ESRF	**Experiment title:** Chemical form of cadmium in the Zn, Cd-hyperaccumulating plant Arabidopsis halleri	**Experiment number**: 30-02-760
Beamline: FAME	**Date of experiment**: from: 06 April 2006 to: 10 April 2006	**Date of report**: 29 Sept 2006
Shifts: 12	**Local contact(s)**: Hervé PALANCHER	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Marie-Pierre ISAURE*, Géraldine SARRET*, Nicolas GEOFFROY*, Stéphanie HUGUET*

*** Environmental Geochemistry Group, LGIT, CNRS-Université Joseph Fourier, BP53, 38041 Grenoble Cedex 9**

Report:

Introduction

Metal hyperaccumulation by higher plants is a rare and interesting phenomenon, which can be the basis of a low cost strategy to extract metals accumulated in soils (phytoextraction). However, a better understanding of the mechanisms responsible for metal tolerance and hyperaccumulation is needed to develop the efficiency of this green technology. This study is part of a National Research Project funded by the CNRS (ECCODYN, 2004-2007), aiming at better understanding the mechanisms responsible for Zn and Cd tolerance and accumulation in the hyperaccumulating species *Arabidopsis halleri*. For that, we use an original approach combining both genetic and physiological investigations and localisation/speciation of metals. Interspecific crosses between the tolerant and hyperaccumulating *A. halleri* and the non tolerant non hyperaccumulating *A. lyrata* give tolerant and non hyperaccumulating progenies F1, and the crosses between F1 and *A. lyrata* provides progenies (BC1) with contrasting properties where tolerance and accumulation are segregated (Fig. 1).

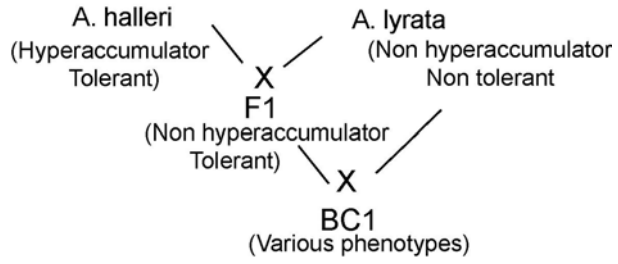


Fig. 1: Scheme of the plant cross studied

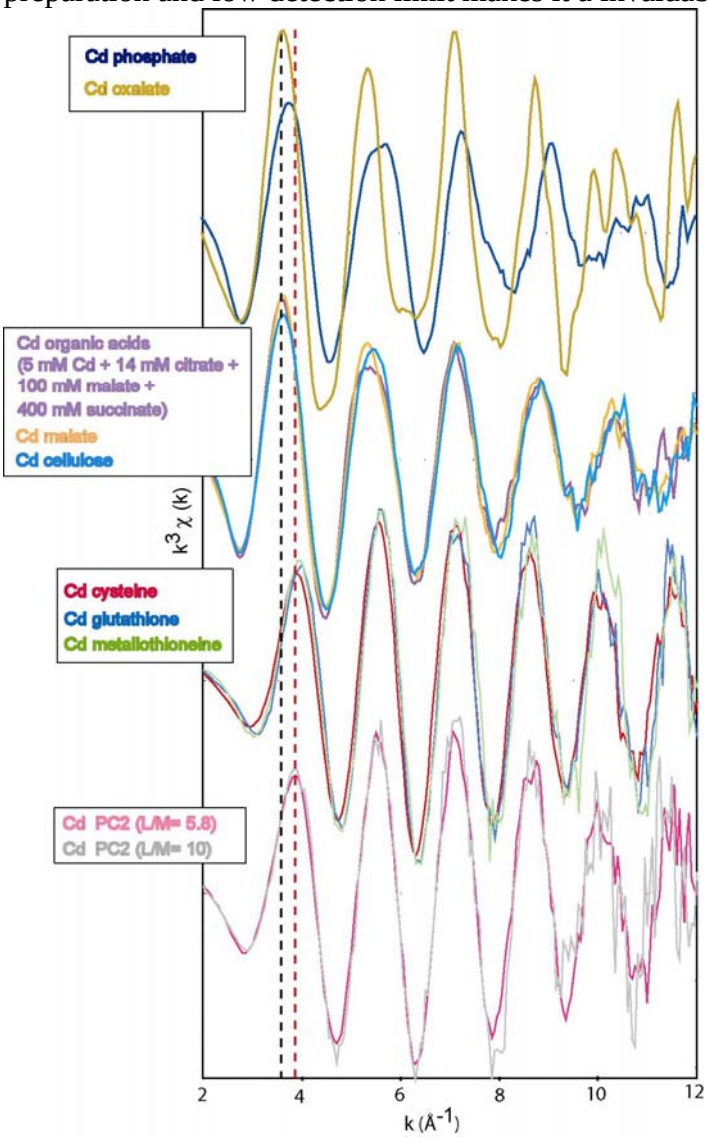
The purpose of this experiment was to determine the chemical form of Cd storage in the leaves of the two parents *A. halleri* and *A. lyrata* and in contrasting progenies (F1 and BC1) in en effort to relate this storage to tolerance and hyperaccumulation traits. We also compared leaves of various ages and trichomes, epidermial hair covering the leaves, to clarify the trafficking of the metal in the plant.

Materials and Methods

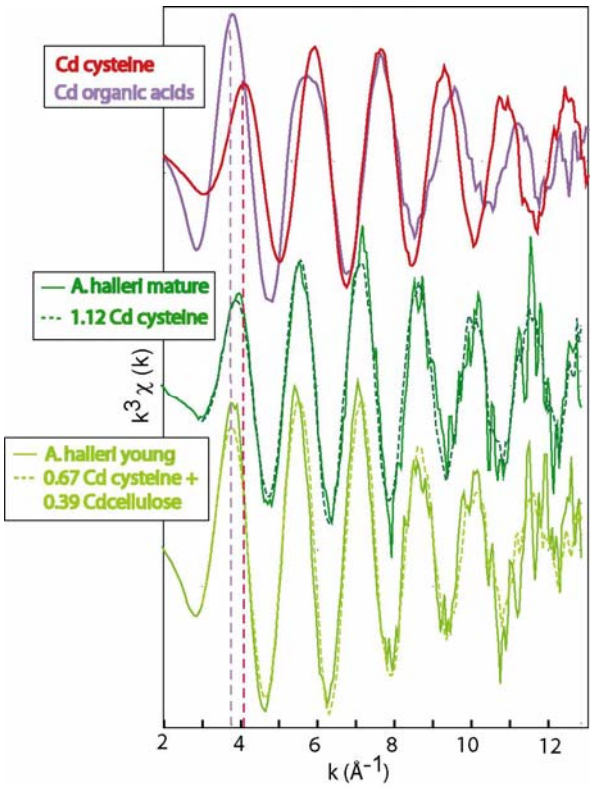
A. lyrata, *A. halleri*, F1 and two BC1 (tolerant BC93, and non tolerant BC121) were grown in hydroponics containing 10 µM Cd in controlled conditions at the Laboratory of Plant Physiology & Molecular Genetics (Brussels). After one and three-weeks exposition, leaves were collected, ground and prepared as frozen hydrated pellets in liquid nitrogen. Samples were then stored in liquid nitrogen. Trichomes, which could not be isolated from hydrated leaves, were collected on freeze-dried *A. halleri* leaves, ground and pressed as pellets. Cd references including various mineral and organic compounds, Cd-phytochelatins, enzymatically synthesized peptides, and Cd-metallothioneins, gene-encoded polypeptides, both enriched with cysteine, were prepared. Cd K-edge EXAFS or XANES spectra were recorded at 20°K using a Helium cryostat available on FAME beamline, and in fluorescence mode using a Canberra 30-element detector.

Results

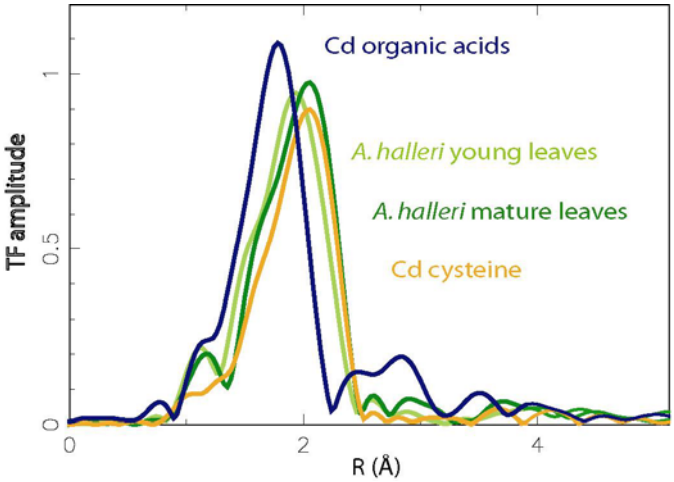
First, spectra obtained on Cd references indicate that Cd K-edge EXAFS spectroscopy is sensitive to the type of ligands : spectra for Cd-S compounds have a clearly higher frequency than for Cd-O compounds (Fig. 2). Although some well crystallized compounds have characteristic spectra (e.g. Cd-phosphate and Cd-oxalate), the sensitivity of the technique seems too low for distinguishing between weakly ordered compounds, e.g. Cd-malate and Cd-cellulose, or Cd-cysteine and Cd-glutathione. Consequently, this spectrosocpic approach should be coupled with analytical chemistry. Still, the very limited sample preparation and low detection limit makes it a invaluable approach for biological samples.



<= Fig. 2 : Cd K-edge EXAFS spectra from Cd references containing O ligands (Cd phosphate, Cd oxalate, Cd organic acids, Cd malate and Cd cellulose) and S ligands (Cd cysteine, Cd glutathione, Cd metallothioneine, and two phytochelatins (Cd-PC2 L/M = 5.8 and 10).



⇑ Fig. 3 : Cd K-edge EXAFS spectra from *A. halleri* young and mature leaves compared to Cd cysteine and Cd organic acids, and linear combination fit.



Leaves	Atom	CN	R (Å)	σ^2 (Å^2)
A. halleri, young	S	3.0	2.55	0.008
	N/O	3.4	2.33	0.008
A. halleri, mature	S	4.4	2.54	0.007
Th. caerulescens, young and mature (1)	S	1.5	2.51	0.008
	N/O	4.5	2.28	0.008
Th. caerulescens, senescent (1)	S	0.3	2.47	0.007
	N/O	5.7	2.28	0.008

CN: coordination number, R: distance interatomic distance
σ^2 : Debye-Waller factor. (1) Küpper et al. (2004).

Fig. 4 : Fourier Transform of Cd K-edge EXAFS spectra for Cd cysteine, Cd organic acids, and *A. halleri* young and mature leaves, and corresponding structural parameters determined by the first coordination shell.

Comparison between *A. halleri* young and mature leaves shows that Cd is only bound to sulfur-containing groups in mature leaves while a small oxygen contribution is observed for young leaves (Fig. 3). This contribution was estimated to 30- 50% using linear combination and FEFF simulation (Fig. 3 and 4). Interestingly, Küpper et al (2004) found that Cd was mainly bound to O/N groups in the hyperaccumulator *Thlaspi caerulescens*, suggesting that *A. halleri*, a less tolerant and accumulating species, would have a different Cd storage. EXAFS analysis performed on undifferenciated leaves (54 µg Cd / g Fresh Weight) showed that the proportion of O/N ligands was not significant (not shown). Investigations on *A. halleri* trichomes indicate that Cd is bound to oxygen groups in this compartment (Fig. 5) as found for the non tolerant and non accumulating species *A. thaliana* (Isaure et al, accepted). Oxygen contribution evidenced for young leaves could result from the high number of trichomes in these leaves.

In the non tolerant and non accumulating parent *A. lyrata* (45 µg Cd / g FW), tolerant F1 (21 µg Cd / g FW), tolerant BC93 (19 µg Cd / g FW) and non tolerant BC121 (4.5 µg Cd / g FW), the Cd fluorescence signal was not sufficient to collect EXAFS spectra. XANES spectra for *A. lyrata* and BC 121 are very close to *A. halleri* and Cd cysteine spectra indicating that Cd is associated to S-containing groups (Fig. 6). Although sulfur is the major ligand observed for F1 and BC93 leaves, we also found a O/N contribution estimated to 20-30% by linear combination.

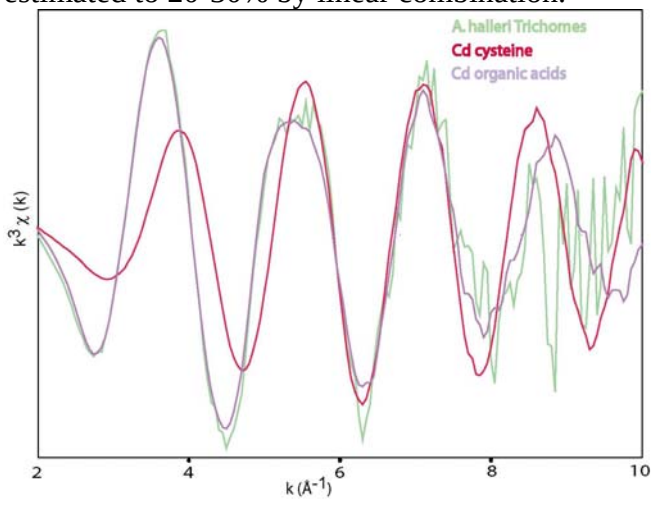


Fig. 5 : Cd K-edge EXAFS spectrum for *A. halleri* trichomes compared to Cd cysteine and Cd organic acids.

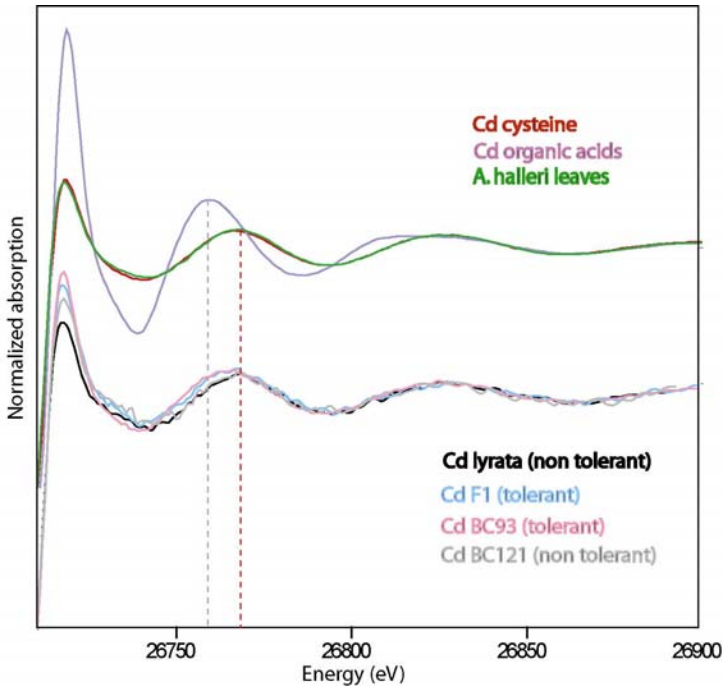


Fig. 6 : Cd K-edge XANES spectra for Cd cysteine, Cd organic acids, *A. halleri, A. lyrata*, F1, BC93 and BC121. leaves.

In parallel, we studied the localization and speciation of Cd in *A. halleri* leaves combining µXRF and Cd L_{III}-edge µXANES at ID21 beamline. We found that Cd was not accumulated in the epidermis but in

mesophyll and veins. High concentrations of metal were also found in a narrow ring of the trichomes where it is bound to O ligands probably provided by cell wall or cuticle (see report n° SC 1768, 24 Feb 2006) corroborating our new results.

Conclusions

Results showed that Cd is mostly bound to S ligands in the leaves of *A. halleri*, *A. lyrata* leaves, and the progenies presented assorted Cd tolerance. This is a new finding compared to results obtained on the Cd hyperaccumulator *Thlaspi caerulescens* (Küpper et al, 2004) who mainly identify O/N ligands, and raises interesting questions : is *A. halleri* can be really qualified as cadmium hyperaccumulator? Is the chemical form of metal storage depends on the accumulation? We also found that trichomes locally concentrate the metal, which is probably associated to functional groups of the cell wall and cuticle. Experiments are still in progress to evidence the chemical form of cadmium in the different compartments and evaluate whether the O contribution could result from the proportion of trichomes in leaves or not.

References

Küpper H., Mijovilovich A., Meyer-Klaucke W., and Kroneck P. M. H. (2004) Tissue- and age-dependent differences in the complexation of cadmium and zinc in the cadmium/zinc hyperaccumulator Thlaspi caerulescens (Ganges ecotype) revealed by X-ray absorption spectroscopy. *Plant Physiology* **134**(2), 748.

Isaure M. P., Fayard B., Sarret G., Pairis S., and Bourguignon J. Localization and chemical forms of cadmium in plant samples by combining analytical electron microscopy and X-ray spectromicroscopy, *accepted in Spectrochimica Acta Part B: Atomic Spectroscopy.*

Scientific production related to this experiment

Sarret G., Isaure M.P., Verbruggen N., Barthes V., Willems G., Manceau A., Saumitou-Laprade P., 2006, Localization and speciation of Zn and Cd in the hyperaccumulating plant *Arabidopsis halleri. 6th International Symposium on Speciation of Elements in Biological, Environmental and Toxicological Sciences (ISSEBETS)*, Bialowieza, Poland, 21-25 June 2006, p. 75.

Isaure M.P., Sarret G., Verbruggen N., Geoffroy N., Barthes V., Palancher H., Susini J., Relationships between Cd chemical form and distribution and Cd tolerance and accumulation : a XAS study of *Arabidopsis halleri* and *Arabidopsis lyrata* interspecific crosses. *In preparation.*