

ENVIRONMENTAL SCIENCE

G. SARRET¹, A. MANCEAU¹ AND J.L. HAZEMANN^{1,2}

- 1** LABORATOIRE DE GÉOPHYSIQUE INTERNE ET TECTONOPHYSIQUE, GRENOBLE, FRANCE
2 LABORATOIRE DE CRISTALLOGRAPHIE CNRS, GRENOBLE, FRANCE

In recent years, environmental problems such as pollution of air and soil have become increasingly important, even for everyday life. For these reasons, the ESRF has been involved in studies concerning environmental science. In particular, absorption experiments are an indispensable complement to laboratory techniques and, sometimes, the only method able to bring unambiguous answers to environmental problems.

Recently, new possibilities to carry out absorption experiments on highly diluted samples have been available on beamline BM32. It is possible to study a diluted sample down to about 100 ppm, which is a very important target, allowing the study of samples which were, to date, inaccessible. The detector is quite user-friendly: it is possible in an easy way and quite rapidly to change the energy selected intervals of detection of the fluorescent photons.

Furthermore, the dynamic focusing of the x-ray beam allows a 300-300 μm^2 spot size to be kept on the sample all over the EXAFS spectrum. This permits us, firstly, to considerably enhance the intensity of incident photons on the sample, which is essential in the case of highly diluted systems, and, secondly, to study small samples even in grazing incidence.

DIRECT DETERMINATION OF THE SPECIATION OF HEAVY METALS IN SOILS BY EXAFS

The input of heavy metals in the environment, and specifically in soils and industrial or domestic urban wastes, endangers living organisms. Assessing the risk associated with their presence is a prerequisite for designing recovery techniques of contaminated sites and preventing future contamination in the case of landfills. In either case, the risk associated with the presence of heavy metals depends primarily on their *speciation*. The notion of *speciation* is

taken in its broadest sense and includes metal characteristics, such as electronic structure (oxidation state, nature of chemical bonds) and chemical state: its association with the organic or inorganic fraction, the nature of the functional groups to which the metal is bonded, fixation by minerals (clays, oxides...) and the crystal chemical mechanism of this fixation (adsorption vs. lattice substitution), or, more simply, precipitation of the pollutant as a salt (sulfate, carbonate, phosphate...), an oxide or a silicate. This chemical state determines the intrinsic toxicity of the host matrix (soil or waste), as well as the mobility of metals in the environment to either surface and ground waters, living organisms (plants, micro-organisms, meso-fauna) or the atmosphere.

Determining the speciation of heavy metals is a difficult task because of their relatively high dilution and the structural and chemical complexity of host materials. Classical approaches are based on the «selective» or the «sequential» dissolution of soils components and can be classified as possibilities offered by EXAFS spectroscopy for determining the speciation of metals. In contrast to previous methods, EXAFS is a «direct method» since it can be applied to non-disturbed, polymetallic and polyphasic materials. The potential of the EXAFS spectroscopy method will be illustrated with the determination of the speciation of heavy metals in lichens contaminated

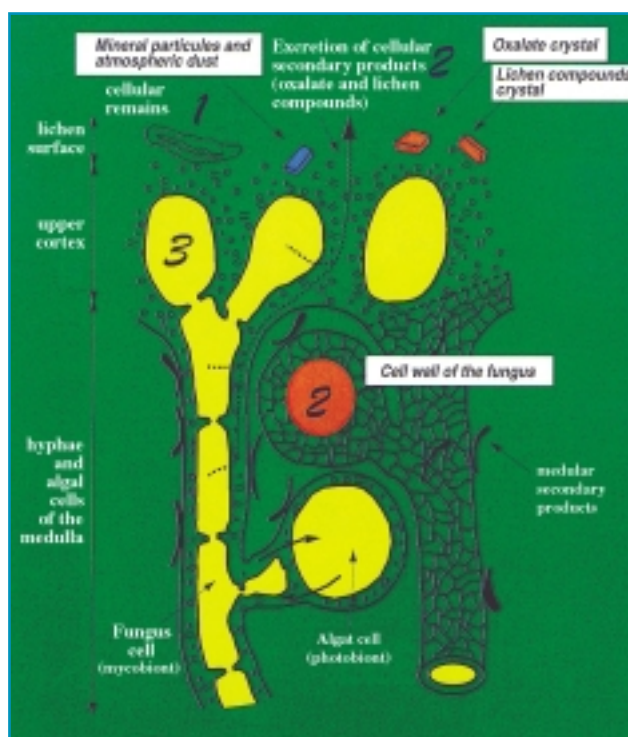
by different sources of pollution of lead and zinc.

UPTAKE MECHANISMS FOR THE FIXATION OF HEAVY ELEMENTS BY LICHENS

Lichens are currently used as biomonitors of atmospheric and soil pollution. The absorbing capacity of lichens for different heavy metals has been widely studied, but little is known about the localisation and retention mechanisms of metals in the thallus. Three different uptake mechanisms are supposed to exist: (1) entrapment of metal-rich mineral particulates originating from atmospheric aerosols (mineral phase) (2) extracellular complexation with polysaccharides of the cell walls, oxalic acid or lichenic acids (3) intracellular uptake by complexation with cysteine-containing proteins (metallothioneins) (Figure 1).

EXAFS measurements on contaminated lichens are a difficult task because (i) metals are generally diluted (500-1000 mg/kg), and (ii) lichens may absorb a variety of metals, thus creating a multi-element system. This study has been possible thanks to the Canberra multidetector and the dynamic focusing allowing a detection limit for trace elements presently

Fig. 1: Three different uptake mechanisms: (1) entrapment of metal-rich mineral particulates (2) extracellular complexation, (3) intracellular.



comprised between 100 and 500 mg/kg. Two lichens from two French sites were exposed to different pollutions: *Xanthoria parietina*, a saxicolous lichen growing on rocks near a tetraethyl and tetramethyl lead factory, and *Diploschistes muscorum*, a terricolous lichen collected on Pb-Zn tailings near a Zn extraction plant.

Comparisons of EXAFS spectra for lichens and for reference compounds (Figure 2) show that in the two lichens studied, metals are not included in mineral phases, but are complexed by organic ligands. The comparison with Pb or Zn-cysteine reference compounds enable us to reject the intracellular trapping mechanism for the two lichens. Therefore, three possible complexing agents could be involved in metal accumulation within the lichens: lichenic acids, macromolecules of the cell wall, or oxalic acid exudates.

• *Diploschistes*: in this lichen, Pb and Zn (Figure 3) were found to be bioaccumulated by the same mechanism, i. e. by precipitation of Pb,

Zn oxalate. The production of oxalic acid is probably enhanced by the pollution stress.

• *Cladonia*: in this lichen, Zn is complexed by lichenic acids but not within the cell wall.

This study reveals that in both lichens, cells are protected from toxicity by extracellular complexation of heavy metals, but the strategies differ: in *Diploschistes*, Pb and Zn are accumulated through an enhanced excretion of oxalate, which precipitates toxic elements such as insoluble salts, whereas in *Xanthoria*, Pb is complexed to carboxylic groups of the fungal cell walls. We conclude that hyperaccumulation of metals results from a reactive mechanism of organic acid production, whereas metallo-tolerance is achieved by a passive complexation to existing functional groups. ■

REFERENCES

A. Manceau, J.C. Hargé, G. Sarret, J.L. Hazemann, M.C. Boisset, M. Mench, Ph. Cambier, R. Prost, 1996, *Direct determination*

of heavy metals speciation in soils by EXAFS spectroscopy, Proceedings of the third International Conference on the biogeochemistry of Trace Elements, 15-19 May, Paris, in press.

A. Manceau, M.C. Boisset, G. Sarret, J.L. Hazemann, M. Mench, Ph. Cambier, R. Prost, 1996, *Direct Determination of lead speciation in soils by EXAFS spectroscopy, Env. Sci. & Technol.*, 30, 1540, 1552.

G. Sarret, A. Manceau, J.L. Hazemann, A. Gomez, and M. Mench, *EXAFS study of the nature of zinc Complexation sites in humic substances as a function of Zn concentration (1997), Journal de Physique IV, C2 799-802.*

G. Sarret, A. Manceau, D. Cuny, C. Van Haluwyn, S. Déruelle, J.L. Hazemann, Y. Soldo, L. Eybert-Bérard, J.J. Menthonnex: *Mechanism of lichen resistance to metallic pollution (1997) submitted to Environmental Science and Technology.*

G. Sarret, A. Manceau, L. Spadini, J.C. Roux, J.L. Hazemann, Y. Soldo, L. Eybert-Bérard, J.J. Menthonnex: *Structural determination of Zn and Pb binding sites in Penicillium chrysogenum cell wall by EXAFS spectroscopy (1997) submitted to Environmental Science and Technology.*

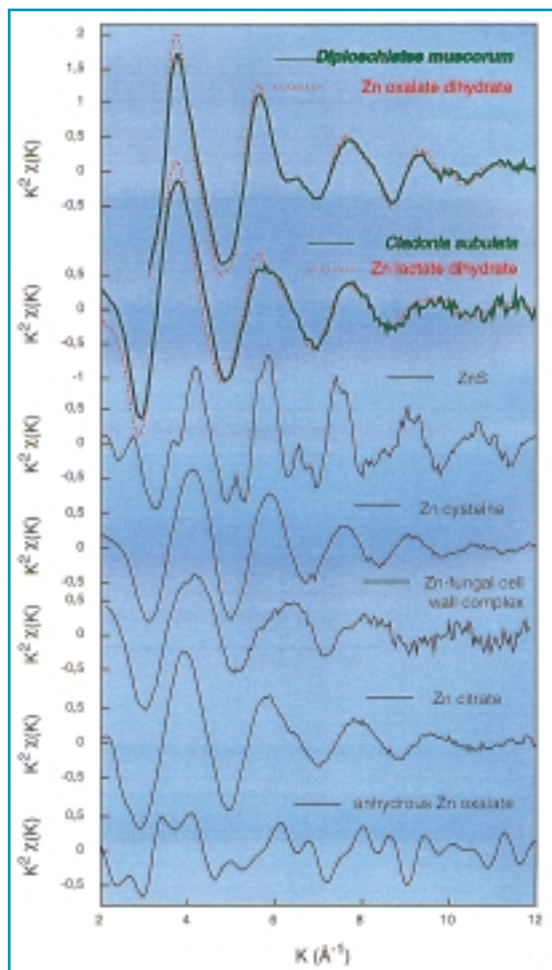


Fig. 2: Comparison of Zn K edge EXAFS spectra for the two lichens and some reference compounds.

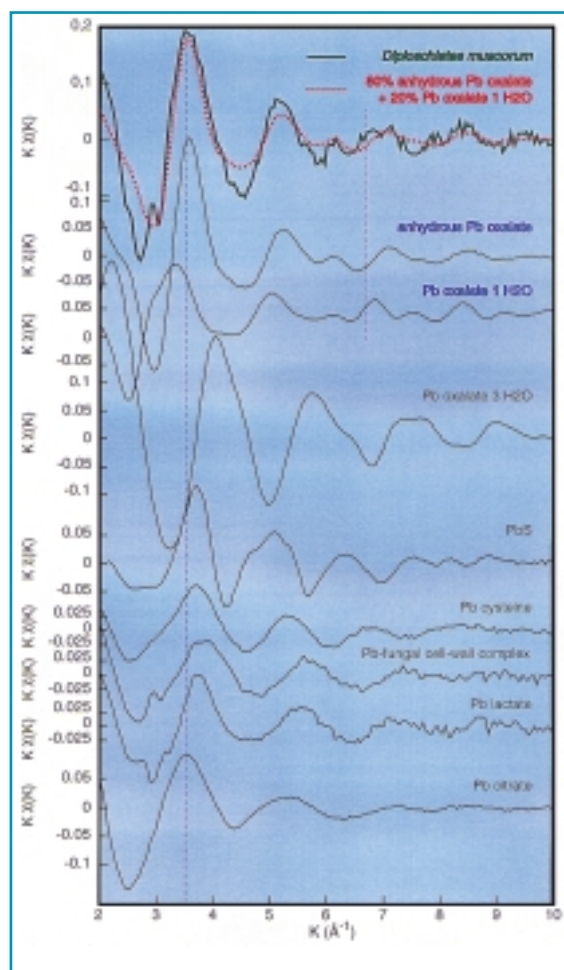


Fig. 3: Comparison of Pb LIII edge EXAFS spectra for a lichen and some reference compounds.