ESRF	**Experiment title:** Speciation of zinc in hyperaccumulating plants	**Experiment number**: 30 02 672
Beamline: BM 30B	**Date of experiment**: from: May, 1, 2004 to: May, 5, 2004	**Date of report**: August, 5, 2004
Shifts: 15	**Local contact(s)**: Olivier Proux	*Received at ESRF:*

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Report:

Introduction

Certain plant species have the ability to survive and reproduce on soils containing high concentrations of metals, and to store large amounts of metals in their aerial parts (more than 10000 µg/g d. w. for Zn). These plants present a great interest for phytoremediation, a soft method in which plants are used for the cleanup of metal-polluted soils. The project aimed at better understanding the molecular mechanisms underlying the immobilization of metals as non-toxic forms in such plants. The Zn and Cd hyperaccumulator *Arabidopsis halleri* is a good model for such studies since its genome is close to *A. thaliana*, the well known genetic model for higher plants, and the molecular tools developed for *A. thaliana* (genetic map, gene ships, putative genes involved in metals homeostasis…) can be used for *A. halleri* (Becher *et al.*, 2004, Weber *et al.*, 2004).
In a previous experiment, we showed that the major accumulation form of zinc is Zn malate in *A. halleri*, whereas this metal occurs as Zn phosphate in the non-tolerant and non- hyperaccumulating species *A. lyrata* (Sarret *et al.*, 2002). The localization of the metal strongly differs in the two species: Zn occurs predominantly in the leaf cells of *A. halleri*, and in the veins of *A. lyrata* (Sarret *et al.*, in preparation). In both species, the base of the trichomes (epidermal hairs) also contain high concentrations of Zn.

We have realized some interspecific crossings between *A. halleri* and *A. lyrata*. in order to segregate the tolerance and hyperaccumulation traits since they are genetically independent in *A. halleri* (Macnair *et al.*, 1999). The first progeny (F1) was tolerant but did not accumulate Zn (Figure 1), and the second generation obtained by back cross (F1 × *A. lyrata*) presented a continuum of phenotypes from non-tolerant to tolerant and from non-accumulators to moderately accumulators, the two traits being independent. Four individuals with contrasted phenotypes were selected : non tolerant non accumulator: BC121, moderately tolerant and accumulator: BC75, non tolerant and moderately accumulator: BC 273, and tolerant but not accumulator: BC5, see Figure 1).

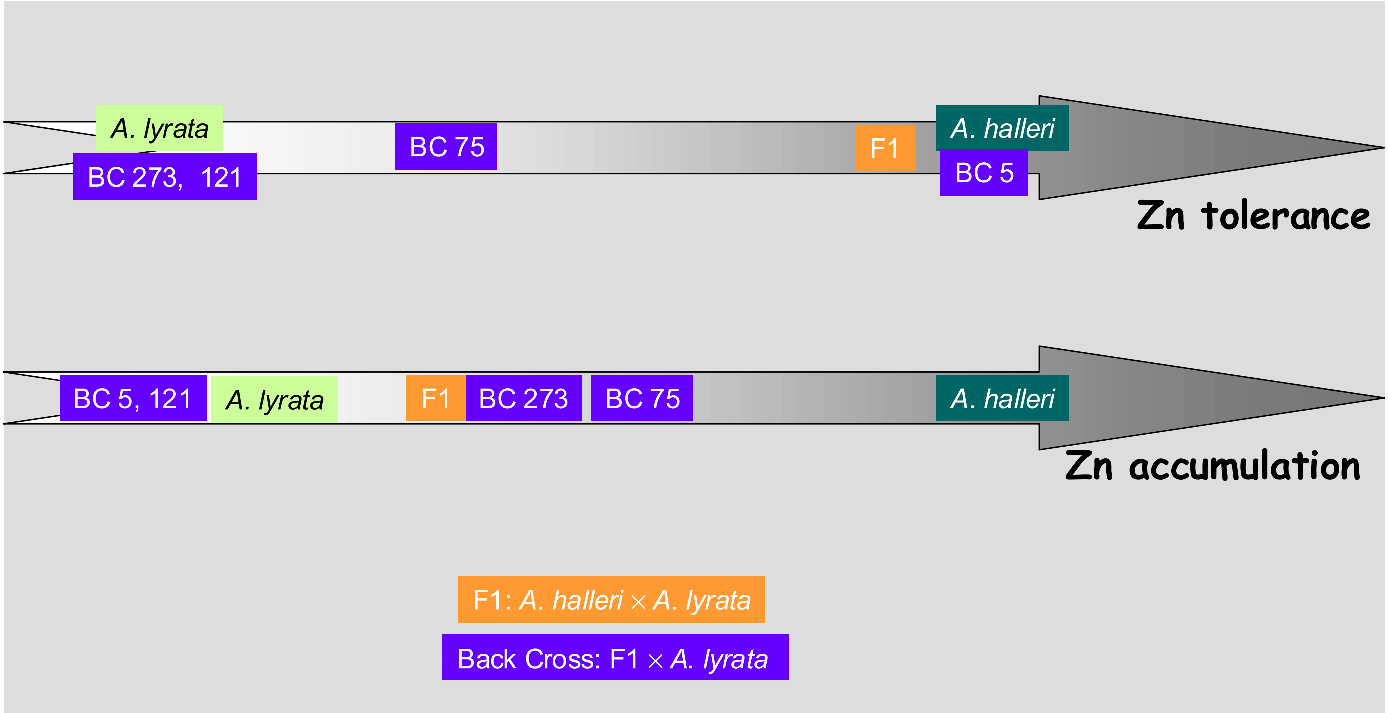

Figure 1: Degree of Zn tolerance and Zn accumulation for the plants studied.

Objectives of this experiment and Methods

The main purpose of this experiment was to determine the relationships between the chemical form of Zn and the tolerance and accumulation phenotypes. Another objective of this experiment was to test various sample conditionnings for the leaf samples (hydrated versus dehydrated, spectrum recorded at room temperature versus 100K). In this purpose, leaves of *A. halleri* were freeze-dried, ground and pressed as pellets, and the spectra were recorded at room temperature and at 100K using a He cryostat. The same leaves were plunged them in liquid nitrogen, ground and pressed as pellets in a cold room (-15°C), and transferred into the cryostat. The EXAFS spectra were recorded on the frozen-hydrated pellets at 100K.

Zinc concentrations ranged between 150 and 3000 mg kg^{-1} (dry weight) for *A. lyrata,* F1 and BC progenies, and reached 1.2 % for *A. halleri*. Spectra were recorded both in transmission and fluorescence mode, using a photodiode and a 13-element Ge detector. Normalized EXAFS spectra were first treated by linear combination fits, using a library of Zn spectra, and then by FEFF simulations in order to determine the structural parameters.

Results

The spectra for the freeze dried plant and frozen hydrated tissue recorded at 100K were identical, so freeze-drying does not seem to alter Zn speciation. At the opposite, some spectral modifications were observed for the spectrum recorded at room temperature, which are probably due to some radiation damage of the high intensity beam. Therefore, EXAFS spectra on plant materials should be recorded at low temperature. All spectra presented in this report were recorded at 100K on freeze-dried samples since spectra for frozen hydrated powders were much more noisy because of the high water content.

Figure 2 shows that the Zn K-edge EXAFS spectra for *A. lyrata*, F1, and the 4 back-crosses are strictly identical, and correspond to Zn phosphate (Zn is in tetrahedral coordination with d(Zn-O) = 1.98 Å, and the second shell contains P). These spectra contrast with *A. halleri* spectrum, corresponding to Zn malate (Zn is in octahedral coordination d(Zn-O) = 2.02 Å, and the second shell contains C). This finding is consistent with the results obtained on the localization and speciation of Zn at the micrometer scale: in a parallel experiment performed on beamline 10.3.2 at the ALS (Berkeley) using µ-XRF and µEXAFS, we found that Zn distribution was identical in the leaves of all these plants, Zn being stored in the veins and in the trichomes (Fiigure 3). The chemical form was Zn phosphate in both locations. In *A. halleri*, Zn was also present as Zn phosphate in the trichomes, but occured as Zn malate in the leaf cells.

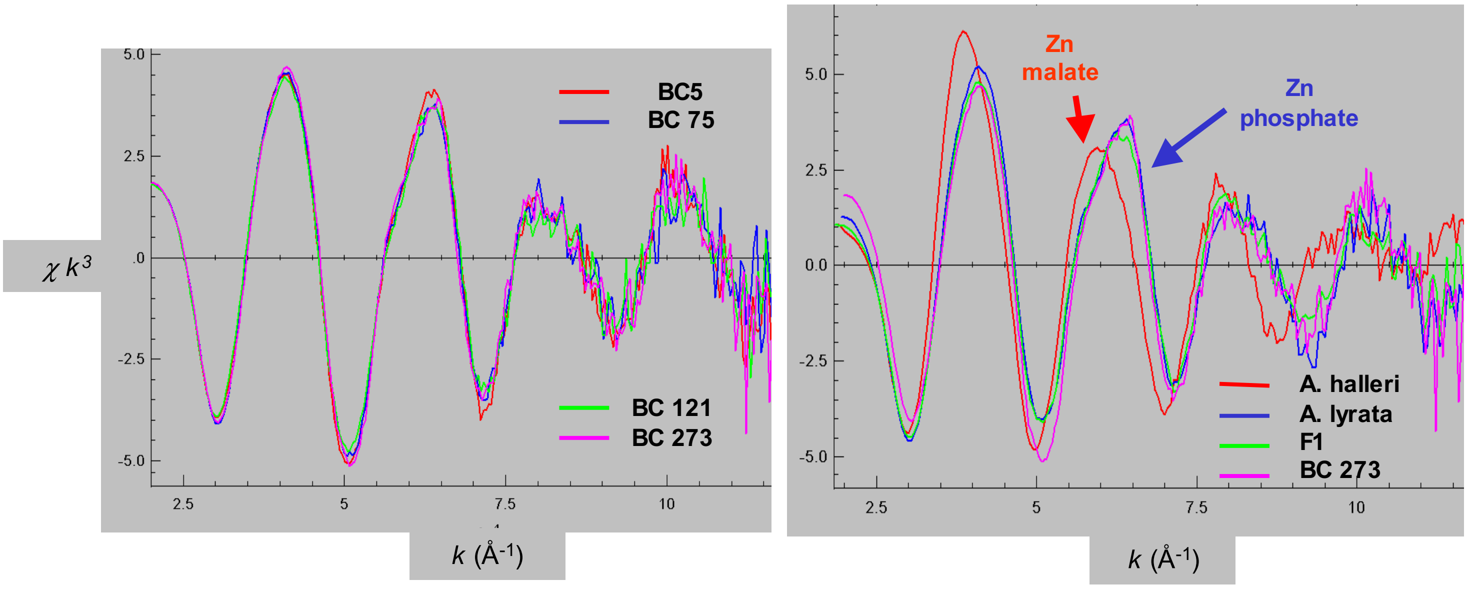

Figure 2: Comparison of the Zn K-edge EXAFS spectra for the leaves of the F1 and back cross progenies with parent plants.

These results are not surprising for F1, BC5 and BC121 since these plants do not accumulate Zn, but they are rather unexpected for the moderately accumulators BC75 and BC273, for which a mixture of Zn malate and Zn phosphate was expected. Moreover, Zn speciation does not change as a function of the degree of tolerance of the plants.

The results show that Zn accumulation can be realized by a mechanism different from the overexpression of Zn transmembrane transporters and the sequestration of Zn in the vacuoles as Zn malate: Zinc can also be stored as Zn phosphate in the veins. A F2 progeny (F1 × F1) is currently being produced. We expect that this progeny will contain real accumulators, and we wish to determine the chemical form of Zn in these plants in order to verify this hypothesis. The fact that no differences in Zn speciation were observed between tolerant and non-tolerant plants suggests that this trait is not determined by the accumulation form of Zn.

Conclusions and Perspectives

The identification of the accumulation forms of Zn by powder EXAFS on diluted samples realized on FAME, and the localization and speciation of Zn by µXRF and µEXAFS at the micrometer scale realized at the ALS provided complementary and consistent informations.

Following this esperiment, we wish to study plants of the F2 progeny, and to start the study of the accumulation forms of cadmium in *A. halleri*. This study is part of a National Research Project funded by the CNRS (ECCO), aiming at better understanding the molecular and genetic mechanisms responsible for Zn and Cd tolerance and hyperaccumulation in this species.

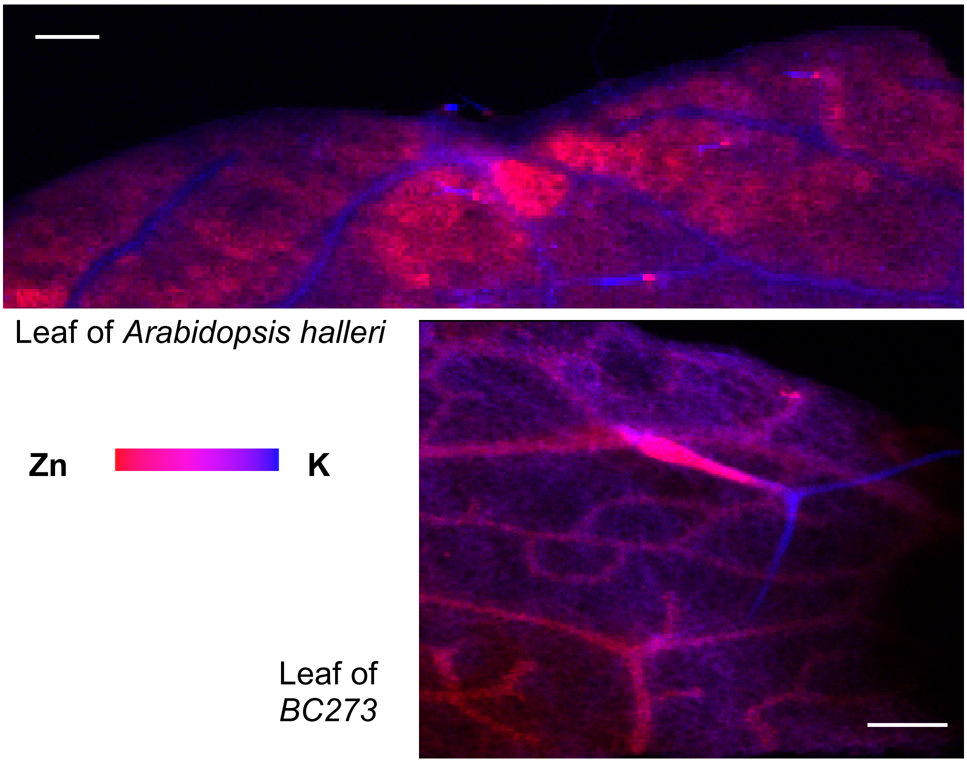

Figure 3: µXRF map showing the distribution of zinc in the leaves of *A. halleri* and BC 273 (non-tolerant, moderately accumulator). Bar = 100 µm. The metal is present in the leaf cells and trichomes in the former, and in the veins and trichomes in the latter.

Scientific production related to this experiment

<u>CONFERENCE (INVITED SPEAKER):</u>

Sarret G., Manceau A., Marcus M.A.M., Saumitou-Laprade P., Willems G., Garnier J.M., Balesdent J., Determining the Chemical Form of Metals in Soils and Plants by Synchrotron Techniques, 87th Canadian Chemistry Conference, London, Canada (May 29 - June 1, 2004).

<u>CONFERENCE (ORAL COMMUNICATION):</u>

Sarret G., Saumitou-Laprade, P., Willems, G., and Manceau, A., Accumulation et excrétion du zinc par les plantes, *Journée scientifique de l'IMBG* "Zinc et Cadmium dans l'Environnement et la Santé", 19 Mai 2004, Grenoble.

<u>ARTICLE</u>

Sarret G., Marcus M.A.M., Saumitou-Laprade P., Willems G., relationships between Zn chemical form and Zn tolerance and accumulation traits: an EXAFS study of *Arabidospis halleri* and *Arabidopsis lyrata* interspecific crosses. *In preparation*

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Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann JL, Traverse A, Marcus MAM, Manceau A (2002). Plant Physiol. **130**: 1815-1826.

Weber M, Harada E, Vess C, von Roepenack Lahaye E, Clemens S (2004). Plant Journal **37**: 269-281.