ESRF	**Experiment title:** **Mechanism of Zn hyperaccumulation in *Arabidopsis halleri***	**Experiment number**: ME-414
Beamline: ID26	**Date of experiment**: from: 16/02/02 to: 19/02/02	**Date of report**: May 2002
Shifts: 12	**Local contact(s)**: Thomas Neisius	*Received at ESRF:*

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Report:

Introduction

Zinc and lead are among the most important metal contaminants in industrialized countries, generally produced by non-ferrous industry. The classical methods for the cleanup of soils are generally unadapted to the treatment of most contaminated sites. Metal tolerant plants have the ability to survive and reproduce on soils containing high concentrations of metals in forms that are toxic or inimical to other plants. Metal hyperaccumulating plants have the additional property to store large amounts of metals in their aerial parts, more than typically 10000 $\mu g\ g^{-1}$ (d. w.) for Zn and Pb. This characteristic makes hyperaccumulators highly suitable for phytoremediation, a soft method using plants for the cleanup of metal-polluted soils. The genetics and the biochemical processes involved in metal uptake, transport, and storage by hyperaccumulating plants are still poorly understood, although these basic types of information are fundamental for the improvement of the technique (Van Der Lelie *et al.*, 2001). Also, the evolution of metals speciation during their cycling, from living plants to decomposing organic debris, humus and bulk soil, is important to evaluate the role of hyperaccumulating plants on the long term evolution of soils toxicity.

Arabidopsis halleri is a Zn and Cd hyperaccumulator. This species is of particular interest because it is one of the closest relatives to *A. thaliana*, whose genome is entirely sequenced. This information, together with the huge amount of literature available on *A. thaliana*, should facilitate our understanding of metal tolerance and hyperaccumulation in *A. halleri*. Pb is not accumulated in high amount in *A. halleri*, but the long term colonization of the soil by this species may strongly influence Pb speciation in the soil.

The purpose of this study was to investigate the mechanisms of Zn and Pb accumulation in *A. halleri*, and to compare the chemical form in the plant and in the contaminated substrate where plants have been growing for years. Experiments were conducted on different beamlines. Zn speciation was studied by EXAFS at LURE (D42) for the most concentrated samples and at the ESRF (BM32) for the diluted ones, and by µEXAFS at the ALS in Berkeley (10.3.2). Pb speciation was studied at the ESRF (ID26). In this report, we will present the results obtained for Pb on ID26.

Methods

Plant and soil samples were freeze-dried, ground, and the powder was pressed into pellets. Various size fractions of the soil were studied. Pb concentrations were about 50 mg/Kg in the plants, and between 500 and 5000 mg/Kg in the various soil fractions. Pb L_{III}-edge spectra were recorded at room temperature in quick EXAFS (qEXAFS) mode to prevent the degradation of the samples under the beam exposure. The incident beam intensity and the fluorescence yield were measured using diodes. Owing to a leakage problem of the cooling system of the monochromator, only 8 shifts were available for data recording.

Results

Pb speciation in *A. halleri*

The study of *A. halleri* exposed to Zn showed that this metal was predominantly accumulated in the leaves of the plant as an organic complex, Zn malate (Sarret *et al.*, subm.). For Pb, the mechanism most often inferred in the litterature is the extracellular complexation of Pb to the cell walls (Verkleij & Schat, 1989). Pb can also form Pb carbonates, as shown for the crop species *Phaseolus vulgaris* (Sarret et al., 2001). Figure 1A compares the Pb L_{III}-edge spectrum for the aerial parts of *A. halleri* and *P. vulgaris*. Pb speciation is clearly different in the two species. *A. halleri* spectrum was compared to a database of Pb reference compounds, and the best match was obtained with Pb acetate trihydrate. Thus, Pb is likely complexed to carboxyl functional groups in *A. halleri*, which may belong either to macromolecules of the cell wall, or to organic acids present in the cells.

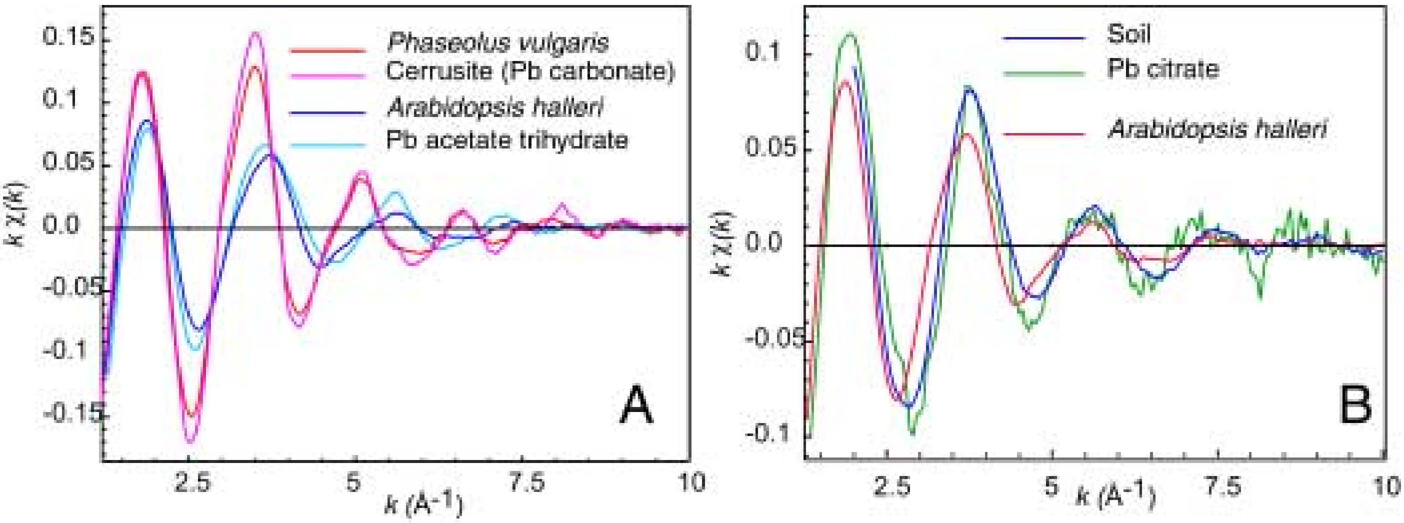

Figure 1A: Comparison of the Pb L_{III}-edge EXAFS spectra for the aerial parts of *A. halleri* and *P. vulgaris* and for two reference compounds. 1B: Comparison of the Pb L_{III}-edge EXAFS spectra for the soil and for the aerial parts of *A. halleri*.

Pb speciation in the soil

In the contaminated soil studied, the biodegradation of plant debris is strongly limited, which results in the accumulation of an organic-rich layer in the topsoil, composed of weakly degraded organic matter, and very rich in metals (about 2% Zn and 0.5% Pb, dry weight). The different fractions of the soil were studied by Zn K-edge and Pb L_{III}-edge EXAFS spectroscopy and XRD. Zn is present as mineral particules originating from the nearby smelting activities (sphalerite, willemite, franklinite mostly present in the fine fraction) and as Zn-organic acids complexes. For Pb, no Pb-bearing minerals were detected by XRD. The Pb L_{III}-edge EXAFS spectra of the different fractions (bulk soil, fine and coarse fraction) were all identical. Pb speciation differed slightly from the one found in the plant, but the best spectral match was also obtained with a Pb-organic acid complex, Pb citrate (Fig. 1B).

This fingerprint analysis of the spectra suggests that lead is bound to carboxylic groups in the plant and in the soil, but that the nature or the structural arrangement of the ligands differs in both compartments. The numerical simulation of the spectra for the determination of the structural parameters is currently underway.

Thus, the complex formed in the plant is not preserved as such after the death of the plant. The modifications of the physico-chemical conditions during plant degradation may release Pb, which can then be complexed by the organic-rich fraction of the substrate. This strong affinity of lead for organic matter explains why this metal generally concentrates in the organic-rich horizon of soils, near the surface, and does not migrate to the bottom horizons and to the ground water (Kabata Pendias & Pendias, 2001).

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Publications

Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann JL, Traverse A, Marcus MA, and Manceau A. Accumulation Forms of Zn in the Hyperaccumulator *Arabidopsis halleri*, submitted to *Plant Physiology*.

Abstract

The chemical forms of zinc in the Zn tolerant and hyperaccumulator *Arabidopsis halleri* and in the non-tolerant and non-accumulator *Arabidopsis lyrata* ssp. *petraea* were determined at the molecular level by combining chemical analyses, extended X-ray absorption spectroscopy (EXAFS), synchrotron-based X-ray microfluorescence (µSXRF) and µEXAFS. Plants were grown in hydroponics with various Zn concentrations, and *A. halleri* specimens growing naturally in a contaminated site were also collected. Zn speciation in *A. halleri* was independent of the ecotype (contaminated or non-contaminated origin) and Zn exposure. In aerial parts, Zn was predominantly octahedrally coordinated and complexed to malate. A secondary organic species was identified at the bases of the trichomes, which contained elevated Zn concentrations, and in which Zn was tetrahedrally coordinated and complexed to carboxyl and/or hydroxyl functional groups. This species was detected thanks to the good resolution and sensitivity of µSXRF and µEXAFS. In the roots of *A. halleri* grown in hydroponics, Zn phosphate was the only species detected, and is believed to result from chemical precipitation on the root surface. In the roots of *A. halleri* grown on the contaminated soil, Zn was distributed in Zn malate, Zn citrate, and Zn phosphate. Zn phosphate was present in both the roots and aerial part of *A. lyrata* ssp. *petraea*. This study illustrates the complementarity of bulk and spatially resolved techniques, allowing the identification of (1) the predominant chemical forms of the metal, and (2) the minor forms present in particular cells, as both types of information are essential for a better understanding of the bioaccumulation processes.