EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** **Local chemical environment and localization of Cd in Cd hyperaccumulator *Gomphrena claussenii* – role of Ca?**	**Experiment number**: EV-159
Beamline: ID21	**Date of experiment**: from: 11/2/16 to: 16/2/16	**Date of report**: 27/03/17
Shifts: 15	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

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Report:

INTRODUCTION – Heavy metal sequestration in specific subcellular compartments allows plants to tolerate as well as to accumulate high levels of heavy metals. Only a small number of plant species has evolved the ability to (hyper)accumulate high concentrations of heavy metals in aboveground parts. Recently, a new metallophyte species with substantial biomass, namely *Gomphrena claussenii*, has been described as the first Zn and Cd hypertolerant species belonging to the Amaranthaceae family. Using SEM-EDX and µ-PIXE we demonstrated that Cd, but not Zn, accumulates in Ca-oxalate crystals. The experiment was conducted to i) unambiguously confirm Cd deposition to Ca-oxalate crystals (overcoming interaction with potassium K edge in SEM-EDX and poorer cross-section for Cd-K line in µ-PIXE), ii) determine Cd ligand(s) in the crystal, and iii) resolve involvement of Ca metabolism in Cd tolerance in this plant species.

EXPERIMENTAL – Plants were grown under controlled conditions in Clark nutrient solution with 100 µM of Cd and three external Ca concentrations (low, 0.1 mM; optimal, 1 mM and high, 10 mM). Fresh plant material was frozen in propane cooled with liquid nitrogen and cryo-sectioned at -25 °C. The measurements were performed using the SXM set-up equipped with cryostat. The excitation energy for the analyses was first set to 4.1 keV to record maps of Ca, K, Cl, P and S simultaneously and then the enery was set to 3.55 keV to access Cd-LIII. Finally, the Cd-LIII edge XANES spectra and Ca-K edge XANES spectra were recorded in different plant tissues (roots, stems and leaves) on Ca oxalate crystals.

RESULTS – Two dimensional maps of roots, stems and leaves were generated first at E=4.1 to determine Ca hotspots which represented Ca oxalate crystals (**Fig. 1A,** Ca in red, K in green and S in blue) and detailed maps on selected crystals were generated at the same energy (Fig. **1B,** Ca in red, K in green and S in blue), followed by mapping at E=3.55 (**Fig. 1C,** Cd in red, P in green and S in blue). At each energy, XANES spectra were measured (40 spectra in total plus Cd compound references; selection of spectra is shown in **Fig 1D**). Linear combination fitting using two references (Ca-Cd-oxalate and Cd-glutathione) confirmed our hypothesis that the majority of Cd was bound to oxalate (95% on average for stems, **Fig. 1E** and 90% on average for leaves, **Fig. 1F**) and the remaining 5% and 10% in stems and leaves, respecively, was bound to S

ligands. In roots, no Ca crystals were found and S ligands dominated (**Fig. 1G**). There was no siginificant influence of different Ca concentration on Cd ligand environment in the crystals.

Figure 1: Localisation of Ca and Cd in *Gomphrena clausenii* tissues and Cd ligand environemnt in roots and in Ca-oxalate crystals in stems and leaves. A) localisation of Ca in cross-section of a stem, B and C) zoom on one of the crystals, D) examples of Cd-LIII edge XANES on the crystals and the reference compounds used for linear combination fits: E) on crystal in a stem, F) on crystal in a leaf and G) in a root.