. Zur Geschichte des Aletschgletscher und des Fieschergletschers, vol. 13, eds. Fürrer G., Keller W.A., Gamper M., Suter J., Geographisches Institut des Universität Zürich,

Huber K. 1997. Altération Minérale et Transferts de Matière Dans des Sols Issus de Moraines Acides d'Âge Croissant (Chronoséquence) des Alpes du Sud. Thèse EPFL n°1714.

Keller C. 1991. Etude du Cycle Biogéochimique du Cuivre et du Cadmium Dans Deux Ecosystèmes Forestiers. Thèse N°916 EPFL, CH-1015 Lausanne.

Legros J.P. and Cabidoche Y.M. 1977. Les Types de Sol dans les Alpes et les Pyrénées Cristallines. Doc. de Cartographie Ecologique19:1-19.

Oess A. Influence of phenols on Cu sorption to a podzol. Chapt.VI.

Oess A. Seasonal Peaks of Cu and Phenols in the Soil Solution.Chapt II

Oess A., Cheshire M.V., McPhail D.B., and Vedy J.C. 1999. Polymerization: a Possible Consequence of Copper-phenolic Interactions. Ed. Berthelin et al.Plenum publisher, London.

Oess A., Cheshire M.V., McPhail D.B., Stoll S., El Alaili M., and Vedy J.C. Elucidation of Phenol-Cu Interaction Mechanisms by Potentiometry, ESR, UV Absorption Spectroscopy and Molecular Simulations. Accepted for publication January 14th 1999 in Science of The Total Environment.

Osol. 1998. Ordonnance sur les Atteintes Portées aux Sols du 1er Juillet 1998. Art.5.

Richard L. 1975. Le Milieu et la Végétation, Etude Ecologique des Massifs des Aiguilles Rouges, au Haut-Giffre et des Aravis (Haute Savoie), Annales du Centre, Université de Savoie, pp100.

Robert M., Cabidoche Y.M., Berrier J. 1980. Pédogénèse et Minéralogie des Sols de Hautes Montagnes Cristallines (Etages Alpin et Subalpin-Alpes Pyrénées), Science du Sol, pp 313-336.

Robert M., Razzaghe M.H., Ranger J. 1986. Rôle du Facteur Biochimique Dans la Podzolisation, Etudes Expérimentales sur les Méchanismes Géochimiques et les Evolutions Minéralogiques, In Podzol et Podzolisation, Ed. Righi D. and Chauvel A. Association Française pour les Sols, 231 pp.

Souchier B.1984. Les podzols et la Podzolisation en Climats Tempérés et Montagnards. AFES, Livre Jubilaire du Cinquantenaire, 1934-1984, 77-96:77-96.

Zech W., Guggenberger G., Miltner A. 1993. Mitteil. DBG, 72:665-668.

Chapter 2

Seasonal Peaks Of Cu And Phenols In The Soil Solution

Abstract

La migration du Cu et des phénols a été étudiée sur trois sols d'une topochronosequence alpine comportant des morphologies de lixiviation croissantes. La solution du sol a été prélevée pendant trois années consécutives à l'aide de lysimètres sans tension placés sous les horizons de surfaces. Les fortes corrélations observées entre le Cu et les phénols totaux dans la solution du sol après les évènements pluvieux ont indiqué que les phénols pourraient être la fraction "active" de la matière organique en tant que vecteur du Cu. Les paramètres saisonniers tels que la fonte des neiges, les apports de litière en automne ont contribué chacun à leur tour au relargage du Cu et des phénols en solution mais aucun de ces paramètres n'a eu une dominance particulière.

Leaching of Cu and phenols was studied on three soils of an alpine topochronosequence with increasing leaching morphologies. Tension free lysimeters placed under the surface horizons were used to sample the soil solution during three years. Strong correlation between Cu and total phenols indicated that phenols could be the "active" metal carrier fraction of organic matter. Seasonal parameters as the snow melting, solution temperature and litter fall all contributed in turns to metal and phenol migration, but none of these parameters was a dominant leaching cause.

Introduction

The implication of Dissolved Organic Carbon (DOC) in metal complexation and transport processes through aqueous ecosystems has been well established (Buffle & Van Leeuwen, 1992; Hering & Morel, 1993; Stumm, 1997). The soil-soil solution interface is a particularly reactive medium for these interactions, as organic matter deriving from plant degradation is abundant and reactive. Furthermore, pedogenic organic matter (POM) could be the dominant transport group of compounds in various aqueous ecosystems, since it has been shown to persist in lakes and outlet of lakes without any major transformations (Zumstein & Buffle, 1989). The activity of DOC has principally been attributed to COOH and phenol-OH groups of soluble humic substances and several models describe the interactions between humic substances and Cu(II) (Sposito & Blaser 1992, Stevenson *et* al., 1993, Tipping *et al.*, 1995, Moolenaar *et al.*, 1998). However, in field experiments divergent correlation's between DOC, pH and metals are often reported. The difficulty in determining dominant complexation parameters can be attributed to diverse factors: competition between cations for complexation sites, variation in water sampling volumes due to preferential flows in the soil, ionic strength, meteorological changes (rainfall, temperature), biological activity and the heterogeneity of the DOC fraction itself.

Different leaching behaviours have also been observed between cations (Tyler, 1981; Keller *et al.*, 1991; Merrington & Alloway, 1994), which is due to their specific activity and affinity with the organic fraction. Investigations on Cu leaching in an alpine podzol indicated that Cu was correlated with insoluble organic matter and mobilisation with organic compounds < 1000 d (Keller and Vedy, 1991; Keller 1991).

Previous studies carried out on molecular interactions of Cu with phenols showed that catechol groups were the most active groups in complexation and that COOH groups only participate in keeping the molecules in a soluble form (Chapt.4 Oess et al., 1999(1); Chapt. 5 Oess *et al.*, 1999 (2)). This implies that only a fraction of the total organic matter could be active.

The aim of this study was to answer three questions: 1) Is there any seasonal parameter (rainfall, snow melting, soil temperature), which controls the translocation? 2) Is there any correlation between Cu and phenols in the soil solution? 3) What is the role of pH in these processes?

A sequence of three soils from the Arpette Valley (Mont-Blanc massive) developed on granitic parent material with increasing leaching morphologies was used for this study (chapter 1). The less differentiated soil was an RANKER ALPIN HUMIQUE (profile named Pierrier I) located at 2,165 m. The second soil of the sequence was an ORTHIC PODZOL (profile 1 and 2 named Barme II) located at 2,071 m and corresponding to a stage where podzolisation is active. This was exemplified by an intense stratification of the horizons. The last soil was an ORTHIC PODZOL with duripan character (profile named Barme I) located at 2,003 m and corresponding to a more advanced stage of podzolisation.

Lysimeters were installed under each profile. Percolating solutions were sampled from O and A-horizons during three years.

Materials and Methods

Sampling of the soil solution

Lysimeters were installed under each of the three profiles. Tension free lysimeters similar to the models used by Keller (1991), Atteia (1992), Della Piazza (1996) and Huber (1997) were used which permitted collection of the percolating water. A simple water collection device was used. A profile was dug perpendicular to the slope and 43 cm long PP plates (∅ 4.5 cm) were inserted in the soil parallel to the slope under the A and O horizons. Silicone tubes were connected on one side to the plates and on the other to 1 L and 500 ml polyethylene flasks. The whole device was then covered with aluminium foil in order to prevent photochemical reactions (figure 2). Finally, the flasks were covered with stones to keep them in the shade and at soil temperature. All the equipment had previously been rinsed with nitric acid (10%) and demineralised water. Figure 1, illustrates the set up on the different moraines and the number of replicates for obtaining the soil solution.

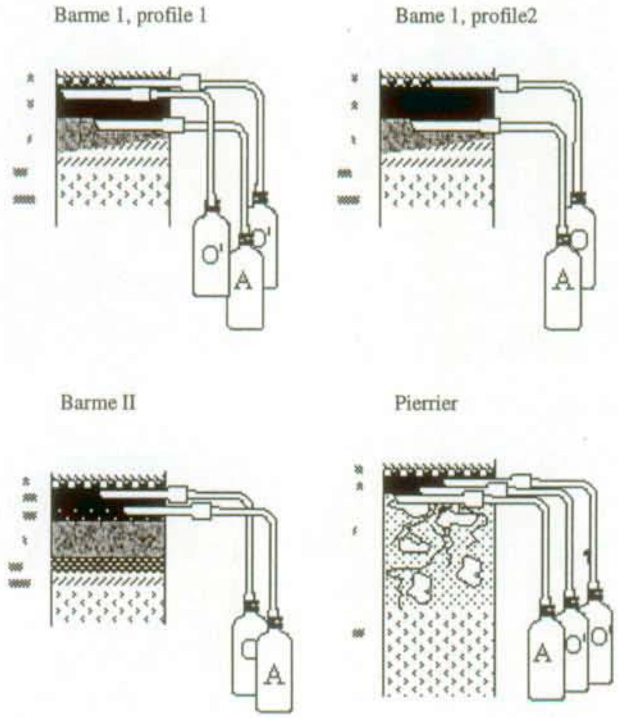


Figure1 lysimeter device installed for each profile on the topochronosequence

An *in situ* temperature device was installed on the Barme I and Pierrier. Grant Squirrel data loggers combined with three thermistor sensors were used to record simultaneously the temperature in the air, the soil and the soil solution.

Oxidative polymerisation of phenols is one of the most important mechanisms in the synthesis of humic substances (Konova, 1966; Stevenson, 1982). Reactions can be accelerated by the presence of inorganic components (Shindo, 1992; Adrian, 1986, McBride, 1990), enzymes (Bollag, 1983, Ruggiero, 1984), alkali (Bremmer, 1950) and of light (Al-Jalal, 1990). Therefore special care was taken in sampling. The solutions were collected after each rainfall event or at least once a week in the summer, but only two times during the whole winter period due to high avalanche dangers. The solution pH was measured on the site and the solutions acidified to pH 2.7 with 0.1M HCl in order to keep all the phenols in the protonated form, which is less reactive to polymerisation. The solutions were brought in a cooler to a freezer within a few hours.

Figure 2. Lysimeter device covered with aluminium and temperature sensors

The solutions were filtered on 0.45µm Nylon filters (Nylon Titon filtration system SRI). TOC was measured on a TOC-5,000 Shimazu Total Organic Carbon analyser. Cu was measured by Atomic absorption (Perkin Elmer 5100).

Total phenols were determined by the Folin Ciocalteux reagent. Gallic acid was used for the standard calibration. 1 ml of sodium bicarbonate 20% and 2 ml of Folin Ciocalteux reagent were added to 7 ml of the soil solution. UV detection was carried out 15 hours later at 760 nm.

The soil solutions originating from the same lysimeter were added together and freeze-dried in order to extract ODP's. The dried material was redissolved in 40 ml water and the monomers extracted with ether following the procedure of Gallet and Lebreton (1995). The monomers were quantified and identified by HPLC following the same method used for phenol identification in plants (Chapt. 3).

Results

Air, Soil and Soil Solution temperature

In order to determine if there were important climatic changes between the highest and the lowest sites temperature sensors were installed at Barme I (2.003 m) and Pierrier (2.166 m). The temperatures were recorded for the summer months of 1996 and 1998. The air temperature increased by more than 1°C per year. The effect was more pronounced at the Pierrier site, which increased from 9.1 to 12.5°C. The average soil temperature was 1.7°C higher at the Pierrier site despite the higher altitude. The difference was less marked in the soil solution (0.9°C). The Pierrier site was less "confined" in the valley and resulted in an increase in solar radiation. Long periods of temperature exceeding 20 °C occurred in the summer of 1998 in particular in August, which is quite unusual at this altitude. Measurements carried out during the winter of 97-98 also revealed that despite the cool air temperatures (-25°C), the soil and the soil solution remained just above 0°C. This was an indication that the solutions continue to percolate under the snow cover. The major factor controlling the freezing of the soil solution seems to be the temperature of the ground just before the first snow falls.

Water tables

No correlation between the important rainfall peaks observed in Monvoisin and the quantity of water collected in our lysimeters could be observed. Higher amounts of water were collected outside the important rainfall events and sometimes no water at all was collected during the rainfall period. This was mainly attributed to the heterogeneity of the soil phase, with a lot of rocks and roots contributing to preferential and random flow paths. Important variations of percolating water were observed between the lysimeters but there was always more water collected under the O horizon compared to the A-horizon. Comparing the rates between the three years higher quantities of water were collected in 1997 due to important snowfalls during the winter (1.70 cm snow were measured at la Barme in April 97 and more than 3 meters at the Pierrier moraine) and to regular rainfalls during that summer. The water fluxes varied in the following order Pierrier > Barme I > Barme II. This was attributed to higher precipitation at the higher sites mostly in the form of snow.

Seasonal trends

The peaks of Cu and total phenols were compared together with the quantity of water collected. As the background levels of Cu in Swiss soil solutions and streams is about 5 ppb, we chose the arbitrary value of 5 µg of detected metal per sampling for water volumes ranging between 100 ml and 1 L, as a migration peak to be interpreted. Phenol values above 2 mg per sampling event were referred to as a total phenol peaks.

Cu and phenol peaks

On the Barme I profile, in the solutions of the O-horizon, the first Cu peak appeared in September 1997 and was correlated to the volume of water, which was the highest in the lysimeters during that period. Similar quantities of water percolated through the A-horizon, but no significant Cu peak was detected. The second peak in the O horizon appeared in August 1998. No correlating factor other than a rise in the

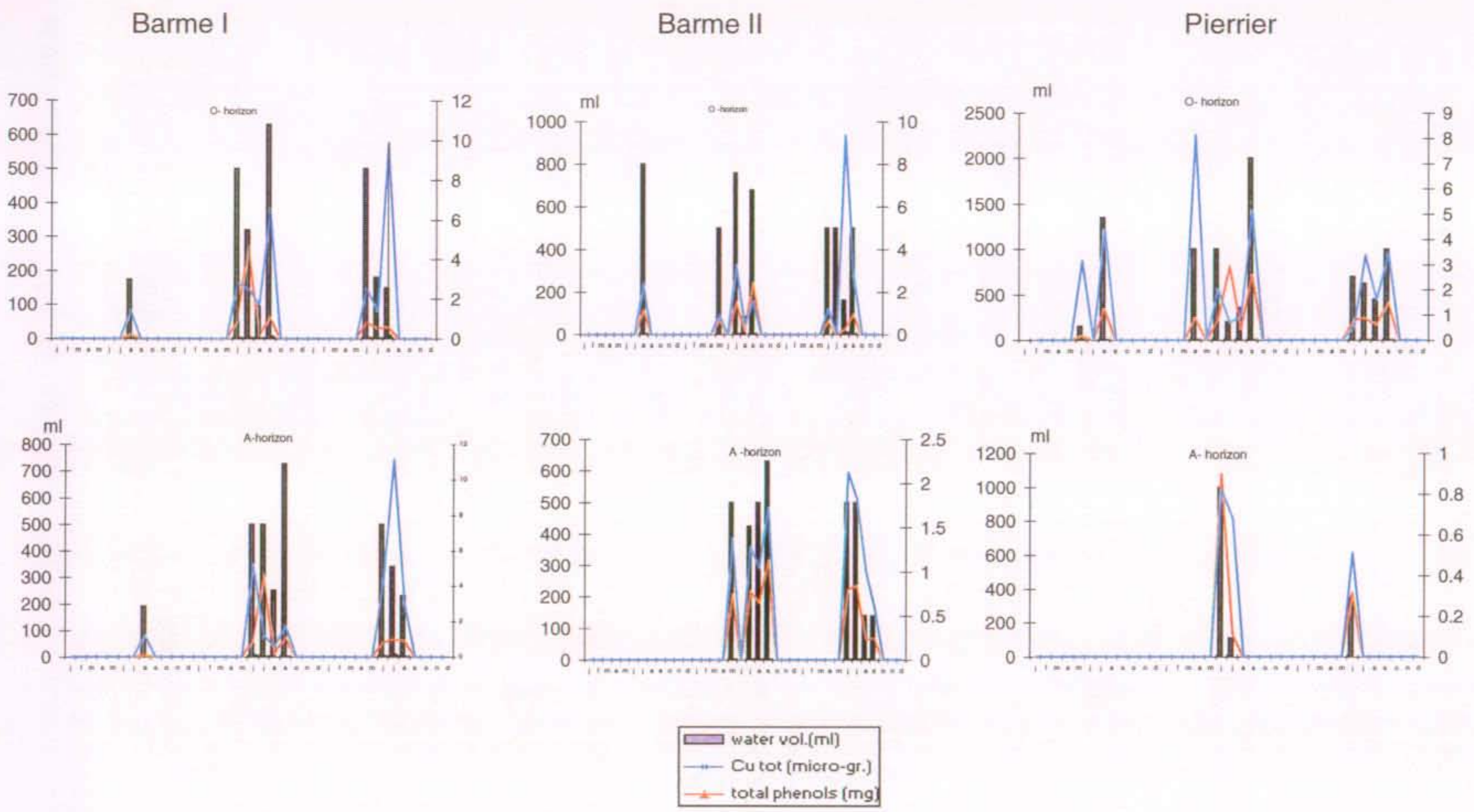


Figure 3. Seasonal variation of Cu, total phenols and percolating water in the lysimeters of the O and A horizons of three profiles (Barme I, BarmeII, Pierrier) between 1996, 1997 and 1998.

air temperature was noted during that period. Different peaks were observed in the solutions percolating from the A-horizon. The first peak appeared in June 1997, corresponding to the end of the snow-melting period and to a rise in pH in the soil solution (pH6). The second peak appeared in July 98 and no other correlating factor than an increase in temperature was observed.

On the Barme II profile, one single high peak appeared in August 98 and corresponding to an increase in pH and temperature. In the O horizon two weak peaks appeared, one in June and one in July 98, which were correlated with total phenols.

On the Pierrier profile a first peak appeared in April 97, which corresponded to the beginning of the snow melting period followed by a peak in September 97. Weak peaks appeared under the A-horizon in July 97 and 98, which also corresponded to high water quantities. Both peaks were correlated with total phenols.

Although important variations in the leaching patterns were observed, most of the Cu peaks appeared in July and August and more peaks were observed in 1997 and 1998 compared to 1996. The highest phenol peaks occurred in June and July and reached maximum values in 1997.

The maximum masses transferred per peak were highest on the Barme I moraine (10 µg Cu and 5mg total phenols), followed by the Barme II site (between 9 and 2 µg Cu and between 2 and 1 mg total phenols), and finally by the Pierrier site (between 8 and 3 µg Cu and between 3 and 1 mg phenols). The highest Cu peaks were not correlated with water volumes but rather with high temperatures.

DOC was highest in the Barme I profile in August for both horizons with maximums of 100 and 45 ppm, but the average concentration ranged between 10 and 20 ppm. On the Barme II profile DOC peaks appeared in September and the concentrations were similar to those of Barme I. At the Pierrier site the concentrations were significantly lower and no specific peak appeared during the year. No particular correlation trend between Cu and DOC was observed.

The pH in the solution was 0.5 to 1 units higher in the soil solution compared to the soil. No significant correlation between DOC and the solution pH was observed. An increase in the pH was rather correlated with high water fluxes.

Total Cu and phenol leaching

The total quantity of Cu and phenols leached through the O and A-horizons between the years of 1996, 1997 and 1998 are presented in Table 1. The fluxes of Cu in the O horizon ranged between 0.08 and 0.95 mg/m^2 per year. The quantities differed within and between stations and years and were lowest in 1996, which corresponded to the year with the least rainfall events. But the sum over the three years remained fairly constant (between 1.13 and 1.82 mg/m^2). The highest fluxes were observed at the Barme station.

The fluxes decreased in the A-horizon in all profiles. The difference was more pronounced for soils for the younger soils.

No ODP's were identified in the soil solution despite pre-concentration. This was attributed, to a lower biological activity at these altitudes and to a higher mineral content in the surface horizons, which can cause the polymerisation and subsequent insolubilisation of phenols in the humus fraction of the soil. Total phenol analyses carried out by the Folin-Ciocalteux method directly on the soil solution were just beyond the limit of detection but presented the advantage of not requiring a pre-concentration procedure. Total phenols fluxes ranged between 0.05 and 2.99 g/m^2 in the O horizons. The fluxes were slightly lower in the A-horizon. The molecular ratio was over hundred times higher for phenols compared to Cu. This indicated that the ideal phenol-Cu complexation ratios (1:4) were respected and one could expect in ideal conditions to have all the Cu in the complexed form (chapter 3). Between stations fluxes varied in a similar trend as for Cu. Less phenols percolated through the younger soils with decreasing leaching morphologies. There was a strong correlation between phenol migration and the phenol content in plants.

Cu. (mg/m^2)

O horizon

year	BI profile 1	BI profile 2	BII	Pierrier
96	0.78	0.08	0.12	0.39
97	0.95	0.70	0.32	0.53
98	0.09	0.71	0.69	0.31
total	1.82	1.49	1.14	1.22

A horizon

year	BI profile 1	BI profile 2	BII	Pierrier
96	0.20	0.06	0.00	0.00
97	1.30	0.46	0.28	0.08
98	out of order	0.96	0.29	0.03
total	1.50	1.48	0.57	0.10

phenols (g/m^2)

O horizon

year	BI profile 1	BI profile 2	BII	Pierrier
96	0.05	0.01	0.06	0.07
97	2.99	0.35	0.25	0.23
98	0.01	0.10	0.10	0.11
total	3.05	0.47	0.41	0.42

A horizon

	BI profile 1	BI profile 2	BII	Pierrier
year	0.09	0.00	0.00	0.00
97	0.24	0.35	0.17	0.05
98	out of order	0.14	0.11	0.02
total	0.34	0.50	0.28	0.07

Table 1. Total fluxes of Cu and phenols between 1996 and 1998

Correlation with plant and soil contents

Cu transferred through the soil solution was compared with Cu distribution in plant and soil determined in Chapter 1.The average Cu concentration in plants remained constant between the three moraines and varied between 7 and 10 mg/kg. The accumulation pattern varied considerably between the three soils (Figure 4).

The highest Cu concentration (173 mg/kg) was observed in the O and A-horizons at the Barme I site and decreased with depth. It indicated a recent Cu contamination probably due to atmospheric inputs.

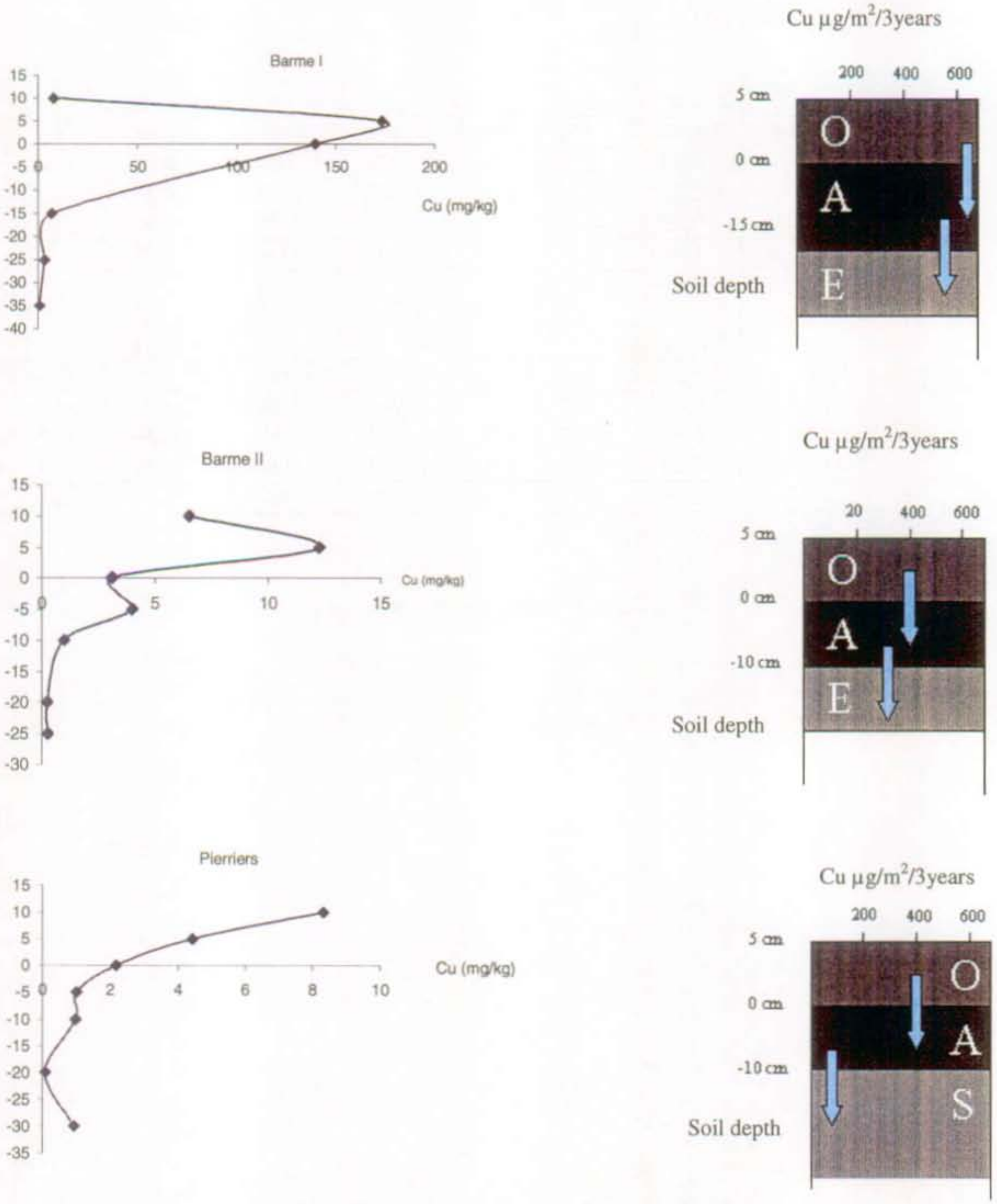


Figure 4. Cu accumulation in the vegetation cover (10cm) and through the soil profile on the left and the corresponding Cu transfers (mg/m^2) over the three years of study on the right.

At the Barme II moraine, the concentrations were tenfold lower in the upper horizons compared to the Barme I profile. The highest concentrations were observed in the O horizon (12.3 mg/kg), which decreased in the-A horizon to reach 3 mg/kg. A little peak (4 mg/kg) was noticed in the E-horizon.

In the Pierrier moraine concentrations were highest in the plants and decreased progressively with depth.

Both in the A- and O-horizons mass transfer of Cu increased in the following pattern: Barme I > Barme II> Pierrier Barme. The fluxes of Cu in the soil solution were strongly correlated to the content of Cu in the surface horizons. The fluxes from the O to the A horizon were always slightly higher compared to the fluxes from the A to the E or S horizon. The highest difference of fluxes between horizons was observed for younger and less polluted soil (Pierrier).

Conclusion

Different patterns of accumulation and migration of Cu was observed for the three soils, which corresponded to increasing stages of podzolisation. The different soil properties associated with the chronosequence were probably responsible for the particular distribution of Cu in the deeper horizons. In the surface horizons and in the soil solutions sampled under O and A, the variations of Cu content were rather caused by differences in atmospheric inputs than by differences in soil properties.

The major factor controlling Cu retention in these soils was attributed to its high affinity with organic insoluble compounds present in the O- and A-horizons. The constant levels of Cu observed in plants, which was below 10 µg/kg despite the variations of content in the surface horizons, indicated that a recycling of Cu by *Vaccinium sp.* or Rhododendrons is weak. The co-migration of Cu and total phenols in the percolating soil solution indicated that complexation with soluble phenol-type vectors is an important step in Cu translocation. The other parameters observed as for instance rainfall events, the snow-melting period, the amount of water in the lysimeters, soil temperature, all contributed in turn to the release of Cu in the soil solution, but none of these factors had a predominant influence on the leaching of phenols or Cu.

In consequence we can say that the dynamic of Cu exchange between the insoluble organic fractions and the hydrosoluble phenolic fraction of our soils, is the determining factor controlling the retention or release of Cu in our soils. Further studies about the mechanisms involved in this exchange should give new insights on the comprehension of these processes.

References:

Adrian P., Andreux F., Metche M., Mansour M., Korte F., Lavollay J. 1986. Autoxydation Des Ortho-Diphénols Catalysée Par Les Ions Fe2+ Et Mn2+: Un Modèle De Formation Des Acides Humiques. C.R. Acad. Sc. Paris, T. 303, 1615-1618.

Al-Jalal, N. 1990. The Synthesis Of Azocine Derivatives By The Cycloaddition Of Phenols To 1-Naphtonitrile Of Phenols To 1-Naphtonitrile And 4-Methoxy-1-Naphtonitrile. Journal Of Photochemistry And Photobiology. A. 1990 vol.54 (19):99.

Atteia O. 1992. Rôle du Sol dans le Transfert des Elements traces en solution. Application à l'étude de quelques Ecosystèmes d'altitude. Thèse EPFLn°1031, CH-1015 Lausanne.

Bollag, J.M. 1983. Synhetic Reactions Of Aromatic Compounds By Fungal Enzymes. American Society For Microbiology, Washington. pp203-207.

Bremner J.M. 1950. Some Observations On The Oxidation Of Soil Organic Matter In The Presence Of Alkali. Journal of Soil Science1 (2):198-204.

Buffle J. 1988. Basic Principles Of Metal Reactivity In Water. *In* Complexation Reactions In Aquatic Systems. Ellis Horwood, pp47-89.

Buffle J. and Van Leeuwen H.P. 1992. Environmental Particles. Volume 1. Lewis Publisher, pp554.

Della Piazza R. 1996. Géochimie des Altérations dans Trois Ecosystèmes Sol Tempérés: Application à l'Acquisition des Caractéristiques Chimiques des Solutés. Thèse EPFL N°1483, CH-1015 Lausanne.

Gallet C. and Keller C. Phenolic Composition of Soil solutions: comparative study of Lysimeter and centrifuge waters. Accepted for publication in Soil Biology and Biochemistry in 1999.

Gallet C. and Lebretton, P.1995. Evolution Of Phenolic Patterns In Plants And Associated Litters And Humus Of A Mountain Ecosystem. Soil Biology And Biochemistry. 27(2):157-165.

Huber K. 1997. Altération Minérale et Transferts de Matière Dans des Sols Issus de Moraines Acides d'Âge Croissant (Chronoséquence) des Alpes du Sud. Thèse EPFL n°1714.

Keller C. 1991. Etude du Cycle Biogéochimique du Cuivre et du Cadmium Dans Deux Ecosystèmes Forestiers. Thèse N°916 EPFL, CH-1015 Lausanne.

Keller C. and Vedy J.C. 1991. Apport De La Lysimétrie Sans Tension Pour L'étude Des Transferts De Cu Et Cd dans les Sols Forestiers Faiblement Pollués. Science Du Sol.29(2): 107-124.

Keller C., and Domergue F-L. 1996. Soluble and Particulate Transfers of Cu, Cd, Al, Fe and Some Major Elements In Gravitational Waters of Podzol. Geoderma, 73:263-274.

Keller, C. and J.-C. Vedy .1994. Distribution of Cu and Cd Fractions In TwoForested Soils. J. Environ. Qual. 23: 987-999.

Konova M.M. 1966. Soil Organic Matter, Pergamon, Oxford.

McBride M.B. & Sikora F.J. 1990. Catalysed Oxidation Reactions of 1,2-Dihydroxybenzene (Catechol) in Aerated Aqueous Solutions. Journal of Inorganic Biochemistry 39:247-262.

Merrington G. and Alloway B.J. 1994.The flux of Cd, Cu, Pb and Zn in Mining Polluted Soils. Water, Air, and Soil Pollution 73:333-344.

Moolenaar S.W., Temminghoff E.J.M., and De Haan F.A.M. 1998.Modeling Dynamic Copper Balance for A Contaminated Sandy Soil Following Land Use Change From Agriculture To Forestry. Environmental Pollution, 103: 117-125.

Morel F.M.M. and Hering J.G. 1993. Principles And Applications Of Aquatic Chemistry.Wiley Interscience.

Oess A., Cheshire M.V., McPhail D.B., and Vedy J.C. 1999. Polymerization: a Possible Consequence of Copper-phenolic Interactions. Ed. Berthelin et al.Plenum publisher, London.

Oess A., Cheshire M.V., McPhail D.B., Stoll S., El Alaili M., and Vedy J.C. Elucidation of Phenol-Cu Interaction Mechanisms by Potentiometry, ESR, UV Absorption Spectroscopy and Molecular Simulations. The Science of The Total Environment, 228:49-58.

Oess A., Cheshire M.V., Spack L., and Vedy J.C. accepted for publication in Analusis 1999. Interaction of plant and soil phenol with Cu pollution.

Ruggiero P. and Radogna V.M. 1984. Properties of Laccase In Humus-Enzyme Complex. Soil Science 138:74-87.

Shindo H., and Kuwatsuka S. 1977. Behavior of Phenolic Substances In The Decaying Process Of Plants. Soil Sci. Plant Nutr. 23:185-193.

Sposito, G. and Blaser P. 1992. Revised Quasiparticle Model of Protonation And Metal Complexation Reactions. Soil Sci. Soc. Am. J. 56 (4):1095-1099.

Stevenson F.J. 1982. Biochemistry of The Formation of Humic Substances: Sources of Polyphenols. Humus Chemistry, John Wiley & Sons pp206-216.

Stevenson F.J., Fitch A. and Brar S. 1993. Stability Constants of Cu(II)-Humate Complexes: Comparison of Select Models. Soil Science155 (2):77-91.

Stumm W. 1996. Aquatic Chemistry, Chemical Equilibria And Rates In Natural Waters. Environmental Science and Technology, Third Ed.Wiley Interscience, 1022 pp.

Tipping E., Fitch A., and Stevenson F.J. 1995. Proton And Copper Binding By Humic Acid: Application of A Discrete Site/Electrostatic Ion-Binding Model European Journal Of Soil Science.46:95-101.

Tyler G. 1981.Leaching of Metals From The A-Horizon of A Spruce Forest Soil Water, Air And Soil Pollution.15:353-369.

Wolt J. 1994. Soil Solution Chemistry: Application To Environmental Science And Agriculture. Wiley & Sons, Inc.

Zumstein J. and Buffle J. 1989. Circulation Of Pedogenic And Aquagenic Organic Matter In An Eutrophic Lake. Wat. Res. 23:229-239.

Chapter 3

Quantification of plant phenols of a sequence of podzols and interaction with Cu

Oess, M.V.Cheshire**, L.Spack*, & J.C. Vedy*. Interaction of plant and soil phenol with Cu pollution. Accepted for publication in Analusis, June 1999.*

Abstract

Les plantes *Vaccinium sp.*, *Rhododendron ferrugineum* ainsi que les mousses sont dominantes sur les moraines de Barme I, Barme II, et Pierriers. La quantité d'o-diphénols présente dans les plantes et susceptible d'être libérée à la surface du sol chaque année a été dosée par HPLC. Les teneurs en acide caféique, catéchol et protocatéchique provenant des feuilles varient entre 50 et 250 mg/m^2. L'impact causé par la pollution du Cu sur ce type d'écosystèmes, a été étudié par le biais de la spéciation chimique. Des solutions phénoliques de synthèse contenant des concentrations croissantes en Cu ont été titrées par potentiométrie alcaline. Les résultats ont montré d'une part, que la complexation ODP-Cu augmente avec le pH et d'autre part qu'un rapport ODP:Cu de (4 :1) était nécessaire pour que tout le Cu soit complexé dans la solution.

Vaccinium sp., *Rhododendron ferrugineum* and mosses are the dominant plant groups present on the moraine soils of Barme I, Barme II and Pierrier in the Arpette valley. The amount of o-diphenols (ODP) available in plants and susceptible to be released each year to the soil surface was determined by HPLC. The quantity of caffeic acid, catechol and protocatechuic acid present in the leaves ranged between 25 and 250 mg/m^2. The impact caused by Cu pollution on this type of ecosystems was studied by a chemical speciation approach. Potentiometric alkaline titrations were carried out on synthetic phenol solutions containing increasing Cu concentrations. Cu complexation increased with an increasing pH, but in order to have all the Cu in the complexed form a minimum ratio of (4:1) ODP-Cu in the solution was necessary.

Introduction

High Cu contents exceeding by tenfold the values recommended by the Swiss legislation (Osubst, 1999) are commonly found in Swiss soils of the lemanic basin. They are mainly due to the extensive use of Cu containing fungicides for the treatment of vineyards and orchards, wastes from the watch industry and the construction field, which uses Cu for the fabrication of roofs and gutters. Unpolluted sites become more and more restricted to the confined mountainous and alpine areas. The only exogenous input of pollutants in alpine valleys comes from atmospheric inputs and so far these inputs are not decreasing. In order to study the impact of Cu pollution on these "natural" sites, we chose to focus our study on chelating agents, which would control the fate of Cu in the soil and soil solution. Hydrosoluble phenols were selected for our study because they are less prone to degradation than other metabolites, as sugars and amino acids, and have a longer life in soils (Stevenson, 1982). A previous study carried out on a range of phenolic compounds bearing different substituents showed that the strongest interaction between Cu and phenols was obtained with o-diphenols (ODP) (Oess et al., 1997). So far, the quantity of these phenols released to the soil by different plant types and their interaction with increasing Cu concentrations is not known. The aim of this study was to determine the impact of Cu pollution on the ecosystem by focusing on the chemical speciation properties of the metal. A two-scale approach including a landscape and a molecular scale was considered. Phenols from different plant species were characterised *in situ* on three different moraine soils in order to elaborate an ODP map of the vegetation. Cu speciation was studied with pure synthetic ODP solutions under controlled temperature and pH conditions.

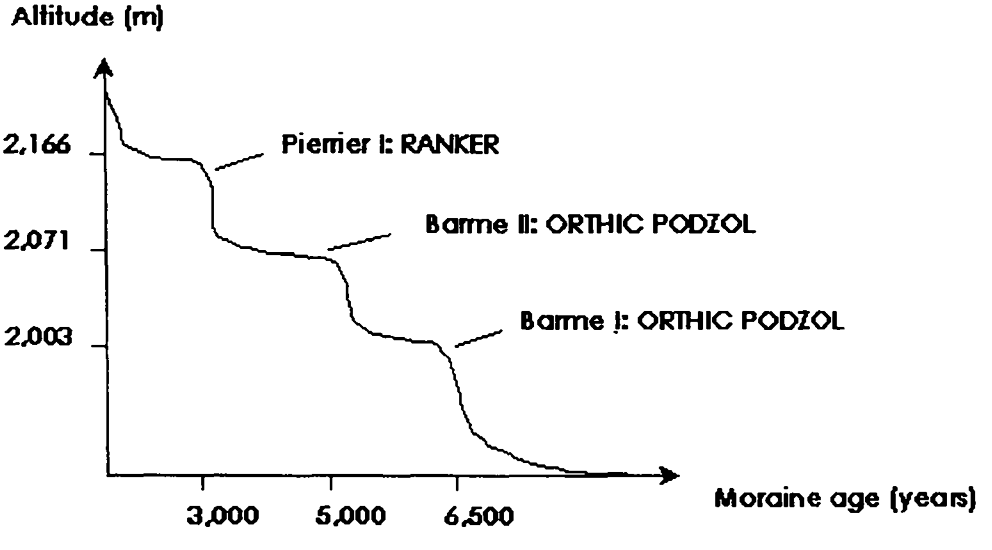


Fig.1. Topochronosequence of the three youngest moraine soils of the Arpette valley

Site description: Alpine Soils and plants

A study site remote from all industrial activities was needed for our study. The Arpette Valley, which was located 15 km south of Martigny, corresponded to this criterion. Three land plots developed on granitic moraines (Barme I, Barme II, Pierrier I) between the altitudes of 2003 and 2166 m on the N-E transect, were delimited (Figure 1). The soils were rankers, and orthic podzols (Référentiel pédologique, 1995) with different intensities of weathering and acidification. At 2,000 m the average annual temperature was 4.5°C, the rainfall greater than 2m and the snow cover persisted during 6 to 8 months per year. A delay in snow melt of about 1 month was observed between the Barme I and Pierrier I plots, due to an elevation difference of 163 m. The main plant associations were subalpine dwarf shrubs such as *RhodoretoVaccinietum* and *Junipero-Arctostaphyletum uva-ursi* (Richard, 1975; Bütler and Domergue, 1997)

Plant biomass and species on the moraines

To establish a phenolic map of the vegetation we proceeded in three different steps. Firstly, we identified the plant species on each selected land plot, secondly we determined their relative abundance, and finally we analysed ODP's in each plant. Land plots of 25 m^2 were delineated on each moraine and the plants identified in August and September 1995, 1996, and 1997. The relative abundance of each species was determined on 25m^2 plots by the normalised method of Braun-Blanquet (1964). The potential mass of plants deposited on the soil each year, was determined from the dry weight on 1m^2 plots for 1995 and 1997. The plants were harvested in August and September by cutting them off at the root and separating them by species. Mosses and lichens were also collected.

Plants	Barme I (dry weight, g)		Barme II (dry weight, g)		Pierriers (dry weight, g)	
	1995	1997	1995	1997	1995	1997
Rhododendron sp.	172	114.8	142	89.5	63	18.2
Vacc. sp.	205	376.13	197	157.95	277	4.35
Herbaceous	13	20.08	13	17.5	30	18
Lichens	0	0	0	2.6	18	5.45
Mosses	241	244.4	202	571.8	31	34.5
Empetrum h.	0	0	80	83.5	50	121.6
Lycopodium a..	79	0	0	0	0	0
Total plants	710	755.41	634	922.85	469	202.1

Table 1. Biomass contribution for each plant group after harvest on 1 m^{2}.

The number of plant species increased from Barme I to Pierrier I, thus increasing with altitude. The abundance-dominance coefficients decreased with altitude. They were highest for *Rhododendron ferrugineum* and *Vaccinium sp.* with coefficients ranging between 3 and 4, and decreasing to 2 on the ranker soil of Pierrier as they were replaced by herbaceous species. Table 1, shows the contribution in biomass of the different plant groups on the three different moraines. *Vaccinium sp.*, *Rhododendron ferrugineum* and the mosses contributed to the highest biomass values. The total biomass decreased from Barme I to Pierrier I, thus corresponding to a transition from a dominance of shrubs to an increase in herbaceous plants, which individually weigh less. This transition was attributed to differences in soil properties, which occur between a podzol and a ranker, but also to the adaptation of plants to cooler temperatures and a longer duration of snow cover. A decrease in moss and an increase in lichen biomass were also observed with increasing altitudes, but the contribution from lichens was negligible. Between 1995 and 1997, the biomass contribution was very similar on the Barme I moraine where the vegetation covers more than 80 % of the soil and looks homogeneous. On the higher plots, the difference was more significant. On the Pierriers moraine, such discrepancies were attributed to a distribution of the vegetation in tufts and a portion of naked soil reaching 25%. For the Barme II station, where the vegetation cover reached almost 100 % and looked homogeneous, climatic and topographic reasons could explain the differences observed. Taking a closer look to the shade curves caused by the surrounding peaks, we noticed that the sun penetration was much higher at this particular site, and therefore could cause fluctuations in the C fixation rate upon important changes in the meteorological conditions.

Phenol analysis

As the abundance-dominance coefficients and the biomass results showed a predominance of the *Vaccinium sp.*, *Rhododendron ferrugineum* and mosses, the other plant groups were discarded for the ODP analysis. The procedure of Gallet & Lebreton (1995) was followed to extract phenols from the leaves and adapted to our high performance liquid chromatography (HPLC) system. After crushing the leaves, 2 g of powder were hydrolyzed in 160 ml 2M HCl during 45 min. at 100°C. Extractions were then carried out with 3 volumes of ether (60 ml, 60 ml, 40ml) in order to isolate the hydrosoluble monomers. The ether was evaporated on a rotavapor at 40°C and a known volume of water not exceeding 30 ml was added. The aqueous phenol solution was analysed with an 1100 HPLC system from Hewlett Packard coupled to an UV detector (270nm). 5µl were injected in a C18-column (Vydac TP54: 250 mm, 46mm, 5µm). The following solvents were used: A: H_2O, B: acetonitrile, C: trifluoroacetic acid. A linear gradient was used increasing from 0% to 20 % B in 80% C followed by a return to the initial conditions in 15 min. The phenolic compounds were identified and quantified by an external standard method using pure caffeic acid, catechol, gallic acid, protocatechuic acid and pyrogallol as reference products. Purity test was carried out with a diode array system.

The total ODP content was two times higher in *Vaccinium sp.* than in *Rhododendron ferrugineum* thus indicating a higher metabolic activity of these phenols in *Vaccinium sp.* Caffeic acid and protocatechuic acid were the most abundant ODP's. Gallic acid and pyrogallol concentrations were too low to be detected. Catechol was present in *Vaccinium sp.* but not in *Rhododendron ferr.* In the mosses only protocatechuic acid was identified and at a much weaker concentration. Comparing the phenol concentrations on the different moraines (Graph 1 and 2), a decrease in concentration with altitude was observed for all phenols, except for caffeic acid, which presented an extremely high value at the Barme II moraine.

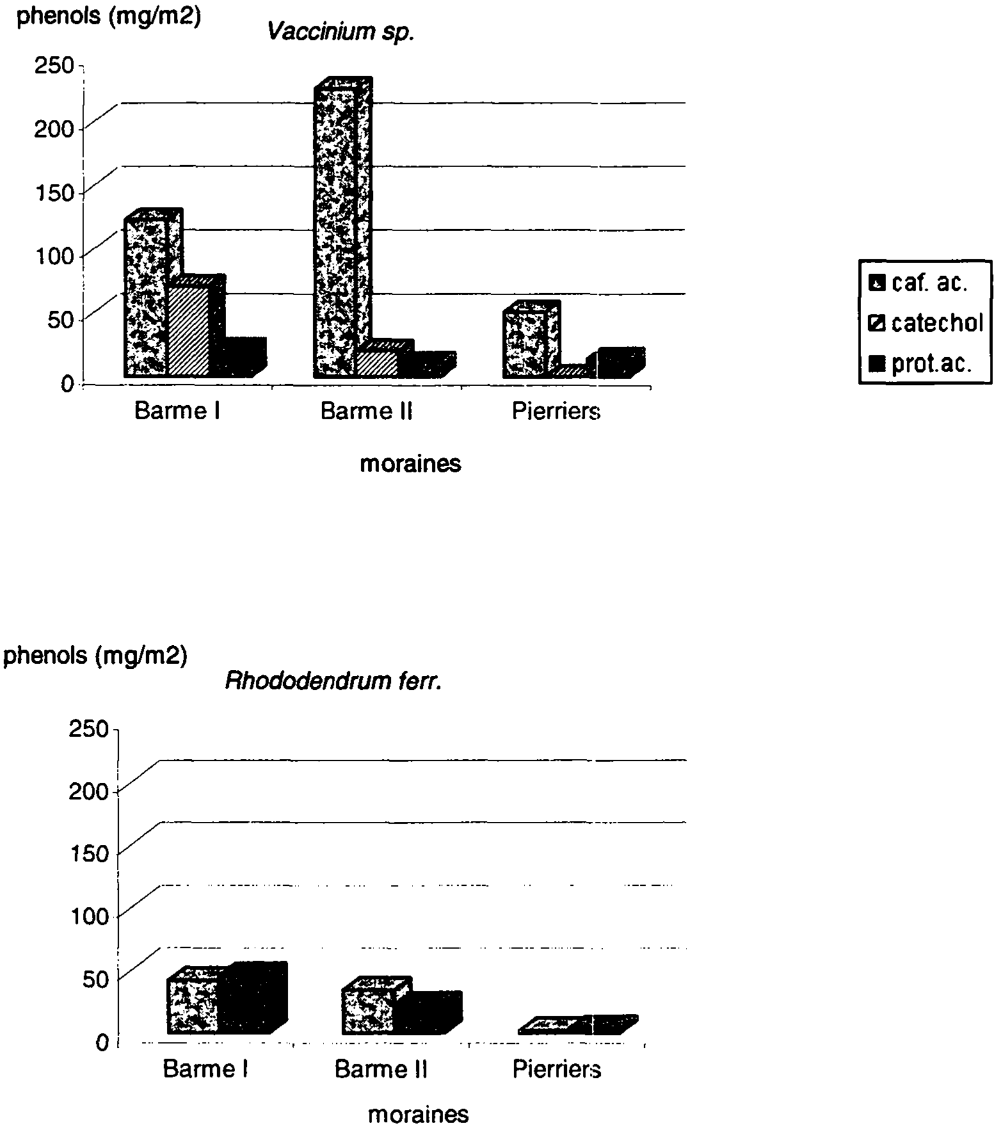


Figure 2. Phenol abundance on the different moraines of the Arpette valley site as extracted from *Vaccinium sp.* and *Rhododendron ferrugineum* on 1m^2 plots.

pH and Cu speciation

Alkaline potentiometric titration was a simple, rapid and reliable method. It permitted to follow Cu complexation with phenols continuously over a whole range of pH. The principle of this method was based on the detection of a shift in the titration curve in response to the release of protons following ODP-Cu interaction. Synthetic phenol molecules obtained from Sigma with a purity degree of 99% were used. Solutions containing 10 mM phenol and 2.5, 10 and 50 mM $CuSO_4$ corresponding to (4:1), (1:1) and (1:5) phenol-Cu ratios were titrated with 0.1 M NaOH using a Metrohm titroprocessor 686 equipped with a dosimeter 665 and a pH electrode.

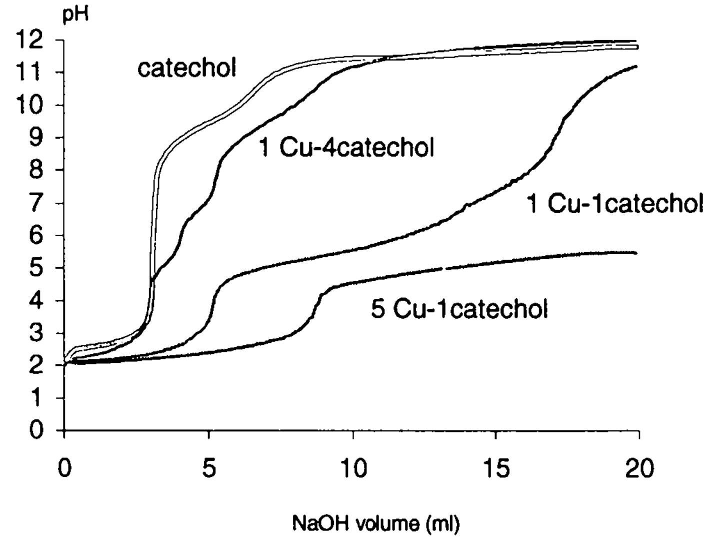


Figure 3. pH complexation domain with increasing Cu concentrations

Figure 3, shows the titration curves of catechol alone and catechol in the presence of increasing Cu concentrations. The shift in the "4 catechol-1Cu" curve indicated that complexation took place between pH4.5 and 11. Two additional shoulders appeared in this curve. They were attributed to the presence of two complex species: a mono-bisdentate complex at low pH and, above pH7, to a di-bisdentate complex. For the "1catechol:1Cu" curve and the "1catechol:5Cu" curve, the shifts indicated a complexation taking place at an even lower pH. The extra shoulders did not appear indicating that only the mono-bisdentate complex was present.

These results where then compared with the phenol-Cu ratios obtained in the field, in order to make predictions on the complexation behaviour. In the Arpette valley, the background level of Cu ranged between $0.4*10^{-8}$ and $5.6*10^{-8}$ mmoles/g. As the phenol concentrations measured on Barme II was 0.045 mmoles/g, an ODP-Cu ratio higher than (1million: 1) was obtained. For the pH range of measured in the soil solution (pH 4 to 6) Cu would be in mostly be in the bound form. On the other hand, if the Cu concentration increased to reach the values recommended by the Swiss legislation (Osubst, 1997), which are 0.159 mmoles/g dry material for composts and 0.93 mmoles/g for sewage sludge, the ODP-Cu ratio would be inverted and reach (1:3.5) and (1:21) respectively. In this case, for the same pH range most of the Cu would be in the free form.

Conclusion

ODP's can make strong interactions with Cu ions. The type and the quantity of these phenols released to the soil depended mainly on the plant species present and on the pedo-climatic properties of the site. *Vaccinium sp.* released the highest amount of ODP's on the Barme II moraine with 250 mg/m^2 and caffeic acid was the most abundant ODP. Complexation of ODP's mainly depended on the initial phenol concentration, on the pH and on the ODP-Cu ratio in the solution. When the solution was saturated in phenols, a single ODP molecule was bound to Cu at low pH, and two ODP's were bound at pH above 7. Predictions were made for the field complexation, nevertheless additional parameters would have to be considered, in order to extrapolate these results. In natural solutions other metals and organic ligands are present and may compete and alter the complexation properties and thus the fate of Cu in the soil phase. Phenols also continue to react and can either disappear or increase in the aqueous medium due to microbial or chemical reactions. At high pH complexation is followed by a polymerisation of phenols, which in turn leads to a precipitation of Cu (Oess *et al.*, 1997). At the opposite, at low pH, even bound to low MW organic ligands, Cu will remain in the soluble phase and thus be able to migrate in soils.

References

Baize D. & Girard M.C. A.F.E.S. 1995. Référentiel pédologique, INRA, Paris.

Braun-Blanquet J. 1964. Pflanzensoziologie : Grundzüge der Vegetationskunde. Springer, Wien.

Bütler R.& Domergue F.L.1997. Vegetation and Soils on a granitic moraine in the subalpine zone described by indicator values. Rev. Ecol. Alp. Grenoble, tome 4, p1-12.

Gallet C.& Lebreton P. 1995. Evolution of phenolic patterns in plants and associated litters and humus of a mountain forest ecosystem. Soil Biol. Biochem. Vol.27, N°2, pp 157-165.

Oess A., Cheshire M.V., McPhail D.B., and Vedy J.C. 1999. Polymerization: a Possible Consequence of Copper-phenolic Interactions. Ed. Berthelin et al.Plenum publisher, London.

Oèss A., McPhail D.B., Cheshire M.V. & Vedy J.C. Copper complexation with plant derived phenols originating from an alpine ecosystem. 1997. Fourth International Conference on the biogeochemistry of Trace Elements. Extended abstract.

Osubst. 1997. Ordonnance sur les substances dangereuses pour l'environnement. Chancellerie Fédérale, 3000 Berne, Suisse.

Richard L.1975. Le milieu et la végétation, étude écologique des massifs des Aiguilles Rouges, au Haut-Griffre et des Aravis (Haute Savoie), Annales du Centre, Université de Savoie:1-100.

Stevenson, F.J. 1982. Humus chemistry; Genesis, Composition, Reactions, Wiley-Interscience, NewYork, 443 p.

PART II

MECHANISTIC APPROACH

Chapter 4

Cu Complexing Phenols and Polymerisation

A. Oess, M.V. Cheshire, D.B. Mcphail & J.C.Vedy. Polymerisation : a possible consequence of Cu phenol interactions. Accepted for publication In Effect of Mineral-Organic Microorganism Interactions on Soil and Freshwater environments. Ed. Berthelin. March 1999.

Abstract

Une quantité considérable de substances phénoliques est libérée à partir des couvertures végétales en sols acides *(Rhus ferrugineum, Vaccinium myrtillus, Calluna vulgaris)* vers la solution de sol.

Dans des conditions de pH et de potentiel redox appropriées, ces substances peuvent réagir avec les métaux traces et participer à leur transfert intrapédique. La spectroscopie RPE (résonance paramagnétique électronique) a été utilisée pour étudier les interactions Cu(II)-phénol à différents pH et les conséquences du cuivre sur la polymérisation des phénols.

A faible pH et en absence de complexes organiques, le spectre ESR du Cu(II) en solution comporte un simple pic pour lequel la structure fine est non résolue. Cela est dû à l'espèce Cu(H2O)$_6^{2+}$ qui diminue lorsque le pH augmente et disparaît à pH 7 par précipitation du Cuivre sous forme Cu(OH)$_2$. A pH>12, apparaît un signal composé de quatre lignes hyperfines bien résolues qui sont attribuables au complexe soluble Cu(OH)$_4^{2-}$.

En présence de catechol (1,2-dihydroxybenzène) et à pH>5, le spectre RPE indique la formation de complexes de Cu de faibles poids moléculaires. La solution initiale reste incolore. A pH 12, un signal correspondant à un radical organique libre a pu être détecté en plus du signal de cuivre. Après 5 heures, les solutions à pH 4.2 deviennent brunes et celles à pH>5 noires. A pH 5, le signal isotrope de Cu(II) est remplacé par un signal anisotrope indiquant un changement de liaison de Cu(II). Les composés organiques de faibles poids moléculaires étant remplacés par des composés de poids moléculaires plus élevés, conséquence probable de la polymérisation du catéchol. En absence de Cu2+, on n'observe pas de noircissement des solutions.

En présence d'acide protocatéchique (acide 3,4-dihydrobenzoïque), le cuivre forme des complexes aux pH supérieurs à 5 et un signal, dû à la formation d'un radical libre, est détectable. Au dessus de pH 7, les solutions deviennent immédiatement jaunes et, après 48 heures, celles à pH>9 deviennent oranges. Contrairement au sytème "catechol", le signal du complexe isotrope de Cu(II) persiste, même après une semaine et bien que le signal du radical libre ait disparu.

La différence de comportement entre le catechol et l'acide protocatéchique en présence de cuivre est discutée en fonction de leur différence structurale.

Abstract

In fluid solution the ESR spectra of copper(II) at low pH in the absence of organic complexes comprises a single peak in which hyperfine structure is unresolved. This originates from the species $Cu(H_2O)_6^{2+}$, which diminishes as the pH is raised and disappears at pH 7 due to the formation of $Cu(OH)_2$, which precipitates. At pH>12 a signal comprising of four well-resolved hyperfine lines appears which is attributable to the soluble complex $Cu(OH)_4^{2-}$.

In the presence of catechol (1,2-dihydroxybenzene) the ESR spectra indicated the formation of a low molecular weight Cu(II) complex at pH > 5. The solution initially remained colourless. At pH 12, in addition to the copper signal, an organic free radical signal could be detected. After 5 hours, solutions at pH 4.2 turned brown and those with pH> 5 had turned black. At pH 5, the isotropic Cu(II) signal is replaced by an anisotropic signal indicating a shift in Cu(II) binding from lower molecular weight to higher molecular weight organic components, a likely consequence of catechol polymerization. In the absence of Cu^{2+}, no blackening of the solution occurred.

In the presence of protocatechuic acid (3,4-dihydroxybenzoic acid), copper formed complexes above pH 5 and an organic free radical signal could also be detected. At pH > 7 the solutions immediately turned yellow and after 48 hours, those above pH 9 turned orange. In contrast to the catechol system, the isotropic Cu(II) complex signal persisted even after a week although the free radical signal had by then disappeared. The difference in behaviour of catechol and protocatechuic acid in the presence of copper is discussed in terms of their structural difference.

Introduction

Although many studies have been carried out on copper interactions with humic and fulvic acids, little is known about its interactions with phenolic compounds. In Alpine podzolic systems considerable amounts of phenols are released from the acidophilic vegetation cover (*Rh. ferrugineum, Vacc. myrtillus, Calluna vulgaris*), together with other primary and secondary metabolites present in leaves (Vaughan and Malcolm 1985). All year-round, and in particular during the period of senescence, these phenols are leached to the soil where they can participate in the humification processes. This study shows that under appropriate pH and redox conditions phenols interact with metallic cations and, therefore, may play a role in their intrapedic transfer. Moreover, the change in redox state as a result of Cu-phenolic interactions may modify the phenol stability itself and provoke the polymerisation of phenolic monomers.

The aim of this study was to apply electron spin resonance (ESR) spectroscopy to: 1) Identify which phenols are able to form complexes with Cu(II) and 2) Determine the effect of copper on phenol polymerisation processes. Since the chemical structure and, in particular, the position of the hydroxyl groups on the benzene ring are important in these processes, a range of phenols which have previously been identified in the vegetation cover (Gallet 1992) were selected for this experiment: (Fig.1)

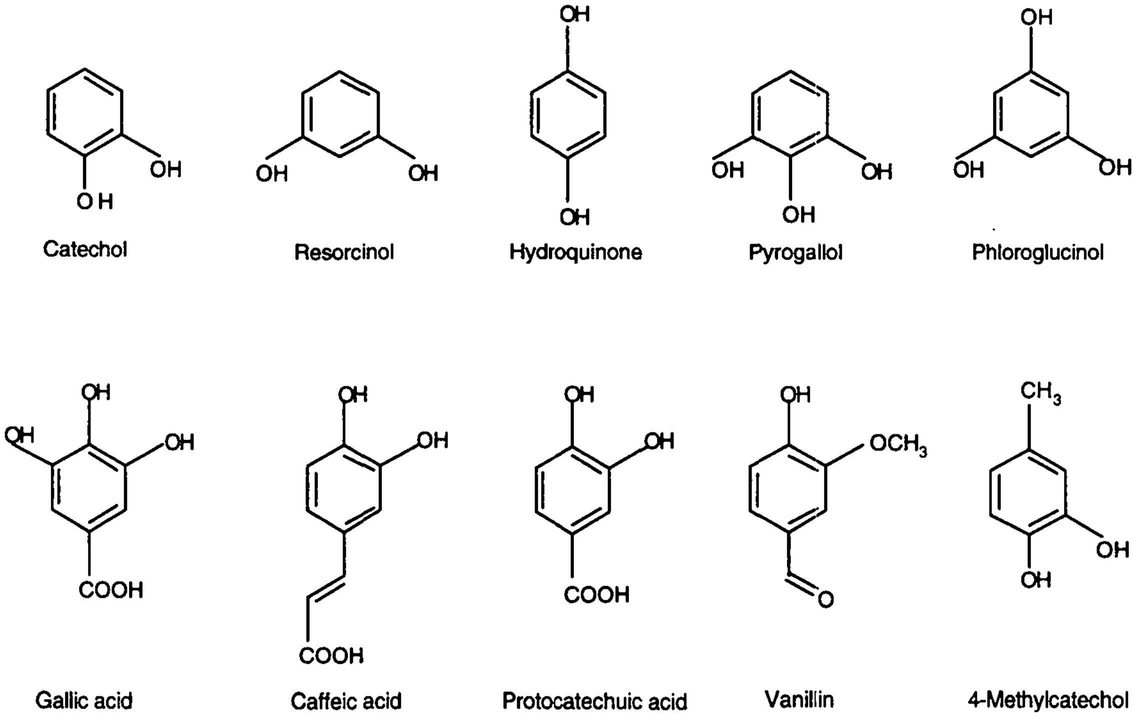


Fig. 1. Phenols selected for interaction with Cu

Determination of copper complex formation by ESR

Cu(II) is a transition metal ion which has a 9d electron configuration. The presence of an unpaired electron results in paramagnetism and makes the ion and its complexes suitable for ESR analysis. Spectra may be obtained from a frozen solution or a fluid solution. To obtain maximum information on the complex under investigation it is often necessary to employ both approaches. Frozen solution spectra represent a summation of spectral contributions for all orientations of the principal axes of the complex with respect to the magnetic field of the spectrometer and are said to be anisotropic. Where the complexes approximate to axial symmetry, the anisotropic spectra display parallel and perpendicular features which can be characterised by $g_{//}$, $A_{//}$, $g_{\perp}$ and A. The g-values are calculated from a knowledge of the microwave frequency at which the experiment was conducted and the magnetic field position of the parallel or perpendicular feature according to the equation:

$$\mathbf{g} = hv/\mathbf{H}$$

where **h** is Planck's constant, **v** is the microwave frequency, is the Bohr magneton and **H** the magnetic field.

The A-values are calculated from the hyperfine structure which arises from the interaction of the unpaired electron with the spin (I = 3/2) on the copper nucleus. In many instances this structure only resolves in the parallel components. In fluid solution where the molecular tumbling rate is rapid in comparison to the timescale of the ESR transition, the anisotropic contribution is averaged out, and an isotropic spectrum results which may be characterised by g_{iso} and A_{iso} parameters. However, where complexes of higher molecular weight exist in solution, the tumbling rate may be insufficient to fully average out the anisotropic contribution which results in spectra intermediate between the isotropic and anisotropic case. Thus, information can be gained about the generation of polymeric copper species. The g- and A-values are sensitive to the coordination environment of the Cu(II) ion and can therefore be used to distinguish the presence of different complexes (Pilbrow,1990).

ESR can also detect and characterise free radical species and thus may be of use in elucidating mechanisms of free radical-mediated polymerisation. Autoxidation of phenols in alkaline solutions in the presence of O_2, which produces free radical intermediates, is well-documented and can lead to the formation of brown polymers. (Taylor and Battersby, 1967).

Materials and Methods

$CuSO_4$ solution (5 ml,10 mM) was added to phenol solution (100 ml, 2.5 mM) in order to obtain a 1:5 Cu / phenol molar ratio. The pH was adjusted to 6 or 10 with NaOH (0.1 M). To prevent polymerisation solutions were frozen at -50 C until examination by ESR. Glycerin (10% by volume) was added to the samples to ensure the formation of a good aqueous glass which needs to be formed to obtain resolved anisotropic (polycrystalline) ESR spectra from the frozen solution. ESR spectra were obtained at 100K, or ambient tempereature, with a Bruker ECS 106 spectrometer operating at about 9.5 GHz and equipped with a TM_{110} cavity. Microwave power was 20 mW and the modulation amplitude 10 G.

Results

Cu(II) in presence of catechol spectrum

At pH 6 the room temperature solution spectrum revealed four peaks with g_{iso} = 2.158 +/- 0.005 and A_{iso} = 64 +/-3 G indicating complexation with a low molecular weight ligand (Fig. 2). The frozen solution spectrum resolves parallel features with parameters different to those from the copper hexaaqua cation indicating complex formation (Fig. 3, Table 1). The isotropic parameters at pH 10 differ markedly from those at pH 6 with g_{iso} = 2.130 +/- 0.004 and A_{iso} = 81 +/- 2 G indicating a change in Cu coordination at higher pH (Fig. 4). The frozen solution spectrum (Fig. 5) shows that two new complexes have replaced the complex at pH 6.

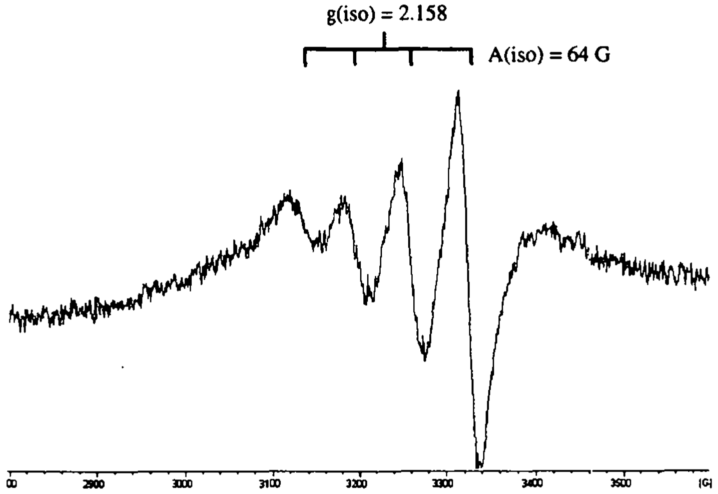


Figure 2. ESR fluid solution spectra of Cu^{2+} in the presence of catechol at pH 6

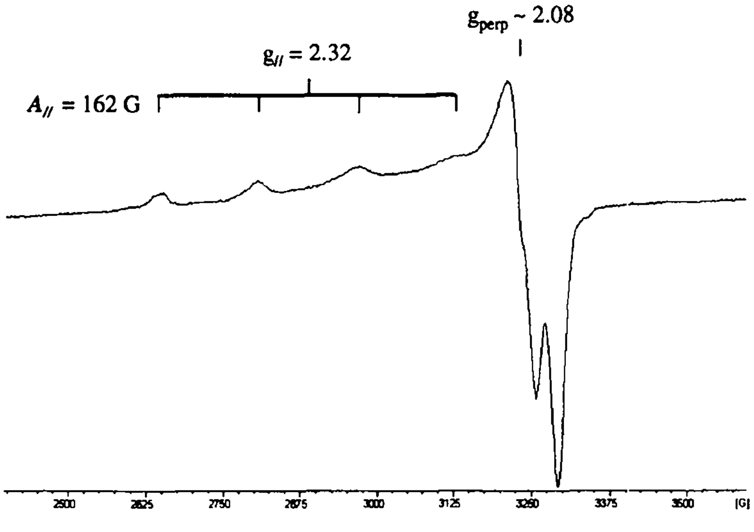


Figure 3. ESR frozen solution spectra of Cu^{2+} in the presence of catechol at pH 6

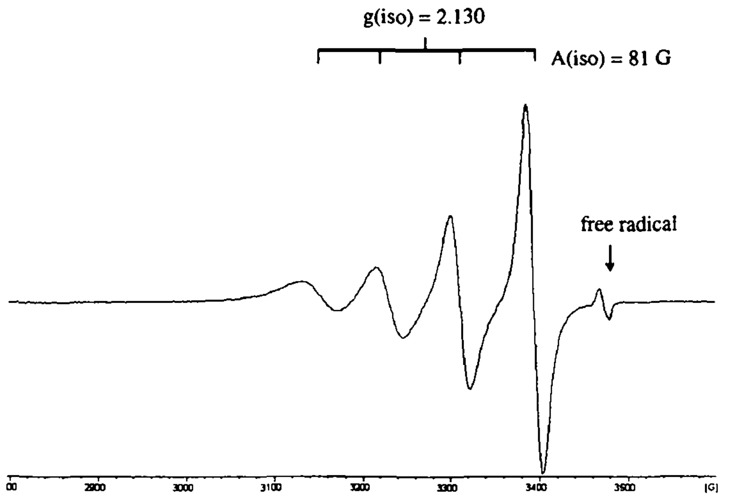


Figure 4. ESR fluid solution spectra of Cu^{2+} in the presence of catechol at pH 10.

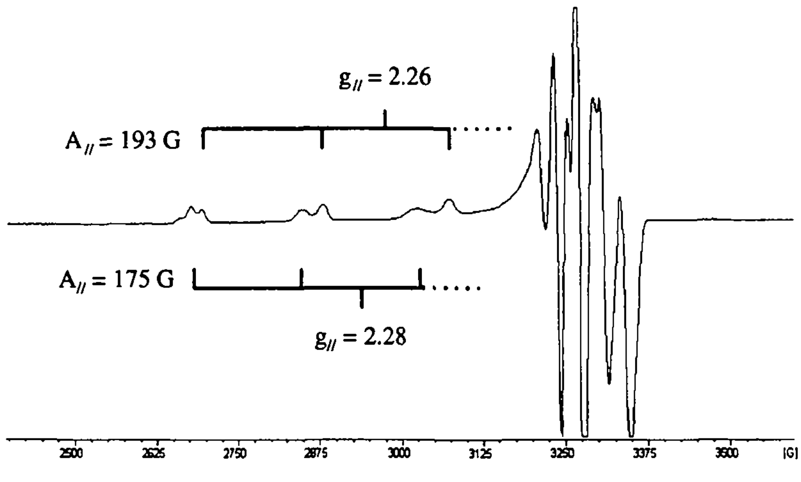


Figure 5. ESR frozen solution spectra of Cu^{2+} in the presence of catechol at pH 10. (The high-field (4th) parallel peaks overlap with perpendicular components of the spectrum).

Sample	pH	number of species	A //	g //	g
Cu(H2O)$_6^{2+}$		1	122	2.42	~2.09
catechol	6	1	162	2.32	~2.08
	10	3	193	2.26	
			175	2.28	
resorcinol	6	2	152	2.36	~2.09
	10		166	2.3	~2.06
hydroquinone	6	1 or 2	122	2.42	~2.08
	10	2	152	2.32	~2.08
pyrogallol	6	1	140	2.36	~2.08
	10		not resolved	not resolved	not resolved
phloroglucinol	6		not resolved	not resolved	not resolved
	10	1	168	2.3	2.06
gallic acid	6	1	142	2.35	not resolved
	12	1	194	2.25	not resolved
caffeic acid	6	1 or 2	124	2.41	~2.08
	10	3	173	2.29	~2.08
			192	2.26	~2.08
protocatechuic acid	6	3	not resolved	not resolved	not resolved
	10	2	158	2.32	not resolved
			163	2.3	not resolved
vanillin	6	1	122	2.4	~2.08
	10		not resolved	not resolved	not resolved
methylcatechol	6		not resolved	not resolved	not resolved

Table 1 ESR spectra frozen solution parameters for Cu2+ with different ligands

Comparing the A and g values for the whole range of molecules, 3 different groups of complexation behaviours were observed: Group 1, for phenols possessing a catechol function, able to form 3 different complexes in presence of Cu(II) at pH 10 as represented by catechol, 4-methylcatechol, caffeic acid, and protocatechuic acid. Group 2, for phenols which are only able to form one complex even at high pH bearing 2 or 3 hydroxyl groups but none in the 1,2 position as for instance resorcinol, hydroquinone, phloroglucinol, gallic acid and pyrogallol. Group 3, for phenols for which no complexes were observed as in vanillin, guaiacol, *trans*-cinnamic acid, *para*-hydroxybenzoic acid and *ortho*-, *para*-, *meta*-, coumaric acids. Generally the most reactive phenols had the greatest increase in $A_{//}$ values with pH and the greatest decrease in $g_{//}$ values.

Free radical signal and spectrum evolution with time

In the absence of Cu(II) most of the phenol solutions turned orange-brown after a few weeks due to oxidation and this behaviour was enhanced at high pH. In the presence of Cu(II) the reaction was considerably faster with the catechol solutions turning black in less than 5 hours. For catechol, gallic acid and protocatechuic acid, organic free radical signals were observed at pH 12 or lower pH, which may indicate the presence of free radical intermediates in the polymerisation of these monomers to polyhydroxy aromatic compounds. For hydroquinone the free radical signal was very stable and could be observed between pH 7 - pH 12. It was attributed to the formation of the stable semiquinone. It must be emphasised, however, that such radical formation can occur in the absence of Cu(II) as a consequence of autoxidation in which molecular oxygen acts as an electron acceptor from the phenoxyl anion. Consequently, further ESR studies need to be undertaken to provide direct evidence that Cu(II) enhances the formation of reactive free radical intermediates which are involved in the polymerisation process.

By following the loss of fluid solution signal intensity of Cu(II) in the presence of catechol with time (Fig. 8), the following deductions could be made:

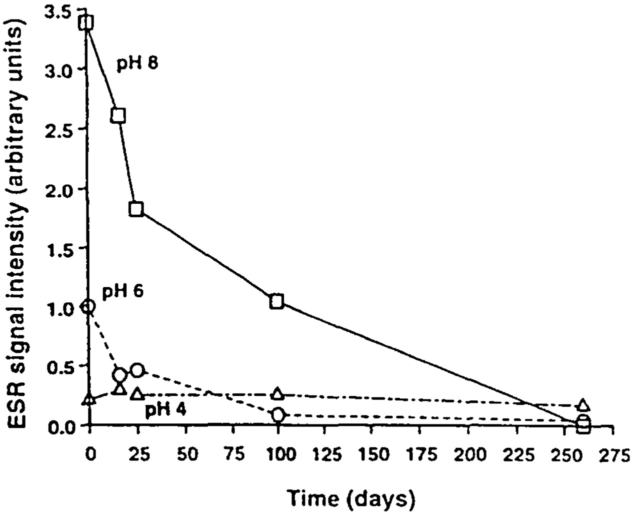
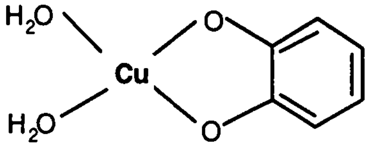


Fig 6. Change of Cu(II) ESR signal intensity with time

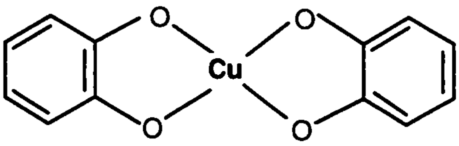
At pH 4, intensity of the uncomplexed Cu(II) signal remains stable with respect to time. At pH 6 the spectral intensity of the dominant peak of the two species present gradually reduced with time which may indicate either that Cu(II) is bound to higher molecular weight species (which the ESR would be less sensitive in detecting) or that Cu(II) is being reduced to Cu(I), which is ESR silent. After 260 hours the Cu(II) signal has completely gone. The same behaviour can be observed at pH 8 although the loss in signal intensity is much more pronounced. The inability to detect an anisotropic signal in the frozen solution spectra and the lack of any precipitate occurring with respect to time may favour the hypothesis that Cu(II) is being reduced to Cu(I). It should be noted that signal intensity has been measured as a function of peak height and therefore accurate quantitative measurements cannot be made between signals derived from different species due to inherent differences in linewidths.

Discussion

The A- and g-values of the fluid and frozen solution spectra of Cu(II) in the presence of different phenols indicates different interactions depending on the nature and position of the functional groups of the benzene ring. Compounds like catechol, caffeic acid, protocatechuic acid and 4-methylcatechol which have adjacent hydroxyl groups on the ring system were most likely to interact with Cu(II) forming up to 3 different species at pH 10. With catechol, two of the species detected may represent the mono- and bisbidentate chelates shown below in which coordination takes place within the equatorial plane of the copper. A third species occurring at higher pH may involve participation of OH^- in the coordination sphere.



monobidentate catechol complex



bisbidentate catechol complex

The presence of a carboxyl group on the phenol ring as occurs with protocatechuic acid, gallic acid and caffeic acid did not lead to the formation of any additional complexes which suggested that the carboxyl groups do not participate in the complexation reactions. The small changes in the ESR parameters as a result of methylation of catechol at the 4-position is thought to result from the weakly electron donating (positive induction) effect of the methyl group. For the 1,2,3-trihydroxyphenols a weaker interaction was observed compared to the 1,2-dihydroxyphenols. This may be a consequence of the third hydroxyl group destabilizing the enolate forms of the compounds.

With hydroquinone, where chelation is not possible, discrete changes in spectroscopic parameters from those of the Cu(II) hexaaqua cation indicates that weak interaction with the ring hydroxyls can occur. This interaction is also sufficient to avoid precipitation of the neutrally charged $Cu(OH)_2$ between pH6 and pH10.

The ESR data indicates a general trend for the $A_{//}$ values of the complexes to increase with pH and the $g_{//}$ values to decrease with pH which can be interpreted in terms of increased ligand - Cu(II) interaction as ligand hydroxyl groups sequentially deprotonate according to their pK_a values.

Polymerisation of catechol was deduced from the loss of signal intensity with time. HPLC analysis confirmed that the chemical structure of the original phenol molecule was modified and exclusion chromatography demonstrated an increase in molecular size (not presented here).

From the colour change of the solutions caffeic acid, gallic acid and protocatechuic acid were less sensitive than catechol to oxidation and subsequent polymerisation. The presence of a substituent in position 4 of the benzene ring, may impede polymerisation through steric hinderance, directly blocking the site of reaction, or altering the unpaired electron spin density, and subsequent reactivity, at other sites.

Although concentrations of phenols used for this experiment were considerably greater compared with the concentrations observed in soil solutions, the differences in affinities observed for Cu(II) with the selected phenols could explain some selective Cu-solute interactions which occur in natural samples.

The formation of higher molecular weight complexes a few hours after interaction between catechol and Cu(II), indicates that Cu(II) is a strong catalyst in the polymerisation process. This has direct environmental implications. In podzol soil solutions, the pH is usually around 4.5 but can sometimes reach 6.5 following rainfalls. The dynamics of polymerisation being very unstable between pH 4 and 8 (Fig.8) the mobility of the complexed metal could also change greatly.

References

Gallet,C.1992. Apports De La Biochimie A La Connaissance Du Fonctionnement Des Ecosystemes Forestiers: Role Des Composes Phenoliques Dans Une Pessiere A Myrtilles, These Numero 278-92 De L'Universite Claude Bernard-Lyon I.

Parish, R.V.1990. NMR, NQR, EPR And Mossbauer Spectroscopy In Inorganic Chemistry, Ellis Horwood Series, P168-194.

Pilbrow, J.R. 1990. Transition Ion Electron Paramagnetic Resonance, Clarendon Press, Oxford.

Taylor, W. I. And A.R. Battersby 1967. Oxidative Coupling Of Phenols, Edward Arnold Publishers Ltd., London.

Vaughan, D. And R.E.Malcolm 1985. Soil Organic Matter And Biological Activity, Developments In Plants And Soil Science, Volume 16, Martinus Nijhoff / Dr W. Junk Publishers.

Chapter 5

Elucidation of phenol-Cu interaction mechanisms by Potentiometry, ESR, UV absorption spectroscopy and Molecular Simulations.

OESS Andrea, CHESHIRE Martin V., MCPHAIL Donald B., STOLL Serge, EL ALAILI Mazen, & VEDY Jean-Claude. Accepted for publication in The Science of the Total Environment, 14 January 1999.

Abstract

Dans cette expérience quatre techniques complémentaires ont été appliquées à l'étude des paramètres controllant la complexation Cu-phénols. Des mélanges réactionnels comprenant du Cu en présence de phénols ont été analysés par potentiométrie pH-métrique, résonance paramagnétique électronique et par absorption UV dans différentes conditions de pH et force ionique. La stabilité liée à la conformation de la molécule a été étudiée en effectuant des simulations dynamiques. A partir des courbes de titration, des constantes A et g isotropiques, de l'évolution du spectre d'absorption et du calcul de l'énergie potentielle de formation des complexes un mécanisme réactionnel comprenant trois étapes a été proposé.

In order to determine the dominant properties, which control the interactions of monomeric hydrosoluble phenols with Cu^{2+} in soil solutions, four different techniques were used. $CuSO_4$ was added to ortho-dihydroxy-phenol (ODP) solutions under controlled temperature, pH, and ionic strength conditions and Cu^{2+}-ODP interactions were studied by alkaline potentiometric titrations, electron spin resonance and UV absorption. Molecular dynamic simulations were also performed for conformational studies. From the titration curves, the shifts in isotropic A and g values, the evolution of the UV absorption band at 400 nm and the calculation of the potential energy of the different complexes, a three step interaction mechanism was proposed.

Introduction

Mobilisation and immobilisation processes of Cu(II) in soils are strongly influenced by the presence of organic ligands in the medium. Various experiments have been carried out on chemical speciation of Cu(II) in relation to humic and fulvic acids (Senesi N. & Sposito, 1989; Stevenson et al., 1993; Goodman et al., 1994; Tipping et al., 1995). But size, heterogeneity and multiplicity of possible interaction sites of humic substances often make it difficult to predict a specific behaviour for metal-organic matter interaction. We chose to investigate a much simpler system by studying monomeric hydrosoluble phenols which are commonly known as precursors of humic substances (Harborne, 1964). A previous study carried out on a range of phenols bearing different substituents on the benzene ring, showed that the presence of two hydroxyl groups in the ortho position was essential for Cu ion complexation (Oess et al., 1997).

The aim of this study was to propose a mechanism which would explain the preferential behaviour of Cu ion to bind to ortho-dihydroxy-phenol molecules (ODP's) by exploiting complementary information obtained by three different analytical techniques: Alkaline potentiometric titration, ESR (Electron spin resonance spectroscopy), and UV spectroscopy. Molecular simulation calculations were also performed to determine the most stable Cu^{2+}-phenol conformation. The different approaches, which contributed to the elucidation of the Cu^{2+}-phenolic interaction mechanism, are summarised as follows:

Potentiometric approach

The interaction of a metal with an electron donor atom of a ligand is usually followed by the release of H^+. Alkaline potentiometric titration was based on the detection of the protons released upon complexation. The main advantage of this technique, compared to other methods, was that from the titration curves it was possible to follow complexation continuously as a function of pH and to detect exactly at which pH complexation takes place. Furthermore, it was possible to calculate the pK_a values, the dissociation constants and the stability constants. The following equilibrium were used for the determination of the pK_a values and the stability constants:

(1) $H_2A \Leftrightarrow HA^- + H^+$
(2) $HA^- \Leftrightarrow A^{2-} + H^+$
(3) $H_2A + Cu^{2+} \Leftrightarrow CuA + 2H^+$
(4) $H_2A + CuA \Leftrightarrow CuA_2^{2-} + 2H^+$

H_2A represents an ODP molecule, which bears 2 dissociable H^+.

		no Cu(II)			with Cu(II)		
phenols	pk_{a1}	pk_{a2}	pk_{a3}	$pk_{a1'}$	$pk_{a2'}$	$pk_{a3'}$	$pk_{a4'}$
caffeic acid	4.50	9.32	11.7	4.63	6.67	10.23	11.92
catechol	9.45	12.80		5.13	6.81	9.38	12.42
gallic acid	4.65	8.70	11.45	4.26	5.87	9.93	11.48
protocatechuic acid.	4.34	8.70	12.2	4.52	6.87	10.43	11.87
vanillin	7.27	11.93		7.2		12.50	

Table 1. shift in pK_a values upon complexation with Cu for a range of phenol molecules.

ESR approach

ESR can detect and characterize species containing unpaired electrons in their atomic or molecular orbitals, such as free radicals and paramagnetic transition metal ions. These electrons can exist in one of two quantum mechanical spin states, which are energetically equivalent (degenerate), and display magnetic moments of the same magnitude, but different sense. In the ESR experiment, an external magnetic field is applied, which interacts with the electron magnetic moments thus lifting the degeneracy of the two spin states and creating a ground and excited state. Radiation at microwave frequencies can promote electrons to the higher-energy state when the energy difference, induced by the magnetic field, equals that of the radiation. In ESR, microwave radiation of a fixed frequency is applied to the sample and the magnetic field scanned. When the resonance condition is fulfilled, absorption of microwave energy is detected and a spectrum obtained. The spectrum can be characterized by the g-value which is calculated from the microwave frequency (ν) and the magnetic field (**H**) at which resonance occurs by the equation:

$$g = h\nu / \beta \mathbf{H}$$

where h is Plank's constant and β the Bohr magneton. Where the unpaired electron interacts with a nucleus of non-zero spin, hyperfine coupling occurs, and the spectrum is split into further components. The magnitude of this splitting is characterized by the A-value, which is usually reported in terms of magnetic field units. The g- and A-values are sensitive to the chemical environment surrounding the unpaired electron. Thus Cu(II) complexes, in which a single electron is located in the d-orbital system (often $d_x{}^2 - {}_y{}^2$), will give rise to spectra of differing g- and A-values depending on the number and type of ligands present, as well as the symmetry imposed by chelates. Consequently, ESR can identify the $Cu(H_2O)_6{}^{2+}$ ion and different ligand complexes formed as a function of pH. In the present work, anisotropic spectra were obtained from frozen solutions and the $g_{//}$ and $A_{//}$ values obtained. The parallel values are those obtained for molecules in which the principal Cu axis is aligned parallel to the applied magnetic field. A change in the oxidation state of Cu(II) to Cu(I) can also be inferred from a decrease in total signal intensity, which is a consequence of Cu(I) being diamagnetic (containing no unpaired electrons). Furthermore, when organic ligands polymerize through a free radical chain reaction, the free radical signal can also be detected by this technique .The three following equilibrium symbolize the type of information which can be obtained by ESR spectroscopy:

(1) Cu(II) + L ⇔ Cu(II)-L	change in Cu coordination
(2) Cu(II) ⇔ Cu(I)	change in Cu oxidation state
(3) 2L ⇔ 2L·⇔L-L⇔	polymerization of the ligand

UV absorption approach

With the UV and visible absorption spectra it is possible to distinguish the organic ligand, from the isolated metallic ion and the metal^{n+}-ligand complex. From the combination of unsaturated ring bonds, and of alcoholic and carboxylic substituents, most phenols have their maximum absorption intensity around 270 nm. For the uncomplexed Cu(II) it is around 700 nm. For a charge transfer complex a precise value has not been attributed, but because of the intensity of the molar absorption, it is normally easy to detect around 500 nm. In the course of this type of interaction the complex absorbs radiation and an electron from the donor atom (phenol) is transferred to the acceptor (Cu(II)).

Molecular simulation

When performing a dynamic simulation, forces are computed and atoms moved in response to these forces. Atomic movements were determined using the classical Newton's equations of motion. The differential equations are integrated numerically in Cerius 2 using a finite difference method. Given atomic positions at time t (and corresponding forces), we obtained their values at a later time t+Δt. The Universal Force Field 1.02 was used in this study to evaluate the total Energy of the metal-ligand system. The Universal Force Field was parameterised for the full periodic table and has been carefully validated for main-group compounds, organic molecules, and metal complexes. It contains all the analytical expressions and data to calculate bond-stretching, angle-bending, torsion, inversion, Van der Waals and Electrostatic contributions. After sketching the ligand molecule and the metal from the builder module, charges are calculated by considering the geometry of the molecule and electronegativity of each atom. After performing a dynamic simulation to determine the most favourable Cu^{2+}-phenol interaction site, a minimisation was performed to determine the lowest state in the potential energy of the new structure. During minimisation the atomic coordinates and unit cells were adjusted to reduce molecular Energy. From the difference in energy between the uncomplexed and the complexed state, it was possible to determine the most stable structure as a function of the number of added ligands. From the total energy it was then possible to determine the most stable interaction site between different molecules or between different sites within the same molecule and the most stable conformation for each structure.

Materials and Methods

Synthetic ODP's obtained from Sigma with a degree of purity of 99% were used for all experiments. Vanillin (4-hydroxy-3-methoxybenzoic acid) was also used as a control. The temperature of the solutions was kept constant at 295 K for the potentiometric and the UV experiments. Ionic strength ranged between 0.056 and 0.077 for the titration experiment and between 0.016 and 0.041 in the ESR experiment. All solutions were kept in the dark to prevent any photochemical reactions but oxygen was not removed. A phenol/Cu(II) ratio of 4:1 was maintained by using 10 mM phenol–2.5 mM $CuSO_4$ M solutions for the potentiometric titrations and 2.5 mM phenol-0.5 mM $CuSO_4$ solutions for the ESR experiment. Potentiometric titrations were carried out at room temperature (22°C) with a Metrohm titroprocessor

686 equipped with a dosimeter 665 and a pH electrode. After one-hour equilibration the solutions were titrated with 0.1M NaOH following the procedure of Timberlake (1957). The pK$_a$ values were determined with the PKAs programme of Martell and Motekaitis (1992). ESR spectra's were run at 100K with a Bruker ECS 106 spectrometer operating at about 9.5 GHz and equipped with a TM$_{110}$ cavity. Microwave power was 20mW and the modulation amplitude 10 G. A Perkin Elmer Lambda 14 UV/VIS spectrometer was used to measure absorption coefficients. For the molecular simulations a Cerius 2 interface was used. Dynamic simulation was carried out by using the UFF (Universal Force Field 1.02) at constant volume and temperature, (300 K), with 500 iterations, $\Delta t=0.001$ ps and for three different dielectric constant values ($\varepsilon=1,10,80$).

Results

Potentiometric titrations

In the classification of soft and hard acids, the intermediary character of Cu^{2+} is probably a good reason for its acting as a good competitor to H$^+$ on the catechol groups. The potentiometric titrations confirmed this behaviour. The titration curve of catechol in the presence and absence of Cu^{2+}are presented in fig1. The shift in titration curves revealed that complexation took place between pH4.5 and 11. In the Cu-catechol curve, two additional shoulders appeared, which indicated the presence of two more stability states. Similar curves were observed for caffeic acid, gallic acid and protocatechuic acid. The corresponding pka values calculated from the titration curve are presented in table 1. The first set of pka's corresponded to H$^+$dissociation of the carboxylic or vinyl-carboxylic group (pKa$_1$), the catechol groups (pka$_2$, pka$_3$) and from the hydroxy-methoxy group in the case of vanillin. The second set of pka's (pka$_1$', pka$_2$', pka$_3$', pka$_4$', pka$_5$') corresponded to the H$^+$ dissociation of the same groups, but in the presence of Cu^{2+}. The additional pka values occurred upon saturation of the free Cu(II) in solution and were attributed to the co-existence of two complexed species: a mono-bisdentate and a di-bisdentate complex. An alternative explanation for these additional values, could be the dissociation of the unreacted phenol, which was fourfold in excess. Only the curves of vanillin, which is not an ODP, did not show any shift in pka values, thus confirming a weak affinity between Cu^{2+} and the hydroxy-methoxy sites, no matter the pH. As the pK$_{a1}$ values of caffeic acid and protocatechuic acid did not decrease in the presence of Cu^{2+}, it indicated that the carboxylic group did not participate in the complexation reaction.

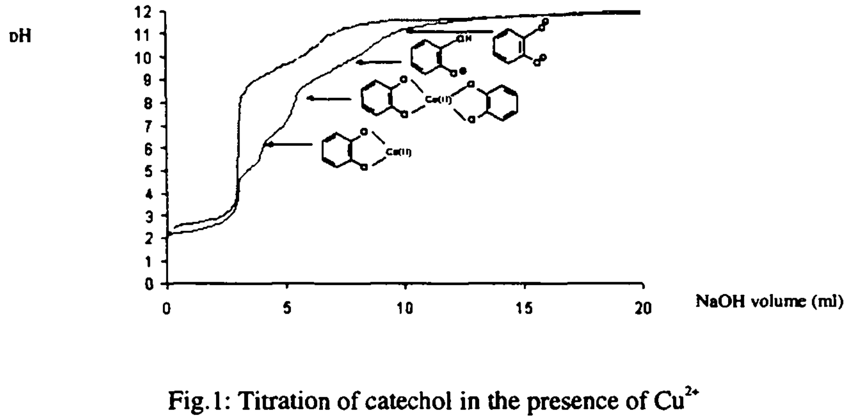


Fig.1: Titration of catechol in the presence of Cu^{2+}

The strong decrease in pka' values observed for the catechol site, indicated that Cu^{2+} was a strong competitor to H^+ even at low pH. An increase in pH, provoked an increase in the shift of the titration curves and thus of the amount of complexed Cu^{2+}. The number of ODP's positioned around the Cu increased with pH. The switch from a dominance mono-bisdentate complex to a dominance in di-bisdentate complexes occurred around pH 7.

ESR

The number of atoms neighbouring Cu^{2+} and thus the number of surrounding ligands was determined from the shift in A// and g// values in comparison to the hydrated Cu^{2+} ion. The values were similar to those obtained by Senesi with fulvic acids (Senesi et al., 1985) and indicated that O was the major atom participating in bond formation. The spectra were run at pH 6 and 10 and the corresponding A// and g// values are summarised in Table 2. At pH 6 the shifts in A// and g// values and the signal intensity indicated the co-existence of two species. The hydrated Cu^{2+} ion was still present, but the mono-bisdentate complex was the dominant specie present. At pH10, a second and a third specie appeared corresponding to the di- and tri-bisdentate complex. In this case the di-bisdentate was the dominant specie present. In accordance with the Jahn-Teller theorem, the most stable structure of Cu(II) is usually found in the tetragonal environment with four strongly bound equatorial ligands and one or two weakly bound axial ligands (Solomon, 1983). As three bisdentate ligands were identified in the Cu^{2+} environment, the two first ligands were expected to lie in the equatorial plane, and the third one in the axial plane. An increase in A// and a decrease in g// were observed for the complexed species. From the studies of Parish (1990) this was an indication of an increase in the degree of covalency between the metal and the interacting ligand.

	Complex	A//	g//	pH
	$Cu(H_2O)_6^{2+}$	122	2.42	6,10
1st species	Cu-catechol 2O	162	2.32	6,10
2nd species	Cu-catechol 2O	193	2.26	10
3rd species	Cu-catechol 2O	175	2.28	10
1rst species	Cu-caffeic acid 1O	124	2.41	6,10
2nd species	Cu-caffeic acid 2O	173	2.29	10
3rd species	Cu-caffeic acid 2O	192	2.26	10

Table 2 : A// and g// values obtained from the frozen solution ESR spectra of Cu in the presence of a range of phenolic ligands.

For caffeic acid, the g// and A// values of the first complex observed at pH 6 were only slightly different from the uncomplexed Cu(II) thus indicating a weak interaction, possibly between the carboxylic O and Cu(II), or originating from an outer-sphere complex. The g// and A// values of the second and third species of caffeic acid were much closer to the first and second species of catechol and indicated that the two ortho-hydroxyl groups were involved to form a more stable inner-sphere complex. For protocatechuic acid no additional inner sphere complex due to the carboxylic groups was observed. Only the catechol group was able to form an inner-sphere complex with the cation. No changes in g// and A// values were observed for Cu(II) in the presence of vanillin, thus indicating that aldehyde and hydroxy-methoxy moieties groups on the benzene ring had little influence on Cu(II) complexation.

A previous study carried out by ESR on fluid solutions showed that for pH>6, a decrease in signal intensity and a broadening of the peaks took place this time thus revealing that Cu(II) had been reduced to Cu(I) in the presence of catechol(Oess et al, in press). A free radical signal was also detected, which indicated that a free radical polymerisation chain reaction was most probable to take place.

UV absorption

The solutions were scanned from 200 to 700 nm. A peak in the 200-300 nm region bearing two maximas (226 and 259 nm) was typical of the uncomplexed catechol spectra. An absorption peak around 500 nm, which was attributed to a charge transfer band, only appeared after 20 hours catechol-Cu contact and could only be observed for the pH 4 solutions. At higher pH and within 10 min. of interaction, the spectra was characterised by a broadening in the 200-300 nm region and the appearance of an absorption peak in the 350-450 region, which only persisted during 1 hrs 30. This was mostly due to the formation of polymers and respectively their quinonic intermediates and hindered the possibility to see a charge transfer band at higher pH. At pH 6 the solutions immediately turned dark green and a precipitate was formed. At pH 10 the solutions turned yellow and a precipitate was only formed after 48 hrs. For the other phenols, which had an enhanced chromophore behaviour, charge transfer bands were hindered by an extended absorption band.

Molecular simulations

Protonated phenol molecules were used for this simulation, thus corresponding to a medium of pH<4.5. For catechol a Cu ion had been randomly placed several times at different sites around the molecule. After minimisation/ dynamic simulation/ minimisation the most stable conformation of the complex was found. This corresponded to Cu(II) being surrounded by the two hydroxyl groups of the catechol, thus forming a five membered chelate ring. In the presence of two ligands, a second five membered chelate ring was formed on the opposite side of the first one, in the same plane. With three ligands, the two first ligands had to undergo a deformation and move closer to each other in order to let the third ligand gain access to the apex of Cu(II) (see fig.1). The differences in energies obtained for the complexed (E2) and the uncomplexed conformations (E1) for $\varepsilon=10$ are presented in Table 3 and indicated the following order of complex stability: $CuL_1>CuL_2>CuL_3$. ε represents the permittivity of the medium (or dielectric constant) and depends on the number of water molecules contained between the metal and the active site. As ε decreases when the metal approaches the active site, the energies calculated for $\varepsilon=10$ represent the transition values between the dry air state ($\varepsilon=1$) and a water solvent state ($\varepsilon=80.37$). Although the « bond terms » were modified upon complexation, the non-bond terms contributed to the major changes around the active site. It was mostly the balance between opposing effects of the electrostatic interactions and the Van der Waals energies, which determined the stability of the complexed molecule. The electrostatic contributions

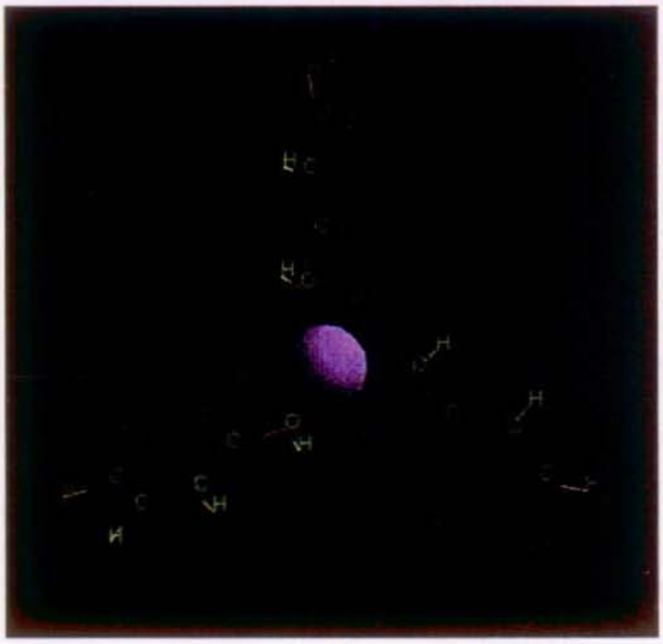


Fig.2: Most stable conformation of Cu^{2+} in the presence of 3 catechol ligands ($\varepsilon=10$)

were favourable for the bonding of one, two, or three ligands and had a stabilising effect on complexation, while the Van der Waals interactions contributed to a decrease in the stability of the complex, in particular beyond two ligands. In order to compare the stability of the catechol complex with other phenols bearing different substituents on the benzene ring, the energy was estimated after placing a Cu(II) atom around the different sites of the molecules. The differences in energies (E2-E1) indicated that the catechol sites (2 vicinal hydroxyls) remained the most favourable binding sites even in the presence of other substituents. The energies calculated for the COOH-Cu structures were favourable for protocatechuic acid but not for gallic acid. Consequently, the 3 OH groups of gallic acid

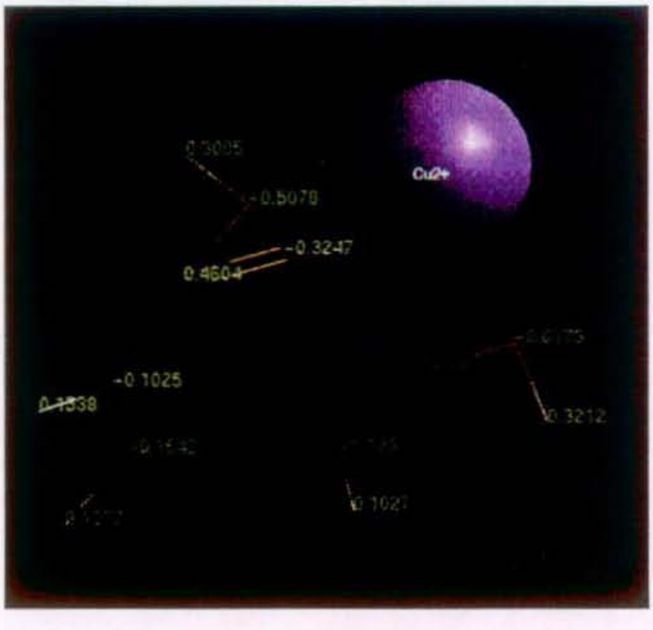


Fig.3. Rearrangement of caffeic acid in the presence of Cu(II) upon Energy minimisation

must have a destabilising effect on the COOH group. For caffeic acid it was not possible to calculate the energies attributed to the vinyl-COOH group, because the molecule would immediately reorientate upon minimisation and adopt a more stable conformation in which the two OH groups participate in the interaction with Cu(II) (Fig.2). For vanillin the energy was favourable at the methoxy-OH site, but it was not possible to carry out a minimisation at the aldehyde site, thus indicating a very low affinity of the Cu(II) at this position.

E2-E1 [kcal/mole] e=10	1catechol 2OH	2 catechol 2OH	3 catechol 2OH
Bond terms			
Bond terms	0.02	1.3	3.05
Angles	-0.08	0.25	1.02
Torsions	-1.87E-08	7.80E-02	1.50E-02
Inversions	-5.64E-10	6.90E-03	3.40E-04
Non bond terms			
VdW	0.93	6.54	21.55
Electrostatic	-8.14	-15.16	-12.48
Total Energy	-7.26	-6.98	13.16

Table 3. Difference in Energy of the structure of Cu bonded to 1, 2 or 3 ligands.

Conclusions

The study of pure solutions allowed us to identify two main parameter, the chelate effect and the pH, which control the interaction process between phenols and Cu. In the presence of mono-dentate sites, low pka values of the substituents can enhance complexation, but as confirmed by Stumm (1981), in the presence of multidentate sites, the chelate effect is much stronger. The driving force of the reaction is the conformational stability obtained when a five membered ring is formed between the Cu^{2+} and the phenol group. Ortho-dihydroxy phenols are particularly suitable for these reactions but other aliphatic compounds bearing multidentate sites could also have the ability to build strong interactions, as for instance oxalic acid or citric acid. pH particularly affects the number of ligands positioned around the Cu and the oxidation of organic matter.

The contribution of different techniques used in this study allowed us to identify a three-step interaction mechanism comprising an initiation, an interaction, and a propagation step.

Initiation: the affinity of Cu^{2+} for the catechol site triggers instantaneously the release of two H^+ from the hydroxyls.

Interaction: During the few minutes which follow the contact between Cu^{2+} and the phenolic solution, conformational stability is reached at the catechol site and leads to the formation of an inner-sphere complex. From pH 4.5 to pH 6, a dark green mono-bisdentate complex is formed and a precipitate can be observed. From pH 6 to pH 10 a switch to a dominance of di-bisdentate complex occurs and the solution turns back to yellow and limpid. In parallel, quinonic intermediates are formed and an oxydative polymerisation reaction starts.

Propagation: as the reaction proceeds with time, Cu^{2+} is being reduced and the course of the reaction mainly depends on the pH and the reactive groups other than catechol on the phenol molecule. For the solutions at pH 6, Cu^{2+} is slowly being removed from the solution by precipitation. For the solutions at pH10, a precipitate appears 48 hours later.

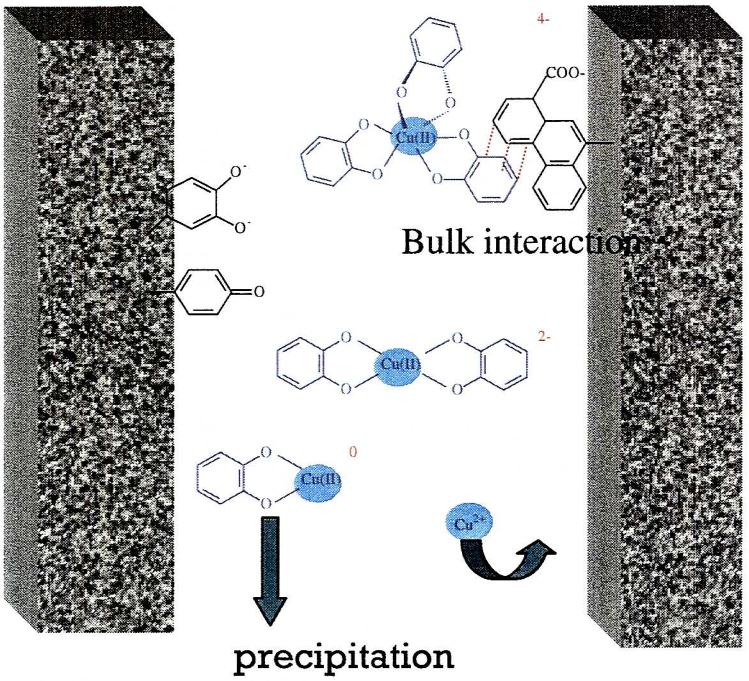


Figure 4. Possible scenarios for Cu^{2+} mobilisation in the soil solution depending on the pH

For caffeic acid and protocatechuic acid, the mechanism was very similar, except that no precipitate was formed. Precipitation was attributed to charge neutralisation upon complexation. For the mono-bisdentate complex, the total charge was equilibrated, but this was not the case for the di-or tri-bisdentate complex. Due to the presence of additional acidic groups on the benzene ring for caffeic acid and protocatechuic acid, charge equilibration was not achieved and could explain why they did not precipitate. The additional functional groups could also hinder polymerisation reactions taking place.

These properties can be used to make predictions for the mobilisation of metals in natural systems. Figure 4, illustrates catechol-Cu interaction at the soil-water interface. Four scenarios are possible. When the pH is below 4.5, most of the Cu^{2+} will be in the free state and move with the water flow. At pH 6, Cu^{2+} will be removed from the solution by precipitation. As the pH increases, Cu^{2+} will remain complexed and soluble. During the first hours bulk interactions with the soil wall components should be the only process able to remove Cu^{2+} from the solution. After 48 hours, Cu^{2+} will eventually be removed by precipitation. In order to check out about the occurrence of these processes in natural solutions several studies on various sites with different soils are still needed

References:

Harborne J B. Biochemistry of phenolic compounds. Academic Press, 1964.

Goodman B A, Cheshire M V, Berrow ML, Deighton N, Identification of paramagnetic species and determination of the copper co-ordination environments in organic matter from soils used for the disposal of distillery wastes, In: Senesi N, Miano T M., editors, Humic substances in the Global Environment and Implications on Human Health Amsterdam: Elsevier, 1994:1025-1030.

Martell A E, Motekaitis R J. Determination and Use of Stability constants. VCH Publisher Inc. 1992.

Oess A., Cheshire M.V., McPhail D.B., and Vedy J.C. 1999. Polymerization: a Possible Consequence of Copper-phenolic Interactions. Ed. Berthelin et al.Plenum publisher, London.

Oess A, McPhail D B, Cheshire M V, Vedy J C. Copper complexation with Plant-derived Phenols Originating from an Alpine Ecosystem, In: Extended abstracts of the Fourth International Conference on the Biogeochemistry of Trace Elements, 1997, 785 pp.

Parish R V, NMR, NQR, EPR and Mössbauer spectroscopy in inorganic chemistry, Ellis Horwood Series in Inorganic Chemistry, 1990, 168-194.

Senesi N, Bocian D F, Sposito G. Electron Spin Resonance Investigation of Copper (II) Complexation by Soil Fulvic Acid, Soil Sci Soc Am. J 1985; 49: 114-119.

Senesi N, Sposito G. Characterisation and stability of transition metal complexes of chestnut (Castanea sativa L.) leaf litter, J Soil Sci 1989; 40: 461-472.

Solomon E I, Bioinorganic Perspectives In Copper Coordination Chemistry, In: Copper Coordination chemistry: Biochemical and Chemical perspectives, Ed.Kenneth D Karlin and Jon Zubieta, 1983, 1-22.

Stevenson F J, Fitch A, Brar M S. Stability constants of Cu(II)-humate complexes: comparison of select models. Soil Sci 1993; 155:77-91.

Stumm W & Morgan J J, Aquatic Chemistry: An Introduction Emphasizing Chemical Equilibria in Natural Waters, 1981, Wiley and Sons, 780.

Timberlake C F, A potentiometric Study of Copper-catechol complexes, J Chem Soc 1957:4987-4993.

Tipping E, Fitch A, Stevenson F J. Proton and copper binding by humic acid: application of a discrete site/electrostatic ion-binding model, Eur J Soil Sci 1995; 46: 95-101.

Chapter 6

Copper Complexation with Plant-Derived Phenols

A.Oess, D.B. McPhail, M.V. Cheshire, and J.C.Vedy. Extended abstract. Fourth International Conference on the Biogeochemistry of Trace Elements. June 23-26, 1997.

Introduction.

At the Arpette valley site, which belongs to the Mont-Blanc massif, the climatic and geological (leucocrate granite) constraints have permitted the development of an acidophilic vegetation cover mainly represented by *Rhus ferrugineum*, *Vaccinum myrtillus*, and *Calluna vulgaris*. Biochemical analysis for primary and secondary metabolites in these plants have shown that phenolic compounds constitute an important fraction which can be released to the soils (1, 2). Their potential activity can be attributed to their high concentration in the plant leaves and their chemical structure. Under appropriate pH and redox conditions phenolic functional groups can deprotonate leaving negatively-charged, oxygen moieties which may interact with metal cations, thereby participating in their intrapedic migration.

The present study had two objectives: Firstly, to characterise by ESR spectroscopy complexes formed between Cu(II) and model phenol compounds bearing different numbers and configurations of hydroxyl groups (Fig. 1). Secondly, to characterise the Cu(II) complexes formed in the presence of a natural mixture of metabolites extracted from blueberries(*Vaccinum myrtillus*) and, by comparison with data from the model systems, assess which structural classes dominate the Cu(II) binding.

Fig. 1. Phenols selected for interaction with Cu(II)

Materials and Methods.

$CuSO_4$ solution (5ml, 10mM) was added to a solution of phenol (100 ml, 2.5mM) to obtain a 1:5 Cu(II) / phenol molar ratio. The pH was adjusted to various pH values between 2 and 12 using HCl (0.1 M) or NaOH (0.1 M). Aliquots were removed and examined by ESR spectroscopy or UV spectroscopy. Prior to ESR examination, glycerol was added to the aliquot (10 % by volume) and the solution frozen at 100K to obtain anisotropic Cu(II) spectra. Phenols were isolated from blueberry vegetation by ether soxhlet extraction followed by a return to the aqueous phase after evaporation of ether on the rotavapor.

Results and discussion.

ESR spectra of Cu(II) complexes are sensitive to the coordination environment surrounding the metal ion. Consequently, the spectroscopic parameters, defined in terms of g- and A-values, can be used to distinguish different ligand interactions (3). Among the different phenols it was possible to categorise three broad classes of interaction with the model compounds: Group 1, consisting of phenols possessing a catechol function, which formed three different complexes as represented by catechol, 4-methylcatechol, caffeic acid, and protocatechuic acid. Group 2, consisting of phenols with two or more hydroxyl groups, but with no catechol moiety, which formed only one complex throughout the pH range studied, as represented by resorcinol, hydroquinone, phloroglucinol, gallic acid and pyrogallol. Group 3, consisting of phenols for which no complexes were observed as in vanillin, guaiacol, trans-cinnamic acid, para-hydroxybenzoic acid and o-, m- and p-coumaric acids. Complex formation was confirmed by UV spectroscopy which showed an increase in absorbance around 500 nm indicating a charge transfer complex. The ESR spectrum of Cu(II) in the presence of blueberry extract was composite in nature with the anisotropic $g_{//}$ and $A_{//}$ values identical to those obtained from catechol and caffeic acid (Fig. 2). Spectra from these model compounds at pH 10 consisted of two complexes with the same spectroscopic parameters. However, the ratio of the two complexes differed markedly between the two phenols. Computer simulation of the blueberry extract composite spectrum is consistent with both catechol and caffeic acid being the dominant Cu(II)-binding phenols.

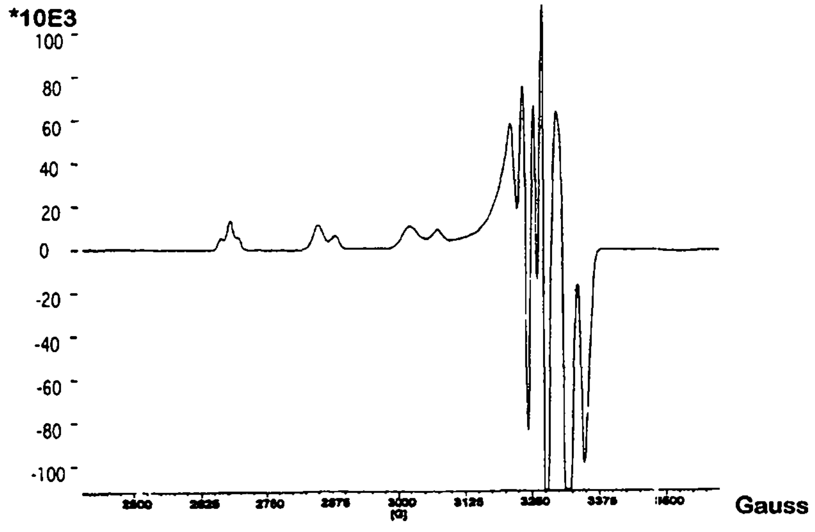


Fig. 2. ESR spectra of Cu(II) in the presence of blueberry extract at pH 10.

Conclusion.

ESR spectra of Cu(II) complexes with model phenol compounds indicate that those bearing a catechol moiety can form stable chelates over a wide pH range. Although complexes could be identified for some phenols devoid of a 1,2 hydroxyl configuration, the catechol moiety appears to dominate complexation in a mixture of phenols extracted from blueberries. Such complexes could be detected at pH values as low as 5 indicating that phenols may play a major role in the intrapedic translocation of copper in our soils where the pH may vary between 4.5 and 6.

Literature cited.

(1) Gallet,C.1992. Apports de la biochimie a la connaissance du fonctionement des ecosystemes forestiers: role des composes phenoliques dans une peessiere a myrtilles, these n°278-92 de l'universite Claude Bernard LyonI.

(2) Vaughan, D. and R.E.Malcolm 1985. Soil Organic Matter and Biological Activity, Developments in Plants and Soil Science, volume 16, Martinus Nijhoff / Dr W. Junk Publishers.

(3) Pilbrow, J.R. 1990. Transition Ion Electron Paramagnetic Resonance, Clarendon Press, Oxford.

Chapter 7

Influence of DOC-analogue o-dihydroxyphenols, and soil parameters on Cu sorption

Abstract

L'influence de la matière organique dissoute (MOD) et en particulier le rôle du catéchol et de l'acide protocatéchique sur les propriétés de sorption préférentielle du Cu ont été étudiés en présence de différents horizons d'un podzol. Les courbes de sorption indiquèrent que la majorité du Cu est retenu sur l'horizon A, suivi de l'horizon Bps et finalement de l'horizon E. L'addition de catéchol a contribué à légèrement diminuer les concentrations de Cu en solution dans l'horizon A. L'addition d'acide protocatéchique n'a pas eu d'effet visible dans cet horizon. Par contre dans l'horizon Bps l'acide protocatéchique a contribué à augmenter la solubilité du Cu d'un facteur 2. L'augmentation de pH de la solution de l'horizon A, a permis la solubilisation du Cu proportionnellement à la quantité de NaOH ajoutée.

The influence of dissolved organic matter (DOC) and in particular the role of catechol and protocatechuic acid, on Cu sorption properties has been investigated in the presence of different horizons of a podzol. Sorption curves indicated that most of the Cu was retained in the A-horizon, followed by the Bps and finally the E horizon. The addition of catechol contributed to decrease Cu concentration in the solution of the A-horizon, while protocatechuic acid had no effect. In the Bps horizon, protocatechuic acid caused an increase in Cu solubility by a factor of two. An increase of pH in the A-horizon solution released both Cu and DOC and was proportional to the quantity of NaOH added.

Introduction

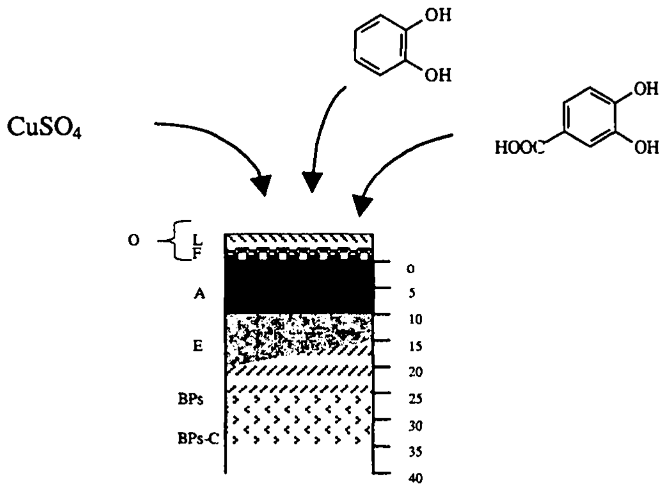
The movement and dispersion of heavy metals in the environment and in particular the transfer from the soil to the soil solution compartment can occur either in the free ion state or in association with organic or mineral colloids. pH, soil texture, the presence of clay and organic matter are the main factors controlling the sorption/dissolution reactions between the soil-soil solution interface (Römkens & Salomon 1998). In general, heavy metal migration through the soil profile is enhanced when pH decreases, but surface accumulations have also been observed in acidic soils and an increase in Cu solubility has also been observed with an increase in pH. The relation between total metal content, metal speciation, pH and DOC can be crucial factors controlling the contamination of drainage water, metal toxicity and uptake by plants. In particular after a change in land use these parameters can change to a high extent.

Investigations on Cu distribution and leaching properties in acid soils corresponding to different stages of podzolisation, showed that the more evolved soil (named BarmeI) had the highest Cu content (Chapt.1). Cu was mostly accumulated in the surface horizons (between 0-15cm). Limited quantities were leached ($20\mu g$ over 3years) and reached the lower horizons leaching was rather correlated to phenol peaks than to total DOC (Chapt.2).

On the other hand mechanistic studies on the interaction of Cu with phenols revealed two types of phenol activities. While ortho-dihydroxyphenols were the major phenol complexing entities due to the "catechol" function, some phenols like catechol were able to precipitate Cu at low pH, while others like protocatechuic acid would keep Cu in a soluble complexed form. The solubility of these complexes was attributed to the presence of a COOH group, which did not participate in the bonding with the metal, but which in the deprotonated form could keep the complexed form in solution (Chapt.4; Chapt.5).

In order to contribute to a better understanding of the soil properties, which control the mobilisation and immobilisation of Cu in these soils, and in particular the role of phenol entities from the soluble and insoluble fraction of organic matter, the following experiment was carried out.

Sorption isotherms were determined for Cu in the presence of the A-, E- and Bps horizons sampled from the Barme I profile. The evolution of Cu and DOC was followed after addition of two different phenols (catechol and protocatechuic acid) and after raising the pH.



Materials and Methods

Soil properties

The soil was an PODZOSOL OCHRIQUE (with a duripan character) (Référentiel Pédologique, 1995) and has previously been analysed and described (chapt.1). It represented the most evolved podzol of a sequence of soils developed on granitic moraines in the Swiss Alps and was dated to about 6'500 yr. The A-horizon underlayed a MOOR humus. In the A-horizon organic matter contributed for a larger part of the soil material (68%) and total CEC was 16.6 me. Organic matter content and CEC decreased in the leached horizon respectively to 5.4% and 4.3. A Bph-horizon was locally present but there was not enough material for sampling. In the Bps horizon organic matter increased slightly again to 12% and CEC to 7.5. pH, organic carbon, humic distribution and texture are presented in table 1 for the A, E and Bps horizons.

Horizon	depth	pH (H2O)	C total (mg/g)	% AF	% AH	%humine
A	0-15 cm	3.79	321	10.3	14.7	75.0
E	15-20 cm	3.87	25	15.3	18.1	66.7
BPs	20-30 cm	4.47	62	69.6	14.4	16.0

		%sand	% sand	%silt	%silt	
Horizon	depth	2-0.2 mm	0.2mm-50µm	50 -20µm	20-2µm	% clay
A	0-15 cm	52.5	15.8	9.6	14.9	7.1
E	15-20 cm	38.9	27	14.4	16.5	3.3
BPs	20-30 cm	45.4	20.6	10.5	16.9	6.6

Table 1. Some characteristics of the podzol profile of the Barme I moraine

The background level of Cu was 139.7 mg/kg in the A horizon, 6.8 mg/kg in the E-horizon and 3.3 mg/kg in the Bps horizon (chapt.1).

The content in phenol monomers was determined by the method of Gallet & Lebreton (1995) for each horizon, but phenols in this form were not present at a detectable level. Most of the phenols occurred in a polymeric form.

Adsorption

The procedure of Spack (1997) used to study the sorption of nitroaromatic compounds was adapted and modified for our experiment. 1 kg of fresh soil was sampled from each horizon. The soil was dried under a hood at room temperature during 3 hours and sieved at 2mm. 5g were deposited in 25ml pyrex tubes from corex ®. An alicot was kept to determine the dry weight of the samples.

The values used for contamination, were chosen from the average content in wine and orchard soils in Switzerland, which represents 238 mg/kg (Quinche, 1985).

Since the dry weight varied between 1.42 g for the humic horizon, 3.92 for the E-horizon and 4.8 g for the BPs horizon, the Quinche contamination was based on a medium dry weight of 2.5 g, which corresponded to 212 mg/L. 10 different solutions were prepared (table 2). For the Freundlich isotherm 80%, 50%, 25%, 10% and 5% dilutions of the solution n°1 were prepared. 10 ml of solutions n°1 to 5 was added to the soils. For the experiment, in which the pH was raised 5ml of solutions n°6 to 10 were added to the tubes. Titration with 0.1M NaOH was performed to raise the pH to 6 and the volume completed to 10ml with distilled water. A Cu-phenol ratio of 1:4 was kept in order to have all the Cu in the bonded form before adding it to the soil. The mixtures were equilibrated in a Heidolph Reax shaker during 1 hour, then centrifuged on a Sorvall®Dupont RC5C rotor during 15 minutes at 3'000 rpm. The supernatant was collected and filtrated on a 0.45µm, 25mm (Nylon Titon filtration systems SRI). DOC was measured on a TOC-5'000 Shimazu Total Organic Carbon analyser. Cu was measured from the supernatant with an Emission Plasma Spectrometer Plasma 2'000 for the higher concentrations and by Atomic Absorption on a Perkin Elmer 5'100 for the lower concentrations.

solution n°	CuSO4 (g/250ml)	added phenol	conc. (g/250)
1	0.053	none	none
2	0.053	catechol	0.093
3	0.053	protocatechuic	0.131
4	none	catechol	0.093
5	none	protocatechuic	0.131
6	0.106	none	none
7	0.106	catechol	0.186
8	0.106	protocatechuic	0.262
9	none	catechol	0.186
10	none	protocatechuic	0.262

Table 2. Different treatments of Cu contamination in the presence of phenols

Results and discussion

Sorption isotherms

Sorption isotherms carried out with the different horizons indicated the following order of Cu retention: A> Bp> E (figure 1.). 99% of Cu was retained to the A-horizon for the whole range of concentrations. For the Bps horizon, saturation was reached at about 45 ppm. The E horizon was less prone to retain Cu since saturation was reached at about 15 ppm.

Adsorption was not correlated with an increase of pH, since the A-horizon had the lowest pH and the highest retention ability.

Organic matter seemed to be the dominant factor to control the sorption process. In the A-horizon humine was the dominant fraction and in the Bps-horizon fulvic acid was the dominant fraction. This suggests that both fractions have a high affinity for Cu, and affect the distribution of Cu along the profile.

Since organic matter was the dominant fraction, the effect of texture was not apparent. Nevertheless, clay content followed the same evolution as organic matter and could play a retention role in the Bps-horizon.

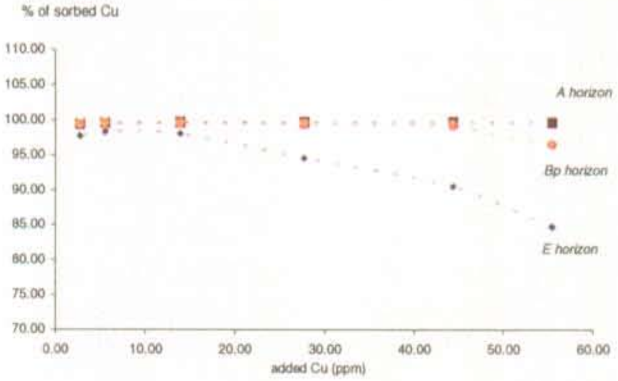


Figure 1. Freundlich isotherms of Cu in the presence of three horizons from an Ocric Podzol.

DOC analysis of the supernatant revealed the following: in the absence of Cu the solutions were dark brown due to soluble macromolecules and DOC evolved in the following order: Bps>A>E. In the presence of Cu the solutions became much clearer and DOC decreased with increasing Cu concentration. This indicated that a fraction of DOC was precipitated by Cu.

Treatment with phenols

Figure 3, shows the supernatant concentrations of Cu and DOC from our batch experiments.

Since Cu sorption to the organic fraction in the A-horizon was very strong, changes brought by the presence of phenols represented weak percentage, but still some variations in reactivities could be observed. The addition of the $CuSO_4$-catechol mixture contributed to increase by tenfold DOC concentration in the supernatant. Cu concentration decreased slightly compared to soil treated with $CuSO_4$ only and indicated that a fraction of the Cu-catechol complex must have precipitated. In the presence of the $CuSO_4$-protocatechuic acid treatment no change in DOC or Cu was observed. It was attributed to reactions of the –COOH groups of protocatechuic acid with some mineral components with higher affinity, for instance Fe, Al and Ca which can cause a co-precipitation.

In the E horizon Cu retention to the soil matrix was considerably reduced and therefore Cu concentration increased about 400 times compared with the A-horizon. DOC was only slightly higher compared to the A-horizon. In the presence of both phenols DOC and Cu increased. Catechol contributed to a higher extent for DOC increase, but this time the matrix interactions of the COOH groups of protocatechuic acid must have been weaker.

In the BPs horizon both phenols contributed to increase DOC in a similar trend as in the E horizon. Cu concentration in the supernatant was much lower compared to the E horizon, but protocatechuic acid contributed almost to double Cu concentration in the solution. In this horizon the markedly higher (4.5) pH was the main reason for keeping Cu in the solution. Since the pKa of the COOH group is between 4 and 4.3 the change in pH could displace the equilibrium to the deprotonated form and solubilise the complex.

Effect of pH

Raising the pH of the A-horizon, both Cu and DOC increased to a high extent in the solution. When either Cu or DOC in the supernatant was plotted versus the amount to NaOH added a linear regression could be drawn with a correlation coefficient exceeding 98%.

Figure 2. Cu and organic carbon dissolution from the A-horizon upon addition of NaOH .

This indicated that Cu is tightly bound to organic matter and its release can only be obtained by the solubilisation or dispersion of the humic fraction. It should thouhgh be noted that these properties are not strictly "pH effects" but can be attributed to the dispersion effect caused by the presence of Na. In the presence of $CaCO_3$, which is commonly used for liming the effect could be just the opposite since Ca is a flocculating cation.

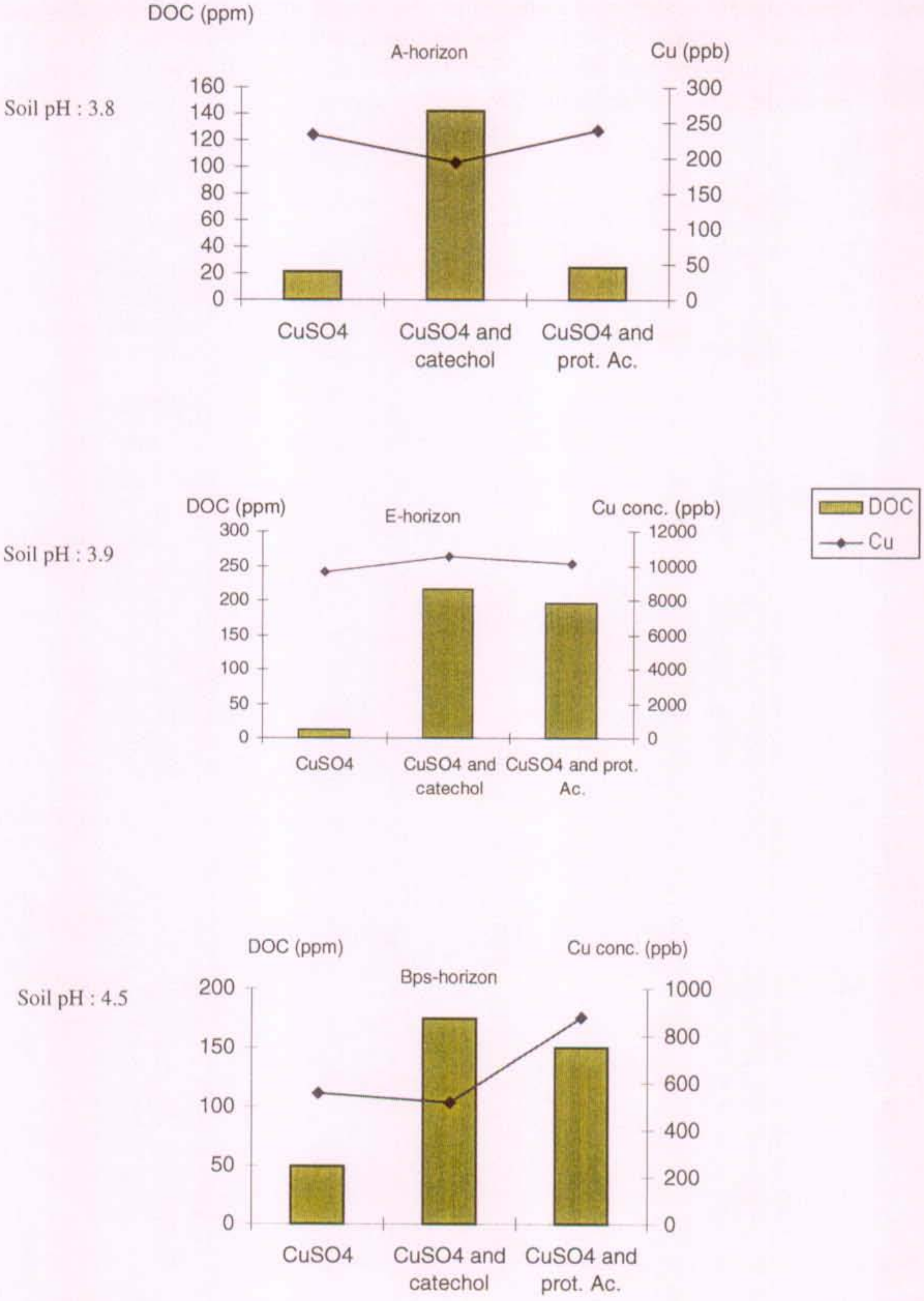


Figure 3. Cu and DOC evolution in soil solutions originating from an A-, E-, and a Bps horizon after different treatments in a batch experiment 1) upon addition of $CuSO_4$ alone, 2) a mixture of $CuSO_4$ and catechol, 3) a mixture of $CuSO_4$ and protocatechuic acid.

Conclusion

Sorption isotherms confirmed that even if physical parameters like soil porosity and texture can play and important role in determining the preferential flow-paths of water and it's organic and inorganic colloids, in this case surface organic matter and in particular the insoluble fraction, was the dominant mobilising parameter. By itself it can explain why very small amounts of Cu are leached from surface horizons of a podzol, despite the high accumulation levels, which exceed the values recommended by the Swiss legislation (Osol, 1998). Cu was instantaneously trapped and insolubilised. The high affinity for the humic fraction can be attributed to "catechol" groups present on macromolecules. Other groups like nitrogen amines could also participate in these interactions, but N is more is more easily metabolised by soil micro-organisms and therefore has a smaller chance to persist in the environment (McKeague et al., 1986).

The use of the two different types of phenols in our batch experiment confirmed the "precipitating" and "solubilising" effect of the functional entities located on a benzene ring, which had previously been observed by utilizing synthetic models (chapt.5 and 6). These properties could also be applied to the humic macromolecules. Depending on the pH, COOH groups become deprotonated and can bring back the complexes to the solution. Between pH 4 and 6 the changes in solubility caused drastic changes in DOC and Cu release to the solution.

The proposed mechanism can also contribute to explain the differential migration properties of metals like Cd, Pb and Zn. For instance Pb can interact with COOH in addition to the "catechol" groups, which is not the case for Cu. The number of COOH groups is often higher, compared to the "catechol" groups. Above the pKa values most of COOH group are therefore blocked by the metal and solubilisation is impeded. Nevertheless, these complexes are much more pH sensitive and with a slight decrease of pH, the metals can be released to the environment.

Literature cited

Gallet C.and Lebreton P. 1995. Evolution of phenolic patterns in plants and associated litters and humus of a mountain forest ecosystem. Soil Biol. Biochem. Vol.27, N°2, pp 157-165.

McKeague J.A., Cheshire M.V., Andreux F., and J. Berthelin.1986. Organo-mineral complexes in relation to pedogenesis. Interaction of Soil Minerals with Natural Organics and Microbes, SSSA spec. pub. N° 17:549-591.

Oess A. Seasonal Peaks Of Cu And Phenols In The Soil Solution Of A Sequence Of Alpine Podzols (Chapt.2).

Oess A. Setting The Scene...Preferential Distribution Pattern Of Cu And Other Cations On Podzol Profiles With Increasing Leaching Capacities In The Arpette Valley (Chapt.1).

Oess A., Cheshire M.V., McPhail D.B., and Vedy J.C. 1999. Polymerization: a Possible Consequence of Copper-phenolic Interactions. Ed. Berthelin et al.Plenum publisher, London.(Chapt.4)

Oess A., Cheshire M.V., McPhail D.B., Stoll S., El Alaili M., and Vedy J.C. Elucidation of Phenol-Cu Interaction Mechanisms by Potentiometry, ESR, UV Absorption Spectroscopy and Molecular Simulations. Science of The Total Environment, 228:49-58.(Chapt.5).

OSOL, 1998. Ordonnance sur les Atteintes Portées aux Sols du 1er Juillet 1998. Art.5.

Quinche J.P. 1985. Teneurs en Cuivre, Zinc, Plomb, Cadmium et Mercure des Sols de quelques Vignes de la Suisse et du Tessin. Revue suisse de Viti. Arboric. Hortic. 17 (6): 341-344.

Référentiel Pédologique, 1995. Baize D. and Girard M.C. Ed. INRA. Paris. pp332.

Römkens F.A.M. and Salomons W. 1998. Cd, Cu and Zn Solubility in Arable and Forest Soils: Consequences of Land Use Changes for Metal Mobility and Risk Assessment.Soil Science. 163:859-871.

Spack L. 1997. Utilisation De CO2 Supercritique Pour L'extraction De Résidus De Pesticides Nitroaromatiques Dans Les Sols. Thèse N°1731 EPFL, CH-1015 Lausanne.

Conclusion

L'étude du rôle des composés phénoliques issus de la lixiviation de la biomasse végétale et des horizons holorganiques sur la dynamique intrapédique du cuivre a été réalisée sur une chronoséquence caractérisée par des couvertures pédologiques à altération hydrolytique acide ou acidocomplexolytique issues de roche-mère cristalline pauvre en minéraux altérables.

Dans cette chronoséquence présentant une évolution des couvertures pédologiques passant de profils morphologiquement peu différenciés à des profils morphologiquement très différenciés le cuivre s'accumule préférentiellement dans les horizons holo- (O) et hémiorganiques (A). Toutefois les accumulations absolues et relatives augmentent en fonction de l'âge des sols donc, dans le cas présent, du degré de leur évolution morphologique mais aussi pédogénétique.

L'évolution saisonnière du pédoclimat de ces horizons O et A imputable, par exemple, à la fonte des neiges, aux variations cycliques de la température, à la dynamique saisonnière de la biomasse et de la nécromasse, etc., présente une influence certaine sur la dynamique intrapédique du cuivre au plan, notamment, de l'importance relative des transferts par rapport aux immobilisations. Mais si ces évolutions saisonnières du pédoclimat semblent jouer un rôle il est difficile de préciser, en l'état de nos recherches, quels seraient de tous les paramètres de ce pédoclimat celui ou ceux qui serait le ou les plus déterminants.

L'analyse périodique de la fraction gravitaire de la solution du sol a mis en évidence la présence conjointe de cuivre et de phénols mais surtout leur interactivité : les phénols représentent donc les vecteurs essentiels du transfert intrapédique du cuivre dans les systèmes sol-végétation étudiés.

Toutefois si les phénols sont abondants dans la biomasse végétale qui présente donc, a priori, un potentiel élevé pour la complexation organo-métallique, la situation apparaît fort différente dans la solution du sol. Dans l'eau gravitaire, en effet, les phénols monomères sont à des concentrations inférieures aux limites de détection telles que permises par les actuelles méthodes d'analyse quantitative. Ceci indique que les phénols issus de la biomasse et des litières en voie de sénescence s'incorporent très rapidement aux horizons hémiorganiques, au fur et à mesure de leur passage dans l'eau gravitaire, ou bien alors, en restant dans la solution du sol, s'oxydent et se polymérisent en donnant naissance à des di-, tri-, ou hexamères phénoliques (Andreux, 1981, Adrian, 1986, McBride *et al.*, 1988 ;1990).

L'étude des mécanismes biochimiques a bel a mis en évidence la formation instantanée de complexes de sphère interne Cu-phénols. En particulier les ortho-dihydroxyphénols communément appelés catéchols ou catécholates peuvent former des complexes mono-bisdenté ou di- voir tri-bisdenté faisant intervenir la fonction catéchol. L'application de la spectroscopie RPE conjointement à d'autres techniques analytiques sur des molécules simples, mais variant juste d'une fonction sur le cycle benzénique a permis de mettre en évidence des propriétés qui jusqu'ici restaient peu connues. En particulier la forte interaction des fonctions « catéchol » ou « dihydroxyphénols » a pu être mise en évidence. Des complexes de sphère internes sont formés et la complexation est indépendante du pK_a des groupes « catéchol » qui varient entre 9 et 12. Des complexes ont même pu être observés à partir de pH3.8. L'activation de la réaction dépend surtout de l'affinité du Cu pour les 2 O et de la stabilité du nouveau cycle à 5 membres qui est formé. La liaison du Cu au ligand contribue elle même à diminuer le pH en déplaçant les protons vers la solution.

L'augmentation du pH contribue à augmenter le nombre de ligand autour du Cu qui peut varier de 1 à 3. Malgré un pK_a beaucoup plus bas pour la fonction COOH (entre 4 et 5) il y a tout au plus un complexe de sphère externe qui est formé, mais en tout cas pas de complexe de sphère interne. Enfin, bien que la fonction COOH ne participe pas directement dans la complexation, elle joue un rôle non moins important sur la solubilité des complexes lorsqu'elle se trouve sur un site voisin du catéchol de la même molécule. La solubilité du complexe est cette fois-ci être fortement dépendante du pK_a et du pH de la solution. Lorsque le groupement COOH se trouve sous forme dissociée le complexe reste en solution. Lorsqu'il reste sous forme protonée le complexe précipite.

Ces propriétés s'appliquent également aux macromolécules humiques et permettent de répondre aux questions que nous nous sommes posés au départ : **comment le Cu migre-t-il dans les sols ? dans quelle mesure la phase humique du sol joue-t-elle un rôle de mobilisateur ou d'immobilisateur ? qu'elle est l'importance des phénols dans ces phénomènes.**

En prenant les questions dans l'ordre inverse de leur formulation, voici les réponses que l'on peut donner :

Il est possible d'affirmer que les phénols de part leur affinité pour le Cu et leur attitude à polymériser constituent une fraction active du sol. De plus les phénols en tant que précurseurs et produits de dégradation de l'humus constituent une fraction active de la matière organique.

Les acides humiques, fulviques ainsi que l'humine peuvent tous être constitués de phénols, comporter des groupement COOH et catéchol, mais dans des proportions variables. Les interactions du Cu avec les différentes fractions de l'humus vont par conséquent être similaires. Cependant les encombrements moléculaires, les degrés d'aromaticité et des densité diffèrent des phénols monomères. Le rapport « fonctions COOH / densité de molécule » sera donc déterminant du comportement du métal. En admettant que chaque molécules soit pourvue de groupements de types catéchol, les réactions peuvent être décrites par trois niveaux d'équilibre.

Dans le premier cas le nombre de groupements COO^- par rapport à la densité de la molécule n'est pas suffisant pour maintenir les complexes en solution. Le métal est alors piégé par la matière organique à la surface du sol.

Dans le deuxième cas le nombre de groupements COO^- par rapport à la densité de la molécule est juste en équilibre et un simple changement de pH peut faire précipiter ou dissoudre les complexes. Dans ce cas on aura une migration opportuniste en fonction du pH.

Dans le troisième cas, le nombre de groupements COO^- par rapport à la densité de la molécule dépassera largement la quantité nécessaire à maintenir le complexe en solution. Alors les conditions de migration seront optimales.

Considérons maintenant ce qui ce passe au Val d'Arpette. La forte accumulation en surface et un degré de sorption avoisinant 100% correspond au premier abord au premier cas avec un nombre insuffisant de groupements COOH des substances humiques de surface dans l'état ionisé. La rétention du Cu en surface malgré les fortes précipitations indique également une forte présence de groupements « catechol » qui permettent de fixer le Cu à la matrice insoluble même a un pH relativement bas. Cependant, comme l'a montré l'expérience d'adsorption (chap.7) une augmentation de pH suffira à libérer le Cu et des substances organiques. Par conséquent nous sommes dans deuxième cas.

Cette situation présente un danger potentiel de migration en cas d'augmentation du pH de l'eau de pluies et illustre bien les problèmes rencontrés lors de changement d'utilisation d'un terrain. Ce n'est pas l'acidité du sol proprement dite, qui est importante dans ce cas mais le comportement opportuniste des fractions organiques présentes.

Ces mécanismes contribuent à expliquer de nombreuses réactions ayant lieu à l'interface plante - sol – solution du sol. Entre autre ils expliquent la migration différentielle des différents groupes d'ETM (Tyler, 1981 ; Keller & Domergue, 1996, Wang & Benoît, 1996). Tous les métaux n'ont pas la même affinité que le Cu pour les fonctions catechol. Certains ETM peuvent interagir plus fortement avec les groupements COOH et donc précipiter des molécules qui resteraient en solution dans le cas du Cu. Reste donc à démontrer quels métaux interagissent avec quels sites.

D'autre part, ces paramètres de mobilisation et d'immobilisation du Cu s'appliquent dans de nombreux contextes comme par exemple, le stockage des déchets et la décontamination des sols. Tant pour le développement de techniques douces comme la biorémédiation ou la phytorémédiation que pour celui des techniques plus sophistiquées (électrodialyse) il est indispensable de connaître la nature des interactions qui maintiennent le métal lié au substrat.

Les équilibres qui maintiennent le métal sorbé aux différentes fractions organiques et minérales sont particulièrement sensibles aux changement d'utilisation des sols. Rien que la transition d'une couverture végétale où la strate herbacée est dominante vers une forêt de frêne ou d'épicéa modifie considérablement les changement de signature des phénols et des polysaccharides dans les sols (Sanger et al., 1997). Dû aux restructuration socio-économiques de l'Europe on assiste actuellement dans de nombreuses régions à une afforestation de sols qui étaient préalablement utilisés pour l'élevage ou l'agriculture. En général l'afforestation conduit à une diminution de pH et une augmentation de matière organique (Moolenaar et al., 1998). Par conséquent les risques de contamination des nappes par des sols qui étaient auparavant traités avec des fertilisants sont énormes.

Les augmentations de CO_2 dans l'atmosphère liées au réchauffement climatique risquent également de perturber l'équilibre des sols. L'augmentation de CO_2 stimule les réactions du sol et peut provoquer une séquestration du C dans le sol et les plantes (Bouwman & Germon, 1998). Les effets sur la distribution de C solide – C soluble , sur le degré d'oxydation de la matière organique, et sur le rapport « groupements COOH – catéchol » restent à être élucidés.

Les perspectives pour l'avenir seraient donc de déterminer par une approche combinée de modélisation et d'expérimentation les domaines limites où les rapports « C solide – C soluble » ainsi que les rapports « groupements ionisables »- « groupements chélatants » et « densité des macromolécules » peuvent faire basculer l'état d'un sol d'une situation où les ETM sont immobiles vers un état de migration et de contamination subséquente des autres systèmes connectés.

Conclusion

The importance of phenols released by plants and organic horizons to Cu mobilisation and immobilisation processes was determined on a sequence of soils characterised by increasing "acid hydrolytic" and "acidocomplexolytic" morphologies.

Soil morphologies evolved from slightly differentiated horizons to an intense stratification. Cu was mostly distributed between the holo-(O) and hemiorganic (A) horizons. The total and relative Cu accumulation increased with soil age and thus corresponded to morphological stratification and soil evolution.

Seasonal parameters, such as soil climate, snow-melting, increase of temperature and biological activity contributed all to some extent to Cu leaching. However, no obvious dominant parameter was observed during the three years of this study. Regular sampling of the soil solution after rainfall events revealed the co-presence and interaction of Cu and phenols. It was therefore possible to affirm that phenols represent the "active" organic matter fraction in the process of Cu translocation.

Phenol quantification in plant biomass revealed a high potential release of these molecules. However, in the soil and soil solution phenol monomer concentrations were just below the detection limit. This was attributed either to a rapid incorporation of phenols in the humic fraction during the release and degradation process and, (or) to an oxidative polymerisation reaction giving place to the formation of dimers, trimers and polyphenolic structures (Andreux, 1981; Adrian *et al.*, 1986; McBride *et al.*, 1988;McBride & Sikora,1990), respectively.

The molecular studies of Cu interaction with phenols confirmed the spontaneous formation of an inner-sphere complex. In particular, ortho-dihydroxyphenols, also called "catechols", were involved in the formation of mono-bisdentate, di- and tri-bisdentate structures. New reactivities of phenols were discovered by applying ESR spectroscopy to a range of simple molecules varying only by the substitution on the benzene ring. The specific role of catechol functions was emphasised. Complexation was independent of the pK_a values of catechol groups, which varied between pK_a 9 and 12. Cu-phenol complexes could be identified for pH values as low as 3.8. The activation of the reaction depended mostly on the affinity of Cu for the 2 O and on the stability of the new formed 5 membered chelate ring. The bonding of Cu itself provoked the release of protons to the solution and contributed to a decrease of the pH. An increase in pH contributed to an increase of the number of complexed ligands. The number of ligands varied between 1 and 3. Despite the lower pKa value of the COOH group (between 4 and 5) no inner-sphere complex was identified. The formation of an outer-sphere complex is though possible. Although the COOH function was not directly involved in complexation, it did participate in the solubilisation of complexes, when positioned on an adjacent site of catechol. In this case the solubility of the complex depended on the pK_a of the molecule and on the pH of the solution. When the COOH group was deprotonated the complex remained soluble. When the COOH group was protonated the complex precipitated.

These properties were also apply to humic macromolecules and permitted to answer the initial questions: **how does Cu migrate in soils? To what extent is Cu migration controlled by complexation with the humic fraction of soils? What is the importance of phenols in these processes?**

Both the affinity of phenols for Cu and polymerisation reactions indicated that phenols constitute an active fraction in soils. As degradation products and precursors of humic substances in soils they represent the active fraction of organic matter.

Humic and fulvic acids as well as humine all bear phenolic components, catechol and COOH functions, to a variable extent. Therefore similar interactions with Cu do occur. However, bulk density, and the number of aromatic compounds are not the same. The ratio of "COOH groups" compared to the bulk density of the molecule is the controlling factor of the fate of the metal. If we assume that each molecule bears at least one catechol group, then the reactions involved can be expressed by three equilibrium situations.

In the first situation the ratio of COO^- groups compared to the bulk density of the molecule is not sufficient to maintain the complex in solution. The metal is then trapped in the organic fraction at the soil surface.

In the second situation the ratio of COO^- groups compared to the bulk density of the molecule is just in equilibrium and a simple change in pH is sufficient either to precipitate or to dissolve the complex. In this case we can qualify migration to be opportunistic.

In the third situation, the ratio of COO^- groups compared to bulk density exceeds largely the amount required to keep the complex in solution. In this case migration conditions are optimal.

Let us consider the Arpette valley case. The high surface accumulation and the high sorption properties of the surface horizons suggest that we are in the case described by the first situation with a deficiency in ionised COOH groups. Surface accumulation of Cu indicates a strong participation of catechol type groups in this fraction which is unable to sorb Cu despite the low pH and the heavy rainfalls. But an increase in pH will release both Cu and dissolved organic carbon to the solution as it was emphasised in the sorption experiment (chapt. 7). The Arpette soils correspond therefore to the third situation.

In the case of an increase in pH of the rainwater or snow, a risk of Cu migration should be considered. Nevertheless, pH is not the main parameter. The origin of the problem lies in the presence of "opportunistic" organic matter.

These mechanisms explain a number of reactions taking place at the plant-soil-soil solution interface. They can explain why different patterns of trace metal leaching have been observed (Tyler, 1981; Keller & Domergue, 1996; Wang & Benoit, 1996). Different trace metals have different affinities with organic matter and in particular with the catechol groups. Some metals can make stronger interactions with the COOH groups and thus precipitate (which would not be the case for Cu). The preferential interaction of other metals with functional groups still remains to be investigated.

Mobilisation parameters have different applications as for example the storing of metal containing wastes and soil decontamination. In order to develop "soft" remediation techniques eg. for phytoremediation and bioremediation or more sophisticated techniques like electrodialysis, it is necessary to understand the nature of these interactions, which keep the metal sorbed to the substrate.

The equilibria, which keep the metal sorbed to the soil surface are particularly sensitive to the change in land use. The change of plant cover from grass to ash or spruce can considerably modify the signature of phenols and carbohydrates in soils (Sanger *et al.*, 1997). An increasing number of land surfaces are currently being reorganised due to economical decisions within the European community. In several regions cultivated soils are planted with trees. In general afforestation leads to a decrease in pH and an increase in organic matter (Moolenaar *et al.*, 1998). The risk is considerable that these soils, which have previously been treated with various fertilisers, become potential contaminants of the groundwater.

Increase in atmospheric CO_2 in relation with climatic changes can also affect soil equilibrium. An increase in CO_2 activates soil reactions and can cause C sequestration in soils and plants (Bouwman & Germon, 1998). The effect on solid and soluble carbon distribution, on the rate of organic matter oxidation and on the "COOH – catechol" ratio still remains to be elucidated.

For future perspectives it would be interesting to determine the limits where "solid C : soluble C", "ionised groups: chelating group" ratios as well as the bulk density can modify a soil and transform it from a state where metals are immobilised to a state where migration and the subsequent contamination of the connected systems occurs.

References

Adrian P., Andreux F., Metche M., Mansour M., Korte F., Lavollay J. 1986. Autoxydation Des Ortho-Diphénols Catalysée Par Les Ions Fe^{2+} Et Mn^{2+}: Un Modèle De Formation Des Acides Humiques. C.R. Acad. Sc. Paris, T. 303:1615-1618.

Andreux F. 1981. Utilisation De Molécules Modèles De Synthèse Dans L'étude Des Processus D'insolubilisation Et De Biodégradation Des Polycondensats Humiques. Bulletin De L'AFES 4:271-292.

Bouwman A.F. and Germon J.C. 1998. Soil Global Change. Oral communication. 16th World Congress on Soil Science. Montpellier. Symposium 26, n° 8026.

Keller C., and Domergue F.-L. 1996. Soluble And Particulate Transfers Of Cu, Cd, Al, Fe And Some Major Elements In Gravitational Waters Of A Podzol. Geoderma 71:263-274.

McBride M.B. and Sikora F.J. 1990. Catalysed Oxidation Reactions Of 1,2-Dihydroxybenzene (Catechol) In Aerated Aqueous Solutions. Journal Of Inorganic Biochemistry 39:247-262.

McBride M.B., Sikora F.J., and Wesselink L.G. 1988. Complexation And Catalysed Oxidative Polymerisation Of Catechol By Aluminum In Acidic Solution. Soil Sci. Soc. Am. J. 52:985-993.

Moolenaar S.W., Temminghof E.J.M., and De Haan F.A.M. 1998. Modelling Dynamic Of Copper Balances For A Contaminated Sandy Soil Following Land Use Change From Agriculture To Forestry. Environmental Pollution 103:117-125.

Sanger L.J., Anderson J.M., Little D., and Bolger T. 1997. Phenolic And Carbohydrate Signatures Of Organic Matter In Soils Developed Under Grass And Forest Plantations Following Changes In Land Use. European Journal Of Soil Science, 48:311-317.

Tyler G. 1981. Leaching Of Metals From The A-Horizon Of A Spruce Forest Soil. Water, Air, And Soil Pollution, 15: 353-369.

Wang E.X., and Benoît B. 1996. Mechanisms Controlling the Mobility of lead in the spodosols of a northern hardwood forest ecosystem. Environ.Sci. Technol. 30:2211-2219.

CURRICULUM VITAE

Andrea Oess

PRESENT ADRESS :

Institut d'Aménagement des Terres et des Eaux (IATE-P)
Département de Génie Rural (DGR)
École Polytechnique Fédérale de Lausanne (EPFL)
CH-1015 Lausanne

Andrea.Oess@ epfl.ch

FAX: +41 (21)-693-56-70
TELEPHONE: +41 (21)-693-37-67

PERSONAL DATA

Date and Place of Birth:	August 2. 1966, in Helsinki (Finland)
Family status:	Single
Nationality:	Swiss

LANGUAGES

French: mother tongue; English: fluent; Swedish: fluent; German: fluent; Italian: almost fluent

EDUCATION

Diploma in Biochemistry	Geneva University 1994
Licence in Biochemistry	Geneva University 1993
Certificat de Maturité	Collège Claparède 1986

RESEARCH ACTIVITIES

Research Assistant in EPFL 1994 - today

Development of field and laboratory methods to study Cu interactions with organic compounds present in plant, soils and the soil solution. Competency in the following domains has presently been acquired : TEM (Transmission electron microscopy) and staining techniques, ESR (Electron spin resonance spectroscopy), Potentiometry, HPLC-UV, Molecular simulations, extraction, adsorption, preconcentration, podzols.

TEACHING ACTIVITY

Teaching soil chemistry and in particular « the interaction of heavy metals with organic matter » for :

- Master's in Rural Engineering at the EPFL,
- Master's in Natural Sciences of the Environment at Geneva and Lausanne University.
- Postgraduate studies in Environmental Sciences at the EPFL,

Supervising a student's research project for a Master's in Environmental Science »
1998-1999

Supervising a student's project for a postgraduate certificate in Natural Sciences of the Environment ».
1996-1997

Teaching chemistry, biology, math's, physics, in the secondary school in Geneva from 1991 to 1993.

OTHER ACTIVITIES, AWARDS AND HONOURS

Aikido training since 1997.
1998 Trekking in the Annapurna range, 1997 Trekking on Mount Kenya
1997 Ovronnaz (Switzerland) first price in the national Telemark race (all women's categories except pro's)
1997 Verbier (Switzerland) second price in the Chinese Telemark race (all women's categories including pro's)
Yoga training since 1995
Member of the Botanic Society of Geneva since 1992
Member of the Zoological Society of Geneva since 1992
Member of the Swiss Alpine Club since 1997

PUBLICATIONS

Oess A., Cheshire M.V., McPhail D., Stoll S., El Alaili M., Vedy J.C. 1999. Elucidation of Phenol-Cu interaction Mechanism by Potentiometry, ESR, UV Absorption Spectroscopy and Molecular Simulations. Science of the Total Environment, 221, 1. Elsevier Science Ireland.

Oess A., Cheshire M.V., McPhail D.B., Spack L., El Alaili M. & Védy J.C. 1999. accepted for publication. Interaction of plant and soil phenol with Cu pollution. Colloque de l'IHSS 1997: Analyse et Diversité des Substances Humiques Naturelles, accepted for publication *in* Analusis.

Oess A. Cheshire M.V., McPhail D.B., & Vedy J.C. 1999. Polymerisation : a Possible Consequence of Copper-phenolic interactions *In* Effect of Mineral-Organic- Micro-organism Interactions on Soil and Freshwater Environments.Ed. Berthelin *et al.* Plenum press.

Oess A., McPhail D.B., Cheshire M.V. & Védy J.-C. 1997. Copper Complexation with Plant-derived Phenols Originating from an Alpine Ecosystem. Extented abstract. Fourth International Conference on the Biogeochemistry of Trace Elements, p. 233-234, Iskandar I.K, Hardy S.E., Chang A.C., Pierzynski G.M Editors, Berkeley CA, USA.

Degueldre C., Grauer R., Laube A. , Oess A. & Silby H. 1996. Colloid Properties in Granitic Groundwater systems.II: Stability and Transport Study. Applied Geochemistry , Vol. 11, pp. 697-710.

ORAL PRESENTATIONS AND POSTERS

Oess A. 1998. Oral Communication. Cu^{2+} Interaction With POM's (Pedogenic Organic Matter), It's Affinity For Phenols And Implications For Mobility In The Environment. Minisymposium On Geochemistry And Surfaces. Nov.1998, Copenhagen.

Oess A., Cheshire M.V., Mcphail D., Stoll S., El Alaili M., Vedy J.C. 1998. Poster Presentation. Elucidation Of Phenol-Cu Interaction Mechanism By Potentiometry, ESR, UV Absorption Spectroscopy And Molecular Simulations. 16ème Congrès Mondial De Science Du Sol. Monpellier.

Oess A. 1998. Oral Communication. Pédogénèse Acide Sur Moraines Alpines (Exemple Du Val d'Arpette) . Présentation À L'association Française Pour L'étude Du Sol. 2 Avril 1998, INRA (Institut National De La Recherche En Agronomie) Paris.

Oess A., Cheshire M.V., Mcphail D.B., Spack L., El Alaili M. & Védy J.-C. 1997 Phenol Release From Plants And Complexation With Cu On Three Alpine Moraines . Colloque De L'ihss: Analyse Et Diversité Des Substances Humiques Naturelles.

El Alaili M., Oess A. & Védy J.-C. 1997. Poster Presentation. Approche Ph-Métrique Des Complexes Cu-Phénols En Solution Du Sol , 5ème Rencontre Du GRAPE: Pollution Majeure En Rhône-Alpes Et Régions Voisines, 10 Juin 1997, Grenoble.

Oess A., Mcphail D.B., Cheshire M.V. & Védy J.-C. 1997. Oral Communication. Copper Complexation With Plant-Derived Phenols Originating From An Alpine Ecosystem . Fourth International Conference On The Biogeochemistry Of Trace Elements, P. 233-234, Iskandar I.K, Hardy S.E., Chang A.C., Pierzynski G.M Editors, Berkeley CA, USA.

Oess A. Cheshire M.V., Mcphail D.B., & Vedy J.C. 1996. Oral Communication. Polymerisation : A Possible Consequence Of Copper-Phenolic Interactions. Second International Symposium On Effect Of Mineral-Organic- Micro-Organism Interactions On Soil And Freshwater Environments.

Oess A., Cheshire M.V., Mcphail D.B. & Védy J.-C. 1996. Poster Presentation. Copper Complexation With Phenols Extracted From Blueberry Leaves . International Workshop On Interactions Of Small Solutes With Materials Of Environmental Relevance, October 20-25, 1996, Monteverita Switzerland.