ESRF	**Experiment title:** **Cd localization and speciation in the hyperaccumulator *Arabidopsis halleri* and genetic traits**	**Experiment number**: EC-798
Beamline: ID21	**Date of experiment**: from: June 20th 2011 to: June 27th 2011	**Date of report**: 15/02/2012
Shifts: 18	**Local contact(s)**: Hiram Castillo-Michel/Murielle Salomé	*Received at ESRF:*

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Report:

Introduction

The Zn, Cd hyperaccumulator *Arabidopsis halleri* is a model plant to investigate the misknown mechanisms of Cd accumuation (Huguet et al., 2012). It is found in both metal contaminated and non-contaminated sites of Europe. The aim of this proposal was to compare the compartments of Cd accumulation in *A. halleri* leaves from a metallicolous origin and in a non tolerant plant *Arabidopsis lyrata* which is non tolerant and non accumulator, and to also compare the nature of the ligands in these compartments. For that purpose, we used a combination of chemical mapping using micro X-ray Fluorescence (µXRF) and micro-focused X-ray Absorption Near Edge Structure spectroscopy (µXANES) using Cd L_{III}- edge. Plants trichomes were also found to accumulate Cd and an objective of the proposal was to progress in the understanting of this mechanism of sequestration using Synchrotron Radiation µFourier Transformed InfraRed spectroscopy (µFTIR).

Experimental

A. halleri and *lyrata* were grown in hydroponics with 10 µM Cd during 3 weeks. Leaves were collected, and prepared as thin-sections using a cryomicrotome. The beam was monochromatized using a Si(111) monochromator and a zone plate focused the beam on the samples with a lateral resolution of H=0,73 µm x V=0,24 µm. Cd distribution was studied by µXRF at 3.57 keV, and chemical ligands were determined by Cd L_{III}-edge µXANES recorded on points of interest. Samples were kept frozen until analysis and all measurements were performed at -170°C using a cryostat. Fluorescence spectra were recorded with a 4 element SDD detector. µXRF spectra were treated and deconvoluted using PYMCA software. XANES spectra were analyzed using Athena software, and compared to Cd references spectra that we had already collected. Single trichomes were also prepared as thin-sections and studied by µFTIR. Different setups and sample preparation were tested. Trichomes have a very thick cell walls and cuticle and are very absorbing, so we had to cut very thin (10 µm) sections and work in transmission mode. In this purpose, portions of leaves containing trichomes were embedded in OCT, frozen and sectioned in the cryomicrotome. Thin sections were deposited on BaF2 discs and placed in a dry atmosphere for dehydration. µFTIR maps were recorded with a 8x8 µm spot size. Maps were analyzed using OMNIC and PyMCA, and spectra were compared to reference compound spectra recorded previously in ATR mode (malate, oxalate, citrate, cell wall , pectin, phosphate, etc...).

Results

Elemental maps collected on *A. halleri* leaves show that Cd is located in the central and secondary veins (xylem and also phloem) and in the mesophyll, particularly in cell walls. The tip of the leaf is most enriched with Cd, maybe due to the smaller size of the mesophyll cells in this area and hence the higher proportion of cell walls. Cd seems to be colocalized with S in the cell walls but this is less obvious in the vascular bundles. The metal is not preferentially located in the epidermis. In *A. lyrata*, Cd seems also localized in the veins but not in the mesophyll, suggesting a different compartmentalization. In this case, the metal is preferentially located in the epidermis. A colocalization with S is observed.

µXANES spectra are displayed in Figure 3 and compared to Cd references compounds. As shown by Cd-malate and Cd-cystein, Cd L_{III}-edge Xanes is powerfull to distinguish a first coordination sphere composed by O ligands and composed by S atoms, respectively (Isaure et al, 2006). Results show that all spectra collected on epidermis and veins of *halleri* and *lyrata* exhibit an absorption edge typical of S ligands. Oxygen ligands are not excluded but are minor. Linear combination are in progress to estimate this porportion. Attempts were done to collect µXanes spectra on cell walls. However, due to the very small size of these cell compartments, it was tricky to maintain the beam on the area during the whole spectrum and no spectrum of good quality could be obtained.

µFTIR spectra recorded in the various regions of the trichome were quite different (Figure 4), and the ring which is enriched in metals, is characterized by an intense band at 1600 cm^{-1} corresponding to COOH groups. This is consistent with the binding of metals by cell wall components. The cell walls (and not particularly the ring) were enriched in lipids. Further data analysis is under way to assign all bands.

The FTIR signal of the OCT was quite strong compared to the signal of the trichome. It is not easy to subtract the OCT background because the amount of OCT varies from one pixel to another. Therefore, other types of resins such as parrafin should be tested.

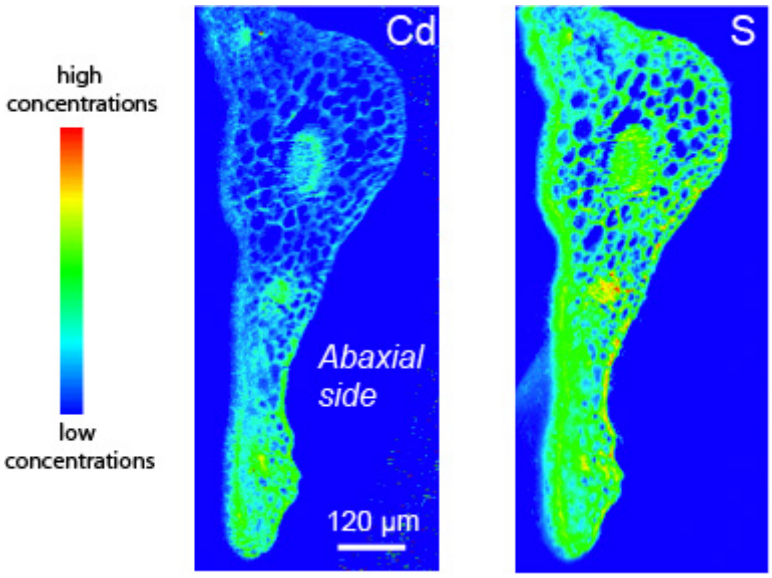

Figure 1 : Cd and S maps collected on a cryo cross-section of *A. halleri* leaves at 3,57 keV, with a beamsize of H=0,73 µm x V=0,24 µm.
Step size : 3 µm, counting time : 100 ms.
Cd is preferentially located in the vascular system and cell walls of the mesophyll.

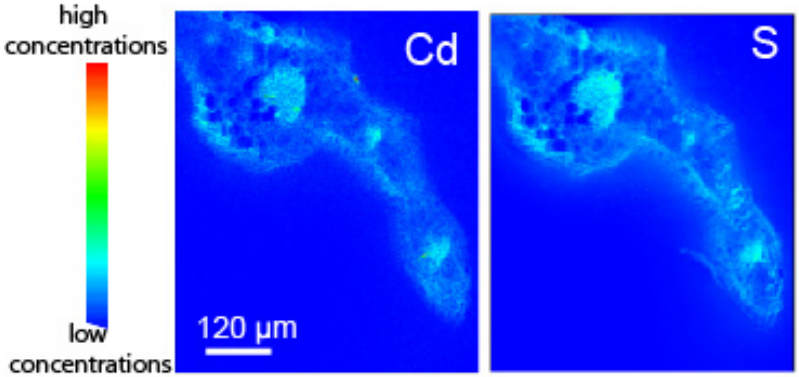

Figure 2 : Cd and S maps collected on a cryo cross-section of *A. lyrata* leaves at 3,57 keV, with a beamsize of H=0,73 µm x V=0,24 µm.
Step size : 4 µm, counting time : 170 ms.
Cd is preferentially located in the vascular system and in the epidermis.

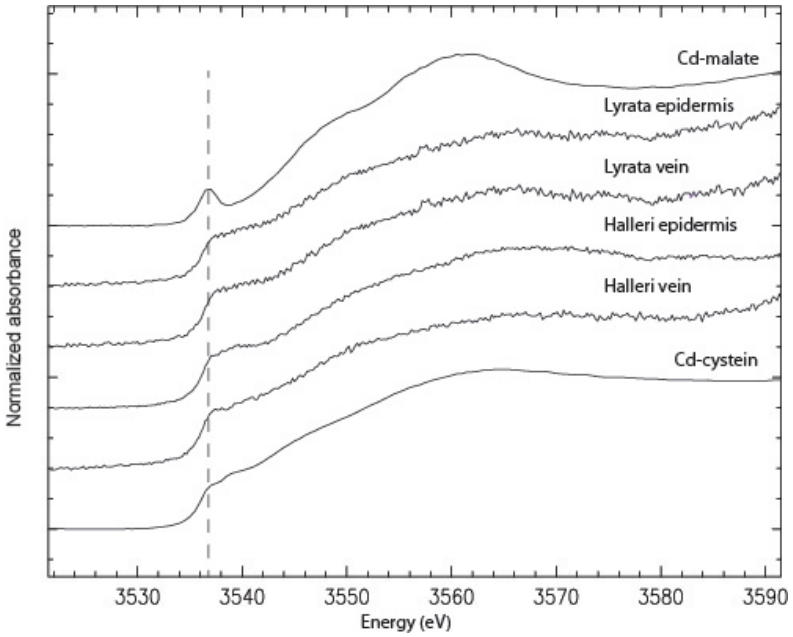

Figure 3 : Cd LIII-edge µXANES spectra collected on veins and epidermis of *A. halleri* and *lyrata* leaves prepared as cryo-cross sections. Spectra are compared to Cd-malate (O ligands) and Cd-cystein (S ligands).

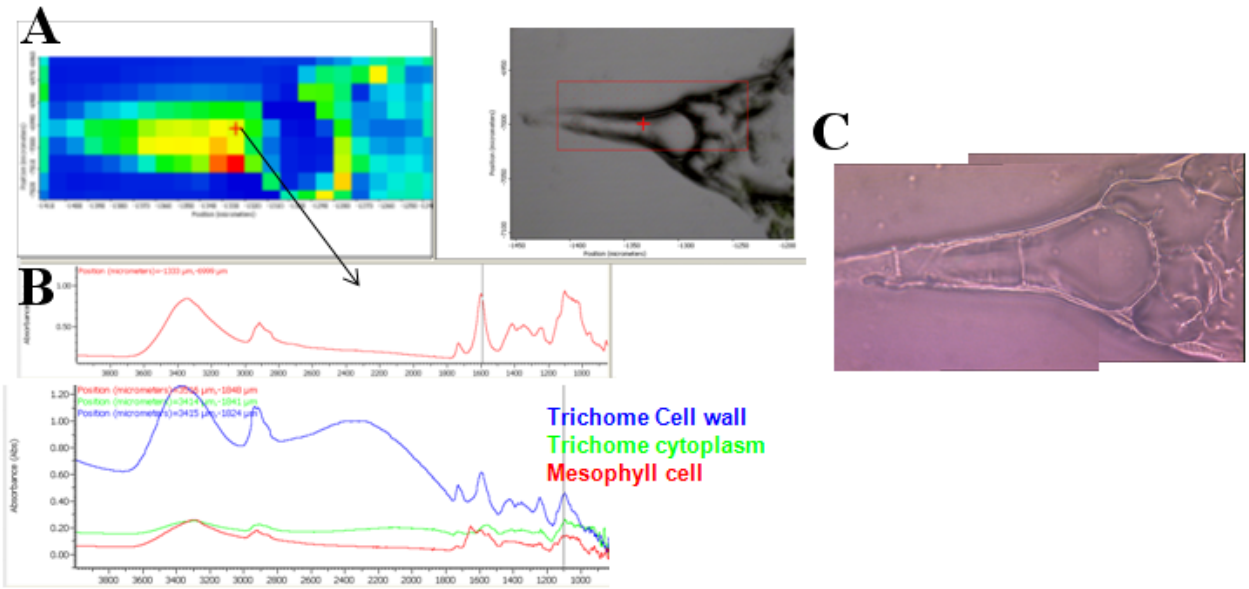

Figure 4: A, µFTIR map of the peak area of the band at 1600 cm^{-1} for a trichome of *A. halleri*. B, spectrum recorded at the position of the ring, in the trichome cell wall and cytoplasm, and in the mesophyll. C, optical images of the trichome.

References :

- Huguet S, Bert V, Laboudigue A, Barthès V, Isaure MP, Llorens I, Schat H, and Sarret G (2012) Cd speciation and localization in the hyperaccumulator *Arabidopsis halleri*. *Environmental and Experimental Botany*, 82, 54-65.
- Isaure MP, Fayard B, Sarret G, Pairis S, Bourguignon J (2006) Localization and chemical forms of cadmium in plant samples by combining analytical electron microscopy and X-ray spectromicroscopy. *Spectrochimica Acta Part B: Atomic Spectrto telated oscopy,* 61, 1242-1252.

Publications related to this proposal:

- Isaure MP, Huguet S, Meyer CL, Verbruggen N, Sarret G. Speciation and localization of Cd in the hyperaccumulator plant Arabidopsis halleri grown in the field and in controlled conditions by XAS techniques, *22nd V.M. Goldschmidt Conference*, June 24-29 2012, Montreal, Québec, Canada (Talk).

- Isaure MP. Combining chemical imaging and XAS techniques to investigate metal localization and speciation, *Speciation Seminar Biological Environmental and Nuclear Speciation,* May 29-31 2012, Montpellier, France (Invited talk).

- A paper is in preparation for *Environmental Science and Technology*.