ESRF	**Experiment title:** **Localization and speciation of Cd and U in *Arabidopsis Thaliana* vegetal cells and plants**	**Experiment number**: SC 1465
Beamline: ID 21	**Date of experiment**: For Cd measurements from: June 16, 2004 to: June 22, 2004	**Date of report**: August 2nd, 2004
Shifts: 15	**Local contact(s)**: Barbara FAYARD	*Received at ESRF:*

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Report: (NB : Experimental report dealing with U experiments on ID22 will be sent separately)

Introduction

Phytoextraction is a remediation strategy using higher plants to extract metals accumulated in soils. This technique requires the understanding of metal accumulation in plants. We study the capacity of *Arabidopsis thaliana*, chosen as a model organism because of the complete knowledge of its genome, to accumulate cadmium (Cd), released into the environment through application of sewage sludge, fertilizers…

The aims of our synchrotron radiation experiments were (i) to identify the distribution of Cd in both *A. thaliana* individual cells and plant tissues, (ii) to evidence association of Cd with other chemical elements entering the composition of the tissues, and (iii) to determine the Cd coordination environment (speciation) in the different tissues.

Samples and experimental setup

Suspensions of *A. thaliana* cells were cultured in controlled conditions in a nutritive MS (Murashige and Skoog) medium contaminated with $Cd(NO_3)_2$ 200µM. After four days of treatments, cells were sampled, rinsed with deionised water, and deposited on an ultralene film. They were then cryofixed in isopentane chilled with liquid nitrogen and freeze dried at –36°C. *A. thaliana* seeds were sown on MS agar in Petri dishes and grown in controlled conditions. After 12 days, they were transferred onto the same media containing 200µM $Cd(NO_3)_2$. Plants were collected after 4 days of treatments, rinsed in deionised water, frozen in liquid nitrogen, and freeze dried at –36°C. Cell and plant samples were maintened between two ultralene films for X-ray measurements.

Measurements were performed on ID21 using the Scanning X-ray Microscope (SXM) in fluorescence mode and under vacuum. Elemental maps were obtained for P, S, Cd, K, and Ca by recording the X-ray fluorescence with a Ge solid state detector. 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=0.9µm x V=0.3 µm.

Cd LIII-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.

Results

Elemental maps recorded on vegetal cells showed that Cd was present in grains (plastes) in association with S, K and Ca (data not shown). Surprisingly, accumulation in vacuoles of the cells could not be evidenced. In plants, the Cd fluorescence signal collected on roots and stems was lower than the signal recorded on leaves, suggesting that this tissue is the main accumulating compartment. Elemental maps collected on leaves and trichomes (epidermial hairs) showed that Cd is mainly present in trichomes in association with Ca (Fig.1). Mesophyll and veins of the leaves were not Cd enriched compared to trichomes.

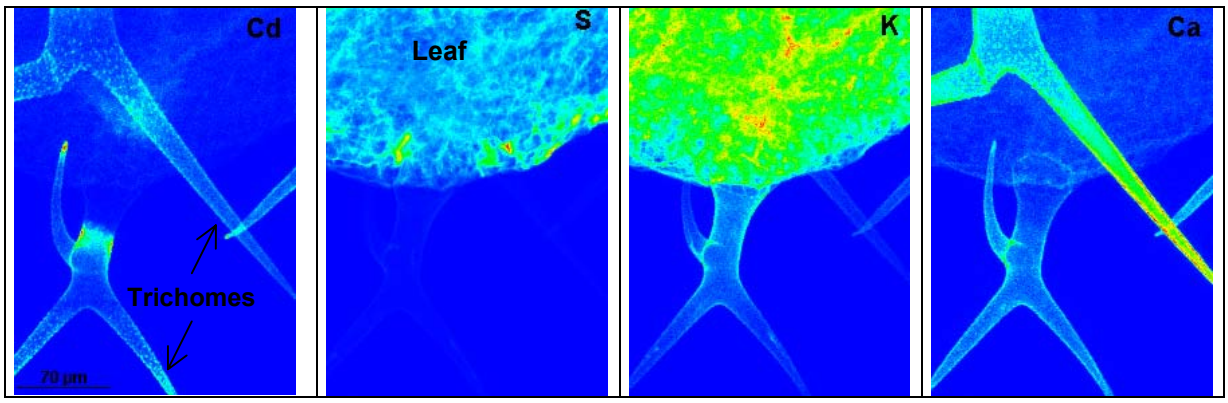

Fig 1 : Cd, S, K, and Ca maps recorded on *A. thaliana* leaves and trichomes. Beam size: 0.9µm x 0.3µm, Energy : 3.55 keV for S and Cd, 4.10 keV for K and Ca, Step size: 1 µm, Dwell time: 500 ms at 3.55 keV, 90 ms at 4.10 keV.

Cd LIII-edge µ-XANES spectra collected on trichomes are cleary different from that recorded on roots and veins (Fig. 2), suggesting that the chemical form of storage in trichome is not the same than the chemical form of transport in roots and veins. These forms have not been identified yet. Cd could be bound to phytochelatine (S ligands) in roots and veins, and to calcium oxalate in trichomes, as observed for tobacco (Choi *et al.*, 2001) but more investigations are required to verify these hypotheses.

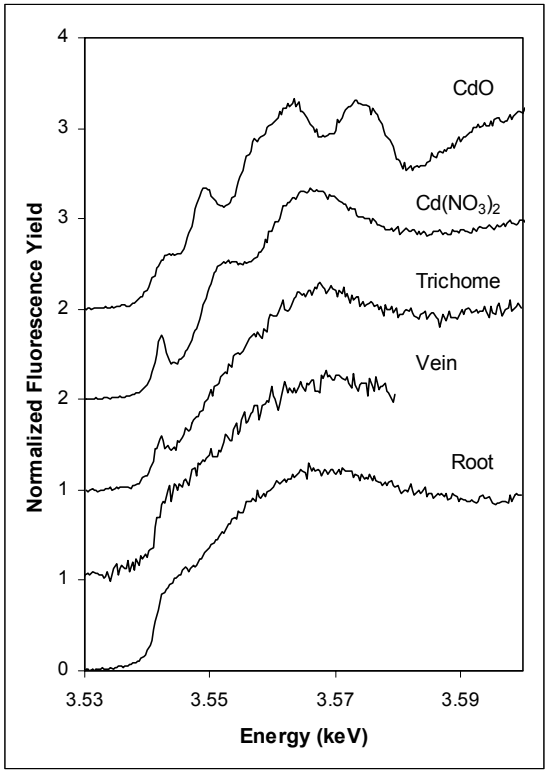

Fig 2 : Normalized Cd LIII-edge µ-XANES spectra from *A. thaliana* and from two references CdO and $Cd(NO_3)_2$. Note that trichome spectrum is clearly different from vein and root spectra, but none of them resembles the CdO and $Cd(NO_3)_2$ spectra.

References

Choi YE, Harada E, Wada M, Tsuboi H, Morita Y, Kusano T, Sano H (2001). *Planta* 213: 45-50.