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ESRF

	Experiment title: Localization and chemical forms of Cd in the hyperaccumulating plant *Arabidopsis halleri*	**Experiment number**: SC 1768
Beamline: ID21	**Date of experiment**: from: 09/14/2005 to: 09/20/2005 (experiment first planned in march 2005 and delayed due to problem in the synchrotron ring)	**Date of report**: 24/02/2006
Shifts: 20	**Local contact(s)**: Jean SUSINI	*Received at ESRF:*

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Report:

Introduction

The use of metal hyperaccumulating higher plants to extract metals accumulated in soils (phytoextraction) might represent a low cost alternative remediation strategy. 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 the beginning of a National Research Project funded by the CNRS (ECCO, 2004-2007), aiming at better understanding the mechanisms responsible for Zn and Cd tolerance and accumulation in the hyperaccumulating species *Arabidopsis halleri*. The purpose of this experiment was to localize Cd in various tissues of *A. halleri*, to determine its chemical form, and to compare these results to the results obtained on the non hyperaccumulating species *Arabidopsis thaliana*, previously investigated (experimental report SC1465). During this experiment, we also checked the influence of sample preparation and conditions of analysis (hydrated sample analyzed using a cryostat *vs* freeze-dried sample or thin section analyzed at ambiant temperature), which appears as a more and more sensitive point in the literature.

Materials and Methods

A. thaliana and *A. halleri* were grown on Cd contaminated medium in controlled conditions. Leaves and roots were collected and immersed in N_2 liquid. Some of the samples were freeze-dried, and others were kept in N_2 liquid. Others were embedded in a cryogenic protective compound (O.C.T.), immersed in N_2 liquid and prepared as 30 µm-thick thin sections using a cryomicrotome.

Thin sections and freeze-dried samples were analyzed under vacuum at ambiant temperature. Hydrated samples kept in N_2 liquid were analyzed under vacuum at -160°C using a N_2 cryostat available on ID21 beamline.

Elemental maps were collected for P, S, Cd, K, and Ca by recording the X-ray fluorescence with a Ge solid state detector (PGT). Because most of the samples contain a large amount of potassium, the K K_α (3.31 keV) overlaps the most intense Cd emission line, Cd $L_{\alpha 1}$ (3.13 keV) and two beam energies were required to map on one hand, P, S, and Cd (3.55 keV), and on the other hand K and Ca (4.10 keV). Samples were scanned with a beam size on the sample of H=0.7 µm x V=0.3 µm. Cd L_{III}-edge (3.538 keV) µ-XANES spectra were collected on points-of-interest selected from the elemental maps. Measurements were performed in fluorescence-yield detection mode using the same Ge-detector. Many XANES spectra were also recorded on Cd reference compounds including mineral and organic, solid and liquid samples.

Results

XANES spectra collected on numerous Cd references indicated that Cd L_{III}-edge XANES was very sensitive to Cd ligands. Indeed, this technique enables a clear distinction between S and O/N ligands since O/N ligands induce a peak at 3539.6 eV (Fig. 1B). Solution complexes and Cd-pectin, Cd-cellulose and Cd-cell wall display poorly structured spectra, and some of them (eg., Cd-malate and Cd-citrate) are hardly distinguishable. On the contrary, mineral Cd references exhibit characteristic spectral features. This is an important result since to our knowledge, XAS at Cd L_{III}-edge was only used in the past by Pickering et al (1999) with a non focused beam.

Microfluorescence maps and µXANES spectra collected on *A. thaliana* leaves and roots showed that freeze-drying did not significantly change the distribution and chemical form of Cd compared to the hydrated sample. In leaves, Cd was found to be accumulated in a ring of the trunk of the trichome, where the metal is associated to O ligands provided by pectin of the cell wall (Fig. 1A,B). In roots, Cd is preferentially located in vascular bundles where it is bound to S-containing groups (probably phytochelatin or metallothionein). A publication reporting these new results and the previous ones on *A. thaliana* was submitted to *Environmental Science and Technology* (Isaure *et al.*) in early February 2006. The abstract is reported at the end of this report.

Results obtained on hydrated samples and thin sections of *A. halleri* indicated that Cd in leaves was mainly found in a ring located in the trichomes as observed for *A. thaliana* (Fig. 2A). µXANES also showed that Cd was mainly associated to oxygen ligands of cell wall but a significant contribution of S ligands was detected (Fig. 2B. This contribution increased when we probed points located a few micrometers away from the ring. In our next experiments (March 2006), mechanisms of Cd sequestration must be clarified in roots and in the tissues of the non hyperaccumulating *A. lyrata*.

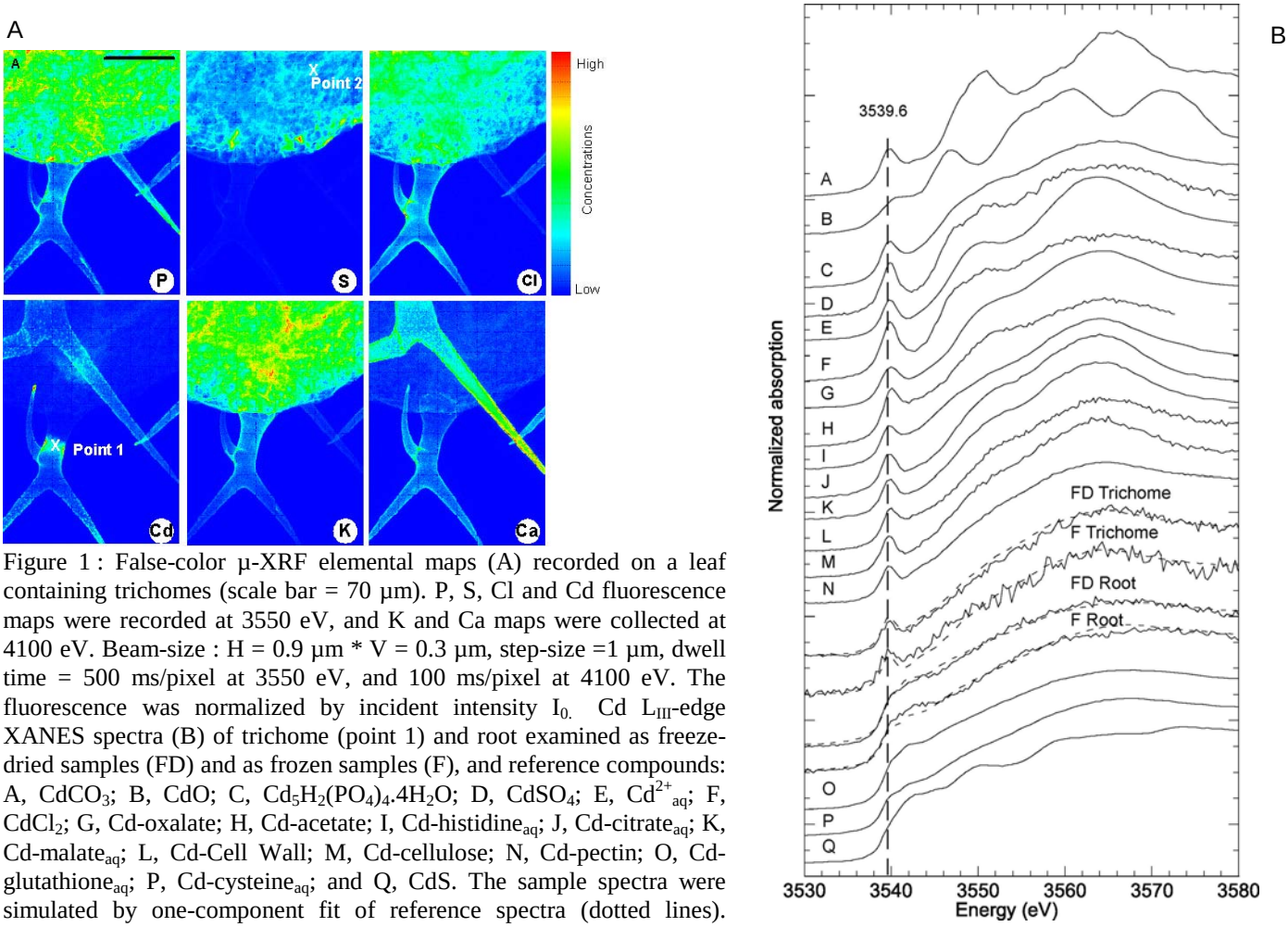

Figure 1 : False-color µ-XRF elemental maps (A) recorded on a leaf containing trichomes (scale bar = 70 µm). P, S, Cl and Cd fluorescence maps were recorded at 3550 eV, and K and Ca maps were collected at 4100 eV. Beam-size : H = 0.9 µm * V = 0.3 µm, step-size =1 µm, dwell time = 500 ms/pixel at 3550 eV, and 100 ms/pixel at 4100 eV. The fluorescence was normalized by incident intensity I_0. Cd L_{III}-edge XANES spectra (B) of trichome (point 1) and root examined as freeze-dried samples (FD) and as frozen samples (F), and reference compounds: A, $CdCO_3$; B, CdO; C, $Cd_5H_2(PO_4)_4.4H_2O$; D, $CdSO_4$; E, Cd^{2+}_{aq}; F, $CdCl_2$; G, Cd-oxalate; H, Cd-acetate; I, Cd-histidine$_{aq}$; J, Cd-citrate$_{aq}$; K, Cd-malate$_{aq}$; L, Cd-Cell Wall; M, Cd-cellulose; N, Cd-pectin; O, Cd-glutathione$_{aq}$; P, Cd-cysteine$_{aq}$; and Q, CdS. The sample spectra were simulated by one-component fit of reference spectra (dotted lines). Freeze-dried and frozen trichomes were simulated by 101% Cd-Pectin, freeze-dried and frozen roots by 100% Cd-Glutathione.

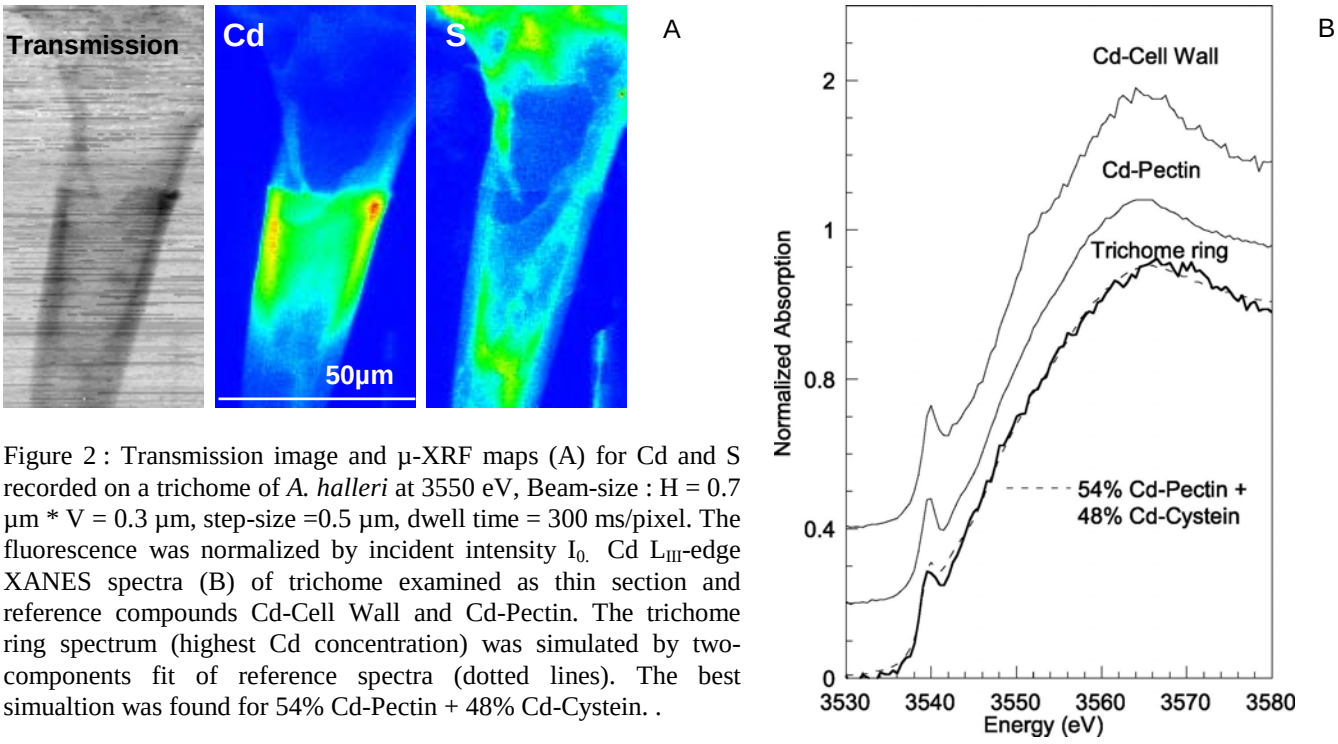

Figure 2 : Transmission image and µ-XRF maps (A) for Cd and S recorded on a trichome of *A. halleri* at 3550 eV, Beam-size : H = 0.7 µm * V = 0.3 µm, step-size =0.5 µm, dwell time = 300 ms/pixel. The fluorescence was normalized by incident intensity I_0. Cd L_{III}-edge XANES spectra (B) of trichome examined as thin section and reference compounds Cd-Cell Wall and Cd-Pectin. The trichome ring spectrum (highest Cd concentration) was simulated by two-components fit of reference spectra (dotted lines). The best simualtion was found for 54% Cd-Pectin + 48% Cd-Cystein. .

References

- Pickering, I. J.; Prince, R. C.; George, G. N.; Rauser, W. E.; Wickramasinghe, W. A.; Watson, A. A.; Dameron, C. T.; Dance, I. G.; Fairlie, D. P.; Salt, D. E. X-ray absorption spectroscopy of cadmium phytochelatin and model systems. *Biochim. Biophys. Acta* **1999**, *1429*, 351-364.

Scientific production related to this experiment

- Isaure MP, Fayard B, Sarret G, Pairis S, Bourguignon J, Localization and chemical forms of cadmium in *Arabidopsis thaliana*, submitted to *Environmental Science and Technology*.

- Isaure MP, Fayard B, Bourguignon J. 2005. Localization and speciation of cadmium in *Arabidopsis thaliana* plants. *International Workshop on Metal Fluxes and Stresses in Terrestrial Ecosystems*, Ascona, Switzerland, 15-20 October (Poster). **Award received for the best poster out of 36**.

- Illustration for **ESRF Highlights 2005**, Introduction of the X-ray Imaging and Optics Session, p. 103-104.

Abstract of *Isaure et al, submitted to Environ. Sci. Technol.*

Cadmium is a metal of high toxicity for plants. The model plant *Arabidopsis thaliana* was exposed under controlled conditions to 200 µM Cd during 4 days. Scanning electron microscopy coupled with energy dispersive X-ray microanalysis (SEM-EDX) and micro X-ray fluorescence (µ-XRF) were used to determine the cadmium distribution in the roots and in the leaves, while Cd L_{III}-edge micro X-ray absorption near edge structure (µ-XANES) spectroscopy was applied to identify the chemical form of the metal. Leaves and roots contained up to 550 mg Cd kg^{-1} dry weight (DW) and 2530 mg Cd kg^{-1} DW, respectively. Our results show that, in the roots, Cd is localized in vascular bundles, and coordinated to S ligands. This chemical form is likely to correspond to an intracellular storage of Cd, and possibly to the form of transport from the roots to the leaves. In the leaves, the trichomes represent the main compartment of Cd accumulation, where the metal is bound to pectin-type O ligands of the cell wall. This work highlights the role of trichomes in Cd detoxification and illustrates the sensitivity of Cd L_{III} XANES spectroscopy, which is applied here for the first time to plants. Combined with µ-XRF, Cd L_{III} µ-XANES is very promising to study Cd storage and trafficking mechanisms in plants.