

Volume II

ENVIRONMENTAL IMPACT of SOIL COMPONENT INTERACTIONS

*Metals, Other Inorganics, and
Microbial Activities*

Edited by
P.M. Huang
J. Berthelin
J.-M. Bollag
W.B. McGill
A.L. Page



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PREFACE

Minerals, organic components, and microorganisms are integral parts of the pedosphere and associated environments. They profoundly affect the physical, chemical, and biological properties of soils and sediments. During the past few decades, scientific accomplishments in individual disciplines of the physics, chemistry, and biology of soils were impressive. However, information on interactions of soil minerals with organic components and microorganisms was fragmentary and scattered in the literature of soil and environmental sciences around the world. Yet these three groups of components are not separate “kingdoms”, but rather a “united kingdom” constantly in close association with each other in the environment.

In view of the significance of the interactions of soil minerals with natural organic components and microorganisms, a special publication entitled *Interactions of Soil Minerals with Natural Organics and Microbes* was published in 1986 by the Soil Science Society of America. The second printing was published in 1989. This special publication provided an examination of these fundamental constituents and processes. It brought together new knowledge on how soil minerals affect the dynamics and transformations of natural organics and metabolic processes, the growth, adhesion, and ecology of microbes, and how natural organics and microbes affect the weathering transformations, properties, and reactivities of soil minerals.

To promote teaching and research on the interactions of soil minerals with organic components and microorganisms and the impact on the production of foodstuffs and fibers, the sustainability of the environment, and human health at the international level, the International Society of Soil Science (ISSS) established the Working Group MO entitled “Interactions of Soil Minerals with Organic Components and Microorganisms” in 1990. With the cosponsorship of the Canadian Society of Soil Science, the ISSS Working Group MO held the first workshop entitled “*Impact of Interactions of Inorganic, Organic and Microbiological Soil Components on Environmental Quality*” in Edmonton, Alberta, Canada, on August 11 to 15, 1992. The workshop focused on the impacts of interactions of soil minerals, organic components, and microorganisms on the dynamics and transformations of metals, other inorganics, xenobiotics, toxicity, microbial activities, pathogenesis, and related environmental quality.

The Organizing Committee received 86 papers from 20 countries located in North America, Central America, South America, western Europe, eastern Europe, Asia, Africa, and Oceania (Australia, Austria, Canada, Chile, China, Congo, Costa Rica, Egypt, France, Germany, India, Israel, Italy, Japan, Mexico, The Netherlands, New Zealand, Poland, U.K., and U.S.). Many scientists of international stature were invited to present key papers at the workshop. It was a major step forward in bringing together the fragmented literature on the environmental impact of the interactions of inorganic, organic, and microbial factors of soils. Such information served to identify gaps in our knowledge and as such provided future direction to stimulate scientific research in this important area of science. Thus, the workshop provided a forum for the exchange of information among soil and environmental scientists from around the world who have common interests and scientific pursuits, and for the subsequent technology transfer for the development of innovative management strategies for environmental sustainability. Furthermore, the workshop established avenues of communication on the subject matter among soil and environmental scientists in developing and developed countries to protect the global environment.

This book, composed of two volumes, is a compilation of 47 research papers and four position papers that were presented at the ISSS Workshop and have been accepted for publication after critical peer review. Volume I deals with the environmental impact of soil component interactions on general soil quality and transformations of natural and anthropogenic organic compounds. Volume II covers the environmental impact of soil component interactions on toxic metals, other inorganics, and microbial activity, pathogenesis, and biotechnology. This externally refereed book arising from the Workshop should be useful for teaching, research, and technology transfer on environmental protection at the international level. It is hoped that the book will serve as the seed for the germination, growth, and flourishing of this exciting area of scientific and educational pursuits for years to come.

We are grateful to the authors who have contributed chapters to this publication and to the external referees who have provided invaluable critical inputs to maintain the quality of this book. Gratitude is extended to the sponsors, Agriculture Canada, Alberta Oil Sands Technology and Research Authority, Canberra Packard Canada Ltd., Cominco Fertilizers, ESSO Chemical Canada, International Minerals & Chemical Corporation Canada Ltd., Natural Sciences and Engineering Research Council of Canada, Potash and Phosphate Institute of Canada, Summer Rain Ltd., and United Nations Environment Programme, who have provided the funding to make the workshop possible. Sincere appreciation is also extended to the Department of Soil Science, University of Saskatchewan, for the excellent support during the preparation of this publication.

**P. M. Huang
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P. M. Huang received his Ph.D. degree in soil science at the University of Wisconsin, Madison, in 1966. He is currently professor of soil science at the University of Saskatchewan, Saskatoon, Canada. His research work has very significantly advanced the frontiers of knowledge on the nature and surface reactivity of mineral colloids and organomineral complexes of soils and sediments and their role in the dynamics, transformations, and fate of plant nutrients, toxic metals, and xenobiotics in terrestrial and aquatic environments. His research findings, embodied in over 180 refereed scientific publications, including research papers, book chapters, and books, are fundamental to the development of sound strategies for managing land and water resources.



He has developed and taught courses in soil physical chemistry and mineralogy, soil analytical chemistry, and ecological toxicology. He has successfully trained and inspired M.Sc. and Ph.D. students, and postdoctoral fellows and received visiting scientists worldwide. He has served on numerous national and international scientific and academic committees. He also has served as a member of many editorial boards such as the *Soil Science Society of America Journal*, *Chemosphere*, and *Advances in Environmental Science*. He is an associate member of the Commission of Environmental Analytical Chemistry of the International Union of Pure and Applied Chemistry and Chairman of the Working Group MO "Interactions of Soil Minerals with Organic Components and Microorganisms" of the International Society of Soil Science. He is a fellow of the Canadian Society of Soil Science, the Soil Science Society of America, and the American Society of Agronomy, and is listed in *Who Is Who in the World*.

Jacques Berthelin is research director at the Centre National de la Recherche Scientifique (CNRS) at the Centre de Pédologie Biologique in Vandœuvre-les-Nancy, France.

He was born in 1940 and received a degree in biochemical engineering (qualified engineer) from the Ecole Nationale Supérieure de Brasserie, Malterie et Biochimie of Nancy, in 1964.

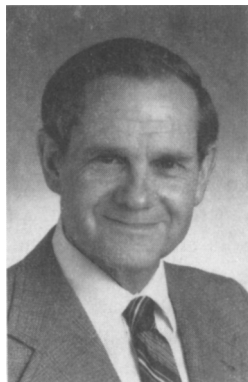
He prepared a thesis in the specialty of biochemistry, presented in 1967, and was also a midshipman and sublieutenant in the navy for 1½ years.

He joined the CNRS in 1968 in the Department of Earth Sciences at the Centre de Pédologie Biologique to conduct research in the field of soil biochemistry and microbiology, and has been a Doctor ès Science since 1976.

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Jean-Marc Bollag is professor of soil biochemistry and co-director of the Center of Bioremediation and Detoxification, Environmental Resources Research Institute, at The Pennsylvania State University. He is the author or coauthor of more than 160 professional papers and serves on the editorial board of five international journals. He is a frequent lecturer at conferences and seminars throughout the world. A recipient of the Julius Baer Fellowship, the Gamma Sigma Delta Research Award, and the Badge of Merit from the Polish Ministry of Agriculture, Dr. Bollag is a fellow of the American Academy of Microbiology, the Soil Science Society of America, and the American Society of Agronomy. He is a member of the International Society of Soil Science, the International Humic Substances Society, and the American Society for Microbiology.



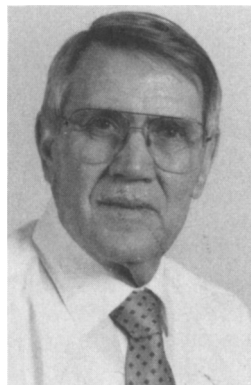
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W. B. McGill is a professor in the Department of Soil Science, University of Alberta, where he has been since 1971 after completing his Ph.D. degree at the University of Saskatchewan and M.Sc. and B.S.A. degrees at the University of Manitoba. He teaches Soil Microbiology and Biochemistry, Advanced Soil Biochemistry, and Waste Management and Utilization. In addition he has taught Soils and People and has contributed to short courses on environmental topics for industry. His research focuses on the fate of organic materials in soil and mechanisms controlling cycles of elements. He has used isotopic tracers to follow elements and simulation modeling to integrate diverse studies. This has led to knowledge of soil-plant systems on one hand, and questions about methods to monitor specific soil organisms on the other. He has supervised 22 graduate students to completion and has been an external examiner for seven dissertations from Canada and Australia; he has published over 65 refereed papers or reports and over 90 additional scientific contributions, and has given numerous invited lectures both nationally and internationally. He has international research/teaching experience in Australia, China, Thailand, and the U.S.



Dr. McGill was chair of the Department of Soil Science from 1979 to 1989, and associate dean, Faculty of Graduate Studies and Research, University of Alberta, from September 1991 through December 1993. He served as president of the Canadian Society of Soil Science during 1992–93. He was awarded the University of Manitoba Gold Medal for highest academic standing in Honors Agriculture upon graduation in 1967. In 1984–85 he received a Senior Research Fellowship from CSIRO, Division of Tropical Crops and Pastures, Brisbane.

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Dr. Page has received several professional awards. He is a fellow of the American Society of Agronomy and the Soil Science Society of America, the recipient of a Guggenheim Memorial Foundation Fellowship, and a Fulbright Fellowship. He has received a U.S. Department of Agriculture Distinguished Service Award, the American Society of Agronomy's Environmental Quality Research Award, the Association of Metropolitan Sewerage Agencies Environment Award, and a University of California, Riverside, Distinguished Teaching Award.



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*Part I: Transformations of Toxic Metals
and Other Inorganics as
Influenced by Soil Colloids and
Microorganisms*



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Effect of Microorganisms on Mobility of Heavy Metals in Soils

J. Berthelin, C. Munier-Lamy, and C. Leyval

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I. INTRODUCTION

The levels of trace metals in all environmental compartments (air, water, soil) are becoming increasingly stressful and toxic with contributions from a wide variety of industrial and domestic activities.¹ On a global scale, the emissions of Pb, Cd, V, and Zn from anthropogenic sources exceed those from natural sources by 12-, 5-, 3-, and 3-fold, respectively.¹ For other metals (Sb, As, Cr, Cu, Hg, Ni, and Se), the industrial emissions are comparable or slightly exceed the natural fluxes.¹ Soils represent the major sink for trace metals released into the biosphere.

In soils, trace metals and, in particular, heavy metals, such as Cd, Pb, Cu, Zn, and Cr, occur in a variety of speciation. They can be present as soluble compounds (e.g., ions, metal complexes) or as exchangeable elements, but they are mainly immobile and associated with different major fractions or soil compartments (carbonates, ferric and manganic oxyhydroxides, organic matter, residual parent materials).²⁻⁵ Hence, they present a risk of toxicity depending on their rate of transfer from these soil compartments to the soil solution, plants, groundwaters, and, more generally, to the food chain.

The mobility and bioavailability of heavy metals are affected by adsorption on mineral surfaces, precipitation as salts, formation of stable complexes with organic compounds, and by different solubilization and insolubilization processes that are influenced by many factors⁵ (pH, redox potential, nature of soil constituents, temperature, cation exchange capacity, etc.). However, soils are interactive systems between plants, soil constituents, and soil organisms (mainly microorganisms), where available energy, already present as organic and inorganic compounds or introduced as plant materials, promotes microbial activity⁶⁻⁸ and microbial weathering processes.⁹

Based on these considerations, it can be suggested that the behavior of heavy metals depends not only on the physicochemical parameters of soil, but also on biological factors associated, to a large part, with the microbial activities of soil–plant systems.

The interactions between microorganisms and heavy metals in soils can be divided into two main types of phenomena:

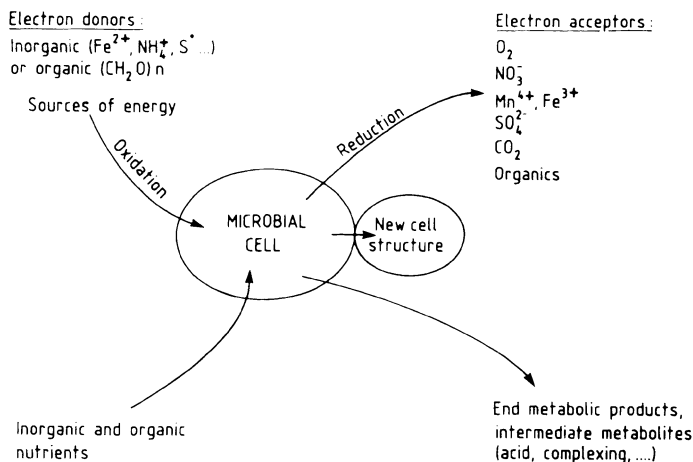


Figure 1 A simplified diagram of general microbial activity.⁹

- the influence of heavy metals on microbial populations, communities, and on their activities (e.g., cellulose and pectin decomposition, nitrogen fixation, ammonification, nitrification), and
- the influence and the role of microorganisms and of their activity on the mobility of heavy metals (solubilization and insolubilization processes), which result in the transfer of heavy metals from the inorganic and organic soil constituents to the soil solution and to plants.

The first type of phenomenon is not the major aim of this contribution and will be presented very briefly, primarily to emphasize the large number of studies that have been performed to determine (1) the toxic effects of metals on microbial populations, communities, and their activities, (2) the environmental factors affecting the toxicity of heavy metals to microbes, and (3) the mechanisms involved in the development of resistant microorganisms.¹⁰⁻¹⁸ Some microbial and enzyme activities in soil (e.g., nitrification, biodegradation of organic compounds, phosphatase activity) seem to be especially sensitive to the addition of heavy metals.^{10,11} In fields contaminated with heavy metals, new fungal species¹⁹ and bacteria resistant and adapted to heavy metals were enriched,^{16,20} apparently as the result of the acquisition of plasmids that coded for resistance of bacteria to the heavy metals.¹⁶ Some specific physiological mechanisms of microbes to methylate, demethylate, reduce, or oxidize mercury (Hg), arsenic (As), tin (Sn), lead (Pb), and selenium (Se) have been determined. However, these aspects have been studied more extensively in sediments and in aquatic and marine environments than in soils.^{21,22} Therefore, they will not be discussed here.

For the second type of phenomenon, a simplified general diagram of microbial activity (Figure 1) suggests that soil microorganisms are able to act directly or indirectly on the dissolution or deposition processes of mineral elements and, more specifically, of heavy metals by mechanisms that involve oxidation, reduction, acidification, complex formation, biosorption, and bioaccumulation.^{9,23}

The major purpose of this chapter is to present and discuss some of the possible participations of soil microorganisms in the mobility of heavy metals in soil-plant systems. Three main aspects that concern the involvement of microorganisms and their activities will be considered: (1) the solubilization (dissolution) in the soils, (2) the retention and accumulation of heavy metals in the soils, and (3) the possible role of

rhizosphere microorganisms, such as mycorrhizal fungi, in the transfer of heavy metals from soil to plants.

II. MICROBIAL SOLUBILIZATION OF HEAVY METALS IN POLLUTED SOILS

Solubilization, dissolution, or leaching means the release of mineral elements, particularly of heavy metals in this chapter, from soil constituents under the direct or indirect effects of microorganisms. Several studies have considered the microbial processes of solubilization of inorganic elements from soils, rocks, and minerals,^{8,9,22,23} but only a few studies have provided data on the microbial dissolution of heavy metals from rocks and minerals,^{24–26} especially of elements that are considered to be soil pollutants.^{12,27,28}

In soil columns previously treated with cadmium [$\text{Cd}(\text{NO}_3)_2$], in order to bind it to soil sorption sites, Chanmugathas and Bollag²⁸ observed that the solubilization of cadmium in the leachates of sterile and nonsterile columns, after 38 days, was 6 and 9%, respectively, when nutrients were not added but reached 16 and 36%, respectively, when nutrients (glucose, sucrose, peptone, yeast extract, NaNO_3) were added to the percolation solution. These results showed that in the presence of nutrients, the mobilization of cadmium from nonsterile soil could be enhanced by the microbiota. In the leachates collected from the soil columns that received nutrients, cadmium was associated with the low-molecular-weight organic fraction. Hence, in this acid sandy loam soil (mesic ultic Hapludalf, pH 4.5, organic matter 23 g kg⁻¹), the cadmium retained in the soil was mobilized (solubilized), despite a higher pH of the effluent (5.0–5.5) than that of the soil, in the form of cationic cadmium and of small molecular weight metal–organic complexes involving unidentified organic ligands produced by the autochthonous microbes.

Microbial solubilization processes have been observed by Munier-Lamy and Berthelin²⁵ in weathering simulation experiments where major (e.g., Al, Fe, Mg, Ca) and trace elements (Cu, U) have been dissolved efficiently in acid environmental conditions, as shown in Figure 2. Organic compounds of microbial origin might promote these dissolution processes. It was observed that the solubilized elements were associated with relatively high molecular weight (2000–2900) metal–organic complexes (Figure 3) that were composed of the solubilized inorganic elements (metals) associated with oxalic, citric, isocitric, succinic, fumaric, lactic, *p*-hydroxybenzoic, and ferulic acids.²⁵ It appeared that simple organic compounds produced by glucose-metabolizing microbes were linked to form polynuclear (i.e., polycations and polyanions) complexes in which metals were bound as chelates, and became unexchangeable with a strong cationic resin.²⁵ Such microbial dissolution processes were much more efficient when the responsible microbiota, selected by natural antimicrobial compounds (thymol, pine resin, soil lipids) under aerobic conditions, produced larger amounts of complexing agents.^{9,23,25} Hence, in aerated or relatively aerobic and acid conditions, soil microorganisms that receive available nutrient and energy sources are able to solubilize, during soil leaching processes, trace elements, and, in particular, heavy metals in a form of metal–organic complexes.^{25,28}

Under anaerobic conditions, when nutrients and energy are available, significant amounts of metals such as cadmium “bound” or “fixed” to soil can also be mobilized from nonsterile soil samples.²⁷ A decrease in the pH and metabolite action of fermentation origin is probably involved, as the dissolution of transition and heavy metal oxides (CdO , CuO , PbO , and ZnO) was observed in the presence of an anaerobic N_2 -fixing *Clostridium* sp.²⁶ However, different results were obtained in waterlogged (anaerobic) and neutral or slightly alkaline conditions.²⁹ Experiments were performed with perfusion-leaching devices using a Luvisol (pH 7.6, organic matter 31.1 g kg⁻¹, C/N 7.6, and CaCO_3 16.3 g kg⁻¹) contaminated by smelter emissions and containing, respectively, 16, 985,

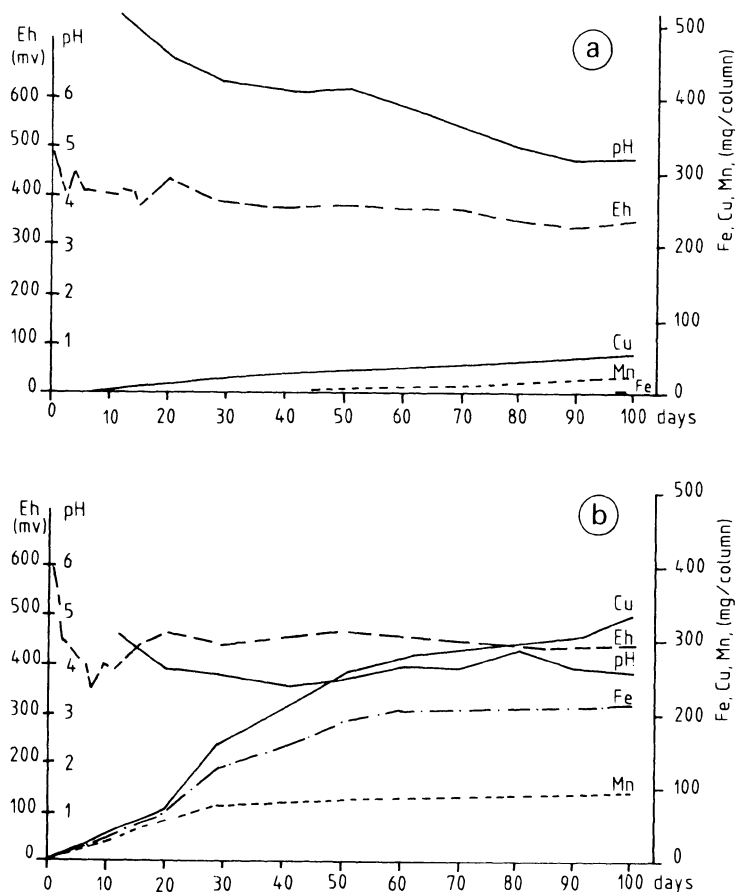


Figure 2 Cumulative curves of net solubilization of Cu, Fe, and Mn and variations in pH and Eh in leaching experiments of granitic rocks under well-drained conditions. (a) Sterile conditions (without microorganism); (b) nonsterile conditions (with microorganisms).²⁵

1330, and $37 \mu\text{g g}^{-1}$ of Cd, Pb, Zn, and Cu. In this soil, only a small part of the metals was exchangeable by ammonium nitrate (1 M, pH 7.0) (respectively, 0.5, 2.3, 2.0, and $0.4 \mu\text{g g}^{-1}$ of Cd, Pb, Zn, and Cu). A basal mineral medium (NH_4NO_3 , 0.286 g; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.101 g; Na_2SO_4 , 0.013 g; KCl, 0.019 g; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.084 g; distilled water 1000 ml) was perfused at a flow rate of 10 to 12 ml per column per day in all the experiments. In these waterlogged columns containing 100 g of soil, the microbiota can use, as carbon and energy sources, depending on the experimental treatments, the soil organic matter, the soil organic matter, and wheat straw (2/100 g of soil, i.e., 10^4 kg ha^{-1}) added as plant residue, or the soil organic matter, the wheat straw, and glucose that was added to the perfusing solution (2 g l^{-1}).

When only soil organic matter was available as carbon and energy sources, the cumulative net solubilization of Pb, after 100 days of perfusion, was greater under sterile (without microorganism) than nonsterile conditions (Figure 4a). The difference was barely significant after 100 days of leaching. When straw was added to the soil, Pb was solubilized at similar levels and always more under sterile than nonsterile conditions (Figure 4b). But the difference in net solubilization between both treatments became

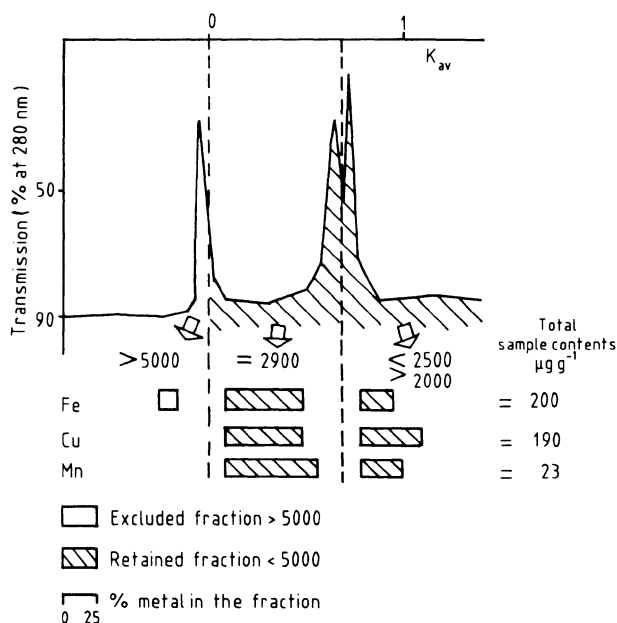


Figure 3 Fractionation of soluble organic–copper compounds formed by selected soil microbiota during leaching of granitic rocks. Exclusion gel chromatography of the leachates on Sephadex G-25. Distribution of Fe, Cu, and Mn within the different fractions. K_{av} , partition coefficient.²⁵

significant ($p = 0.05$) in the final period of the leaching. Thus, in soil columns with microorganisms, using soil organic matter or soil organic matter and added straw as carbon and energy sources, the solubilization of Pb was less than in sterile conditions. Similar results were observed with Cd, Zn, and Cu, either in waterlogged or in well-drained soil columns.²⁹ The addition of an easily available energy source, such as glucose, to the perfusing solution resulted initially in more solubilization under sterile conditions than in the presence of soil microorganisms (Figure 4c). However, after 60 days of perfusion, the solubilization of lead was greater under nonsterile conditions (Figure 4c) and the difference in net solubilization was significant ($p = 0.05$) after 90 days of leaching. Hence, the supply of some easily available carbon and energy source resulted in a significant increase in the microbial leaching of lead.

The involvement of microorganisms in the mobility of heavy metals by solubilization processes has to be considered not only on the bases of energetic, nutrient, or some environmental conditions, such as pH and Eh, but the speciation of heavy metals and their association with soil constituents (e.g., clays, organic matter, carbonates, oxyhydroxides) must also be considered. The metal availability will vary with its speciation in the solid phase. Different types of processes are involved in forming soluble metals. Exchangeable metals are easily mobile, but heavy metals associated with organic matter, such as insoluble complexes, can become mobile through biodegradation processes. If the metals are present in carbonates, acidification and complex formation would be involved in dissolution, whereas reduction and complex formation would mobilize metals associated with ferric oxyhydroxides. In all these processes, direct proton exchange and/or complex formation with the metals, or the biodegradation, or the dissolution of the “matrix” bearing the metals are involved.

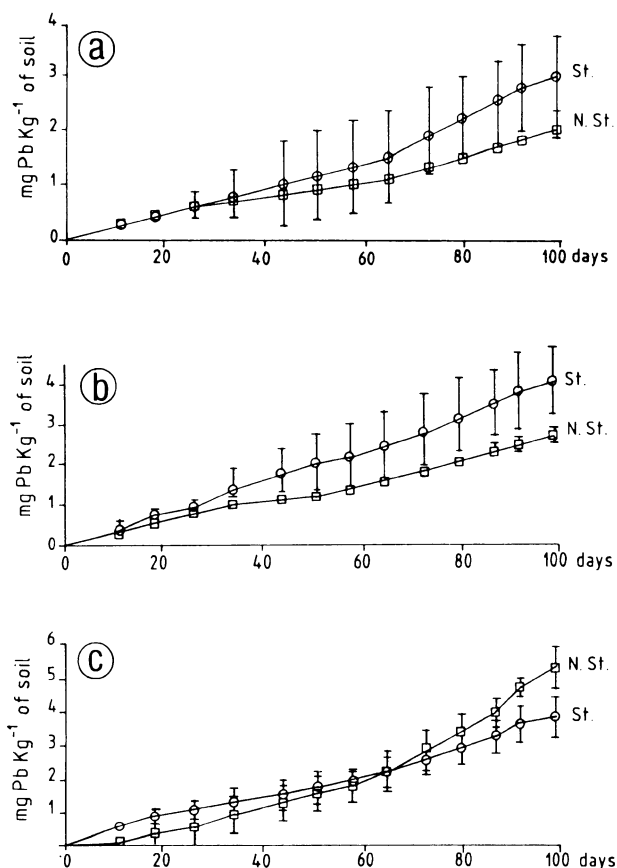


Figure 4 Dissolution of lead from a soil contaminated with heavy metals originating from a smelter. Cumulative curves of lead collected in leachates of sterile (St.) and nonsterile (N. St.) soil columns. Experimental treatments: (a) leaching with a basal mineral solution; (b) leaching with a basal mineral solution + addition of straw in the soil; (c) leaching with basal mineral solution + glucose + addition of straw in the soil.²⁹

III. INVOLVEMENT OF MICROORGANISMS IN INSOLUBILIZATION AND ACCUMULATION PROCESSES OF HEAVY METALS IN SOILS

It is now well recognized that microorganisms are involved in insolubilization and deposit processes of metals by different mechanisms: (1) biosorption or bioaccumulation by cell constituents and by exopolymers, (2) reduction or oxidation of metals, and (3) biodegradation of ligands of soluble metal-organic complexes.^{9,12,23,30,31} Some of these processes have been studied to determine their possible participation in the behavior of heavy metals in soils, and are presented and discussed below.

A. BIODEGRADATION OF ORGANO HEAVY METAL COMPLEXES

The specific chemistry and fate of heavy metals in soils are affected by formation of stable complexes with soil organic compounds.^{12,32-35} However, few studies have dealt with the formation and the behavior of such complexes, although some experiments have been performed to study their biodegradability and the fate of the metals during biodegradation.^{35,36}

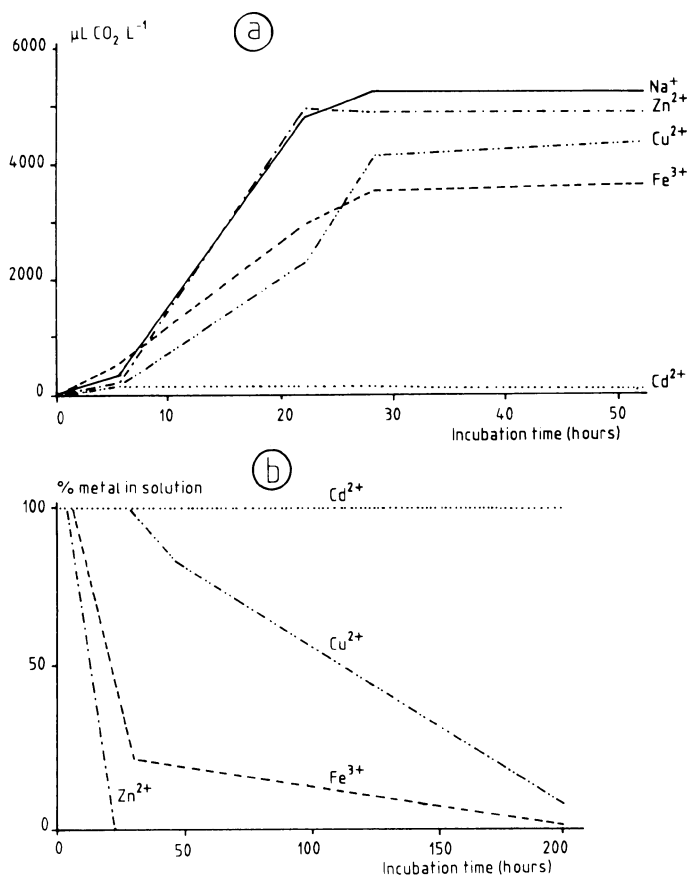
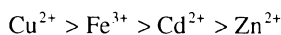


Figure 5 Biodegradation of metal–citrate complexes by a rhizobacterium. (a) Measurement of the biodegradation of the ligand by CO_2 released; (b) metal in solution related to biodegradation of the ligand. Different scales of time were used to present, respectively, the biodegradation of the ligand and the metal precipitation.³⁵

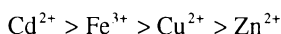
The comparison of the biodegradation of soluble metal–organic complexes of heavy metals (Cd^{2+} , Zn^{2+} , Cu^{2+}) or of reference metals (Fe^{3+} , Na^+) with simple ligands (citrate, protocatechuate) by bacteria and fungi isolated from maize rhizosphere showed different kinetics of ligand biodegradation and of metal precipitation.³⁵ In a medium containing $3 \times 10^{-3} \text{ M}$ of organic ligand and 10^{-3} M of metal, the biodegradation of well-defined soluble metal–organic complexes, such as citrate complexes, by a rhizobacterium (Figure 5) was evaluated by CO_2 release (as a measure of the gross microbial activity), the concentration of metal and carbon in solution, and biomass production. The biodegradation activity of the bacterium varied with the metal (Figure 5a):³⁵ it was more pronounced with Na^+ -, Zn^{2+} -, Cu^{2+} -, and Fe^{3+} -citrates, whereas it was insignificant (close to zero) with Cd^{2+} -citrate. The microbial activity increased with time, particularly from 5 to 22 h, and then stabilized. Biodegradation of Cu^{2+} -citrate was higher than that of Fe^{3+} -citrate after 50 h of similar incubation, but it began more slowly. Similar effects of metals on the biodegradation of polysaccharides were previously observed by Martin et al.,³⁷ who noted a lower biodegradation of Cu, Zn, and Fe complexes than of Al complexes. The biodegradation of protocatechuate (3,4-dihydroxybenzoate)

complexes, also at pH 7.0, by another bacterium and a fungus gave different results.³⁵ While Cu^{2+} - and Cd^{2+} -protocatechuates were not biodegraded, Zn^{2+} -protocatechuate appeared to be toxic to the bacterium but not for the fungus.³⁵ The degradation of the complexes resulted in the precipitation of the metals (Figure 5b) owing to the formation of hydroxides or their uptake by the microbiota. During the biodegradation of the citrate complexes, precipitation of the metals increased in the following order: $\text{Zn}^{2+} > \text{Fe}^{3+} > \text{Cu}^{2+}$. Cadmium (Cd^{2+}) was not precipitated, as a result of its toxicity to the cultures and of the nonbiodegradation of the Cd^{2+} -citrate complex. Such a toxic effect was also observed with Cu^{2+} , but to a lesser extent, during the lag phase of the respiratory activity and in the curve of precipitation of the metal. Therefore, the Cu^{2+} -citrate complex appeared to be more stable toward biodegradation than the Fe^{3+} -complex. The release of CO_2 and the content of soluble organic carbon in the culture flasks, when compared with Fe^{3+} -citrate, suggested that Cu^{2+} was more strongly bound to citric acid than was Fe^{3+} . At pH 7.0, the logarithm of the stability constants for the Cu^{2+} - and Fe^{3+} -citrate complexes are 32.9 and 21.2, respectively.³⁵

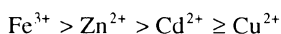
The logarithms of the stability constants of the citrate complexes at pH 7.0 and 20°C are 4.98 for Cd^{2+} , 3.75 for Zn^{2+} , 21.2 for Fe^{3+} , and 32.95 for Cu^{2+} , in the form of MA, MA, $\text{M}_2(\text{OH})_2\text{A}_2$, and M_2A_2 complexes, respectively, where M is the complexed metal and A is the organic ligand.³⁵ It is interesting to note that the chemical stability of citrate complexes increased in the order



whereas the biological stability determined in citrate biodegradation experiments was observed to be



In contrast, the biodegradation of protocatechuate complexes at pH 7.0 by a rhizobacterium or a fungus isolated from a soil contaminated with heavy metals from sludges provided different results. For both nonidentified microorganisms, the biodegradation increased as³⁵



As shown by the diagrams of the distribution of soluble metal–organic complexes vs. pH,³⁵ the species of complexes and their chemical stability differed according to the ligands, the metals, and their concentration.³⁵ Furthermore, the biodegradation of such complexes was also different and was dependent on the ligand, on the microorganisms, and on the toxicity of the metal.

B. BIOSORPTION–BIOACCUMULATION OF METALS

Accumulation of metals by microorganisms can correspond to the absorption of elements by living organisms for their mineral nutrition usually in excess of their requirements.⁹ Such processes were mainly studied in cultures of microbes in the presence of metals in solution to observe, for instance, the sorption of cadmium.³⁰ Experiments performed in the presence of minerals, rocks, or soil constituents bearing phosphorus, calcium, uranium, potassium, etc. showed that bacteria and fungi were able to take up elements from solution and therefore to displace very significantly the chemical equilibrium of these elements between mineral and solution. Thus, in increasing mobilization, i.e., uptake of

elements (e.g., K, P, U, etc.) from rocks and minerals, they promoted not only weathering but also the insolubilization and concentration of elements in or on their cells.^{31,38}

Bacterial and fungal cell constituents and particularly cell walls in aqueous systems can act as a sink for metal ions.^{31,39,40} Proteinaceous, but mainly polysaccharidic, exopolymers are efficient agents of metal fixation and accumulation.^{31,41,42}

The sorption of soluble cadmium by microorganisms in competition with other soil constituents was studied in laboratory batch experiments³⁰ using dead and living cells of microorganisms. The removal of Cd^{2+} from a liquid medium by dead bacteria was greater than by live bacteria and by the clay, montmorillonite, or sand. There were considerable dissimilarities among the various species, and it was not possible to establish significant obvious differences in sorption pattern between bacteria and fungi, but the sequence of sorption and uptake of Cd^{2+} from solution by bacterial cells, clay, and sand can be classified as³⁰

$$\text{dead bacteria} > \text{live bacteria} \approx \text{montmorillonite} > \text{sand}$$

To study the mobility and the availability of trace elements under field conditions and to examine directly the behavior of heavy metals in contaminated soils, a “bag method” was developed and used to determine the transfer, the fixation, and the accumulation of heavy metals on microorganisms as compared with soil constituents (clays, oxyhydroxides) under natural environmental conditions.^{43,44} Inorganic soil constituents (Ca-saturated montmorillonite and synthetic ferric oxyhydroxide: goethite), biodegradable organic compounds (cellulose), and dead microorganisms (filamentous and unicellular fungi, *Aspergillus* and *Saccharomyces*, respectively) enclosed in a nonbiodegradable porous bag (pore size 0.2 or 10 μm) made of recalcitrant material were placed in a surface soil horizon (calci Luvisol) contaminated by sewage sludges.⁴⁴ The A horizon (pH 5.3) of this soil contained 8, 96, 234, and 820 $\mu\text{g g}^{-1}$ of Cd, Cu, Pb, and Zn, respectively. After 70 days of exposure in the contaminated A horizon, the contents of Cu, Pb, and Zn reached 10, 0.5, and 20 $\mu\text{g g}^{-1}$ (dry weight), respectively, for ferric oxyhydroxides, 85, 35, and 320 $\mu\text{g g}^{-1}$ for *Saccharomyces*, and 38, 18, and 170 $\mu\text{g g}^{-1}$ for *Aspergillus*.⁴⁴ Montmorillonite and Cd were not analyzed in this first experiment, but in samples collected after a longer time of exposure (2.5 years), Cd content was 0, 4, 11, and 19 $\mu\text{g g}^{-1}$ dry weight for the montmorillonite, the *Aspergillus* residue, the synthetic ferric oxyhydroxide (goethite), and the residue of biodegraded cellulose, respectively.⁴⁴ Hence, in natural conditions, microorganisms growing on plant material (cellulose) and microbial constituents or microbial cell residues of dead microorganisms are able to take up, accumulate, and concentrate relatively large amounts of heavy metals from contaminated or polluted soils and at larger concentrations than the mineral soil constituents (clays and ferric oxyhydroxides), which are usually considered by soil scientists and geochemists to act as sinks for metals. An attempt to determine the kinetics of transfer to and accumulation of metals by dead microorganisms, i.e., the biosorption by fungal residue during a winter and spring period of exposure has allowed us to observe the time function of an increase of Zn, Cu, and Cd content of dead mycelium enclosed in porous bags that were placed in the contaminated calci Luvisol previously mentioned (Figure 6).⁴³ During these times of exposure, the content of Cd and Cu reached those of average soil contents and Zn was also fixed in large amounts. But a better knowledge of such metal mobility, uptake, and accumulation, in natural conditions, needs to be related to environmental parameters such as metal speciation, rainfall, soil moisture, and temperature.

The extractability of the metals that were “sorbed” by the fungal residues differed with the extraction reagent used and gave a scale of the binding efficiency of microbial cell

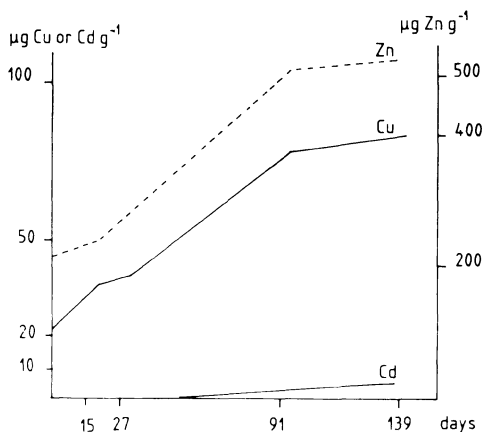


Figure 6 Accumulation of heavy metals by dead fungal mycelium (*Aspergillus*) in a field experiment in a soil polluted with sewage sludges: metal content of dry mycelium residue after different times of exposure.⁴³

constituents. The extraction by a 0.5 M MgCl_2 solution (i.e., the exchangeable metals) was highest for Cu (about 50% of the metal), but no more than 20% of Zn, and only very low amounts of Pb and Cd were exchangeable.¹² The exchange by a strong cationic resin (Amberlite, IR 120) was very low, but the extractability by NaOH (0.1 M), corresponding to metals associated with fulvic- and humic-like compounds, was a little higher than that with MgCl_2 . Only Na_4EDTA (0.1 M) was able to extract 90% or more of Cu and Zn and also relatively large amounts of Pb and Cd.¹² Hence, metals appear to be strongly fixed by fungal cell constituents, particularly, as also observed by Venkateswerlu and Stotzky,⁴⁵ by the cell walls¹² that contain efficient chemical compounds such as chitin.⁴⁰ Other chemical structures, e.g., glutamic, aspartic, and diaminopimelic acids, that can be present in large amounts in resistant bacterial strains are also able to retain metals such as Cd.⁴⁶

IV. INFLUENCE OF MYCORRHIZAE ON HEAVY METAL UPTAKE BY PLANTS

Plant root systems are associated with rhizobacteria, and most of the roots (about 90%) are also associated with fungi to form mycorrhizae, which are known to promote plant growth and plant nutrition. Numerous studies have demonstrated the ability of the rhizospheric microbiota^{47,48} to weather minerals and to promote the transfer of mineral elements from the soil to the plants.⁴⁷⁻⁴⁹ The effect of bacteria has been attributed, in some experiments, to the production of organic acids that can be involved in the dissolution of mineral elements and in the formation of soluble metal-organic complexes.⁴⁸ However, both bacteria and fungi can also act by other, not well-defined, reactions, such as proton-exchange, production of chelating agents, and hormonal indirect effect, that by promoting plant growth or by modifying exchange-uptake of nutrients increase metal transfer to and uptake by plants.

High amounts of heavy metals in soil were reported to decrease mycorrhizal infection.⁵⁰ However, soils polluted with heavy metals contain mycorrhizal fungi, and contradictory results on the effect of mycorrhizae on heavy metal uptake (Zn, Cu, Cd) by plant roots have been reported.⁵¹⁻⁵⁴ Some results have shown that mycorrhizae increased the uptake of heavy metals by plants,⁵¹⁻⁵³ but others have shown a decrease.^{54,55}

The possible involvement of endomycorrhizae (arbuscular mycorrhizae or formerly vesicular-arbuscular mycorrhizae) in the transfer of heavy metals to plant was studied using sterile maize seedlings in disinfected soil that has been polluted with sewage sludge.⁵⁵ Disinfection of the soil that has received sewage sludge was done by fumigation. Three levels of various heavy metals were used in the experiment as indicated in Figure

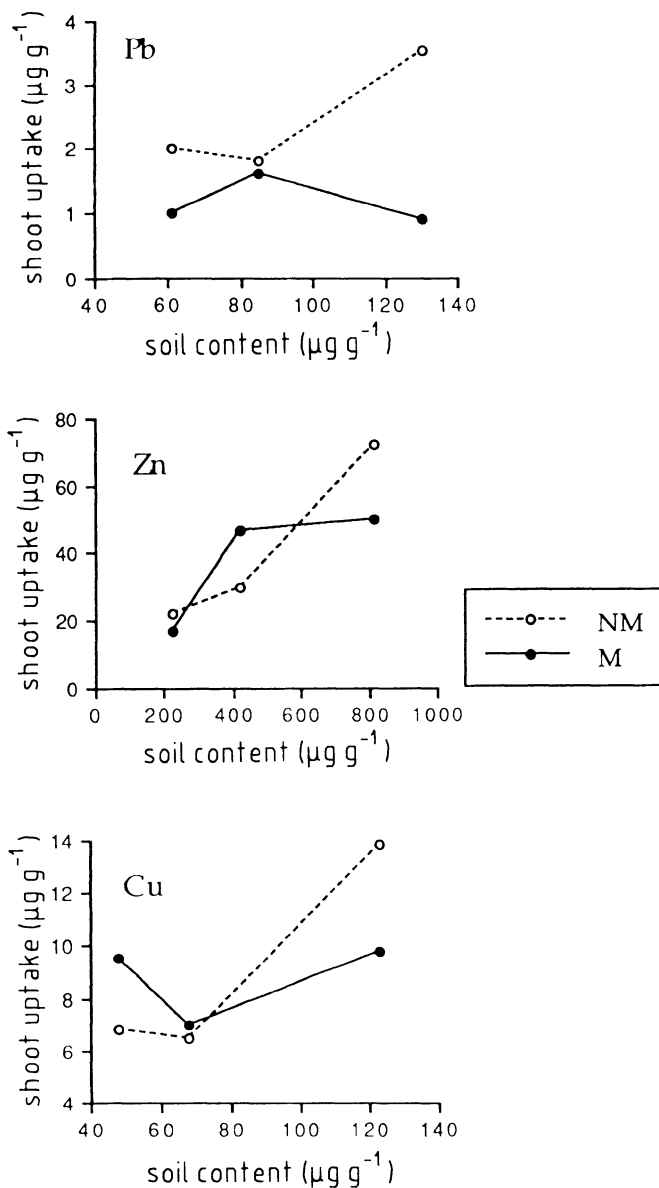


Figure 7 Heavy metal contents in the shoot of maize colonized (M) or not colonized (NM) by endomycorrhiza (*Glomus mosseae*) after 11 weeks of growth on soil polluted with different amounts of metals added as sewage sludges.⁵⁵

7. Half of the pots were inoculated with the arbuscular mycorrhizal fungus, *Glomus mosseae* (25 spores and 5 sporocarps per plant), and the soils were covered with 600 g of sand (0.5–1 mm) and 400 g of fine sand coating with silicone to allow gas exchange but to protect against contamination from liquid or solid particles. There were five replicates per treatment. After 11 weeks in a greenhouse, the plants were removed from the pots, dried, and digested to determine the contents of Zn, Pb, Cu, and Cd. Mycorrhizal infection was verified by microscopic observation of stained roots.⁵⁵ The presence of the heavy metals and *G. mosseae* did not significantly ($p > 0.05$) modify shoot dry weight of

the maize. However, differences were observed in the heavy metal contents of the shoots that were modified by the presence and the content of heavy metals in the soil and, especially, by mycorrhizal infection of the roots. In the nonpolluted (or lower polluted) soil, mycorrhizae seem to increase Cu uptake significantly and to decrease the uptake of Zn, and, especially, of Pb. In the soil with high levels of heavy metals, the uptake of Cu, Zn, and Pb was significantly lower for mycorrhizal than for nonmycorrhizal plants (Figure 7).⁵⁵ The Cd content of the shoots was too low to be measured accurately.⁵⁵ Comparable results were observed for plants cultivated on soils contaminated by mineral salts of heavy metals.¹² These results suggest, at least under these experimental conditions, a regulative effect of mycorrhizae on heavy metal uptake. Such effect could be associated with different processes, such as the binding or adsorption of metals by the mycorrhizal fungi, or with interactions between phosphorus accumulation as polyphosphates in the roots and heavy metal fixation, but need to be further investigated.^{55,56}

The involvement of rhizobacteria in the transfer of heavy metals to the plants has not been studied, even though it is well recognized that bacteria participate in the rhizospheric weathering of minerals and in the mobilization of different elements, such as phosphorus and potassium, from rocks and minerals.^{9,23,47,48}

V. SUMMARY AND CONCLUSIONS

The fate of trace elements, and particularly heavy metals, in soils is affected by adsorption on mineral surfaces, precipitation of salts, formation of stable complexes with organic matter, desorption, dissolution by proton-exchange reactions, etc. Therefore, their behavior depends not only on the physicochemical parameter of soil, but also on biological factors, mainly on microbial activities that have been already recognized to be involved either in the solubilization or in the insolubilization of inorganic elements, the weathering of minerals, and the formation of deposits.

The interactions between soil microorganisms and heavy metals have been studied mainly to determine the toxic effects of metals on microbial populations and communities, their activities, and the development of resistant organisms. Except for some mechanisms, such as oxidation, reduction, and methylation of some elements, such as Hg, As, Se, and Pb, observed especially in sediments or waters, there have been few studies on the effect of microorganisms on the mobility of trace elements in soil.

When nutrient and energy sources are available, microorganisms solubilize heavy metals (e.g., Cd, Pb, and Cu) that were fixed or bound to soil constituents or present in the parent material by the production of acid and/or complexing agents as observed in experimental and natural conditions, either in bulk soil or in the rhizosphere.^{34,48,57} Modifications of physicochemical conditions (e.g., pH, Eh) that act directly on metal speciation or on the matrix in which they are contained can explain some solubilization processes. However, biological mechanisms, such as bacterial reduction of ferric iron and bacterial oxidation of sulfur species involved in the dissolution of ferric oxyhydroxides and of sulfides containing heavy metals, respectively, can also promote their release.

Insolubilization and, therefore, deposition and accumulation of heavy metals can be associated with the biodegradation of the organic ligands of soluble metal-organic complexes.³⁵ The metal may then be precipitated as hydroxide and/or adsorbed by microorganisms. Such processes are related to the nature of the ligand, of the cation, of the microorganisms, and of the soil environmental conditions, in particular the pH and Eh.

Biosorption and bioaccumulation, which have been studied mainly in aquatic systems, were also observed under natural soil conditions, where dead or living microbes are able to retain and accumulate large amounts of metals.^{43,44} Sorption and complexation prop-

erties of microbial cell constituents, especially cell walls, are involved in this, but other mechanisms, e.g., bacterial oxidation of iron in aerobic environments or bacterial sulfate reduction in anaerobic environments, have to be considered, because they participate extensively in the formation of ferric oxyhydroxide and sulfide deposits.

Rhizosphere microorganisms, in particular mycorrhizae, may have an important role in the uptake of heavy metals and their transfer from soil to plants. A regulative effect of the mycorrhizae that would depend on the amount of available metal (the transfer to the shoot of the plants being increased at low metal availability and decreased at high metal availability) is suggested, but must be carefully considered and needs further investigations. Rhizobacteria, which are known to be involved in the weathering of minerals and the mobilization of major elements, may also participate in such processes, but their role remains to be defined.

Consequently, microbes are involved in solubilization and insolubilization processes and in the transfer of heavy metals from bulk soil to the soil solution and to plants by different mechanisms. It is essential to consider the role of microorganisms in heavy metal behavior and, more particularly, in the mobility of metals in the soil-plant system. However, there are some major gaps in knowledge of "microorganisms-heavy metal interactions" that need further investigations, in particular on the mechanisms involved, such as the transfer to plants and the role and the importance of environmental parameters that control the microbial activities.

The involvement of soil microorganisms in the bioavailability of heavy metals and in the modification of their speciation and mobility has to be studied to determine "diagnostic" and "pronostic" of heavy metals behavior and risk and to consider remediation of contaminated soils.

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