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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:** **EC-959 Potential mechanisms involved in Cr(VI) reduction in sunflower roots**	**Experiment number**: EC-959
Beamline:	**Date of experiment**: from: 29/June/2012 to: 02/July/2012	**Date of report**: 25/03/2013
Shifts: 12	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Guadalupe de la Rosa, Universidad de Guanajuato

Huetzin Pérez-Olivas, Université of Versailles at Sn Quentin

Report

In order to gain knowledge on the biotransformation of Cr(VI) in *Helianthus annnuus* L. (sunflower), a potential Cr phytoremadiator, microXAS experiments were performed. For the first time, using µXRF, we demonstrated Cr reaches the root stele and is located in the walls of xylem vessels. Bulk and microXANES results showed that Cr(VI) is reduced to Cr(III) in the roots, and is mostly present as Cr(III) phosphate (80%), with the rest bound to carboxylic groups. Our results suggest this plant species may serve for Cr(VI) phytofiltration purposes.

We are especially interested in exploring options for the rhizofiltration of tannery wastewaters containing Cr. Thus, in an effort to identify new alternatives for Cr remediation in central Mexico, as well as to gain information on mechanistic aspects, the aim of this research was to study the effects of growth stage and sulfate concentration on Cr(VI) uptake and tolerance by *H. annuus* L. Different experiments were set to determine the effect of Cr(VI) on seed germination and plant growth. Oxidation state of Cr in roots was studied by bulk X-ray absorption spectroscopy (XAS) and the microdistribution of this element was observed using X-ray Fluorescence microscopy (microXRF). These studies were set in hydroponics to explore the potential use of *H. annuus* L. for the phytofiltration of Cr(VI).

Methodology

Experiments were done at beamline ID21. Roots of *H. annuus* L. exposed to 0.19 mM Cr(VI) and 1 mM sulfate were rinsed with 0.01M HNO_3 and deionized water, immersed into Tissue Tek resin, and rapidly frozen in liquid nitrogen. After embedded in resin, the samples were axially sectioned at 25 µm thick using a cryomicrotome, freeze dried and mounted in between Ultralene window film. MicroXRF mapping of Cr K-edge was performed with an incident beam at 6.1KeV during the continuous operation mode. This experiment was promoted from reserve list due to cancellation of another experiment and for this reason we had to adapt to the short notice and perform the experiments on freeze dried samples. The XRF data was processed using the PyMCA software (Solé *et al*. 2007). The Cr, S and P images were obtained by fitting each pixel in the XRF maps. Thus, the net counts in the images are those of the elements of interest. The S to Cr and P to Cr ratios were calculated using the fitted images. For microXANES data acquisition, the energy was selected using a Si111 monochromator and scanned from 5980 to 6090 eV. The zone plate was translated in the beam axis in order to maintain the beam focus. XANES data analysis was carried out using the Athena software (Ravel and Newville 2005). XANES spectra from samples were fitted using the linear

combination procedure provided in the Athena software. Reference materials were chemical grade reagents analyzed as fine powder pellets in transmission and fluorescence mode.

Results and Discussion

Our results showed Cr is mainly accumulated in the root epidermis and the casparian strip, but it also moves across the root reaching the stele (Figure 1C and 2A). The co-localization of Cr, P and S can also be observed in the images from a xylem vessel in the stele (Figure 2A, B and C). The highest accumulation of Cr was observed in the epidermis and casparian strip, which suggests these might be precipitates of inorganic Cr(III) compounds (highly insoluble at plant physiological pH 4.5-7.0) or Cr(III) bound to carboxylic groups from the suberinized cell wall in the casparian strip. The casparian strip is an impermeable waxy layer between the endodermal cells that stops water and solutes from entering the xylem, except by passing through the cytoplasm of adjacent cells. Despite of this, Cr is reaching the root stele and is located in the walls of xylem vessels. Xylem vessels are strengthened by rings of lignin; as it can be observed in Figure 2A Cr is likely to be bound to lignin in xylem vessels.

The microXANES spectra from several spots in *H. annuus* L. roots confirmed the full reduction of Cr(VI) to Cr(III) in roots (Figure 2F). Linear combination fitting (LC-XANES) of the bulk and microXANES Cr spectra was performed using a set of model compounds (Cr(III) phosphate, sulfate and acetate). The LC-XANES results from bulk roots and spots 1-5 showed the main contributions are given by Cr model compounds $Cr(PO_4)_3$ and Cr(III) acetate (Figure 3). The LC-XANES results suggest Cr(VI) is rapidly reduced to Cr(III) and 80% of this is most likely precipitating as Cr(III) phosphate and the rest is either bound to cell wall components or mobilized through the roots by complexation to organic acids. Cr(III) acetate was used as proxy for Cr(III) bound to carboxylic groups from cell wall components and organic acids in an octahedral geometrical arrangement. Cr in the root stele (Figure 2A) is either bound to lignin and cell wall components or precipitated as Cr(III) phosphate. However, a small fraction should be forming complexes to organic acids and transported to the aerial plant tissues. Cr(III) complexation to organic acids should enhance Cr(III) solubility in the root environment and facilitate the passing of Cr through the casparian strip layer.

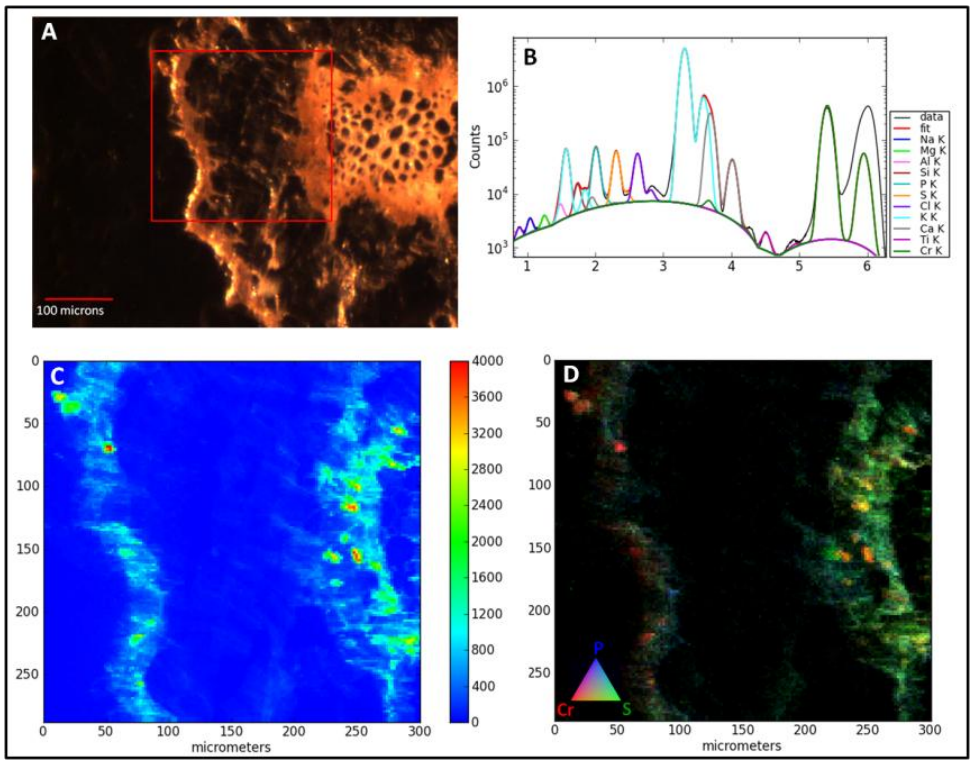

Figure 1. *Cr distribution in the roots of seedlings germinated and grown for 15 days in 0.19 mM Cr(VI) and 1mM sulfate. A) Video microscope image of the root cross section (25µm thick). B) Fit of the map X-ray fluorescence sum spectrum. C) Cr K-edge X-ray fluorescence image acquired at 6.1KeV (2µm² pixel), color bar scale in raw counts. D) Red Green Blue (RGB) image of Cr, S and P.*

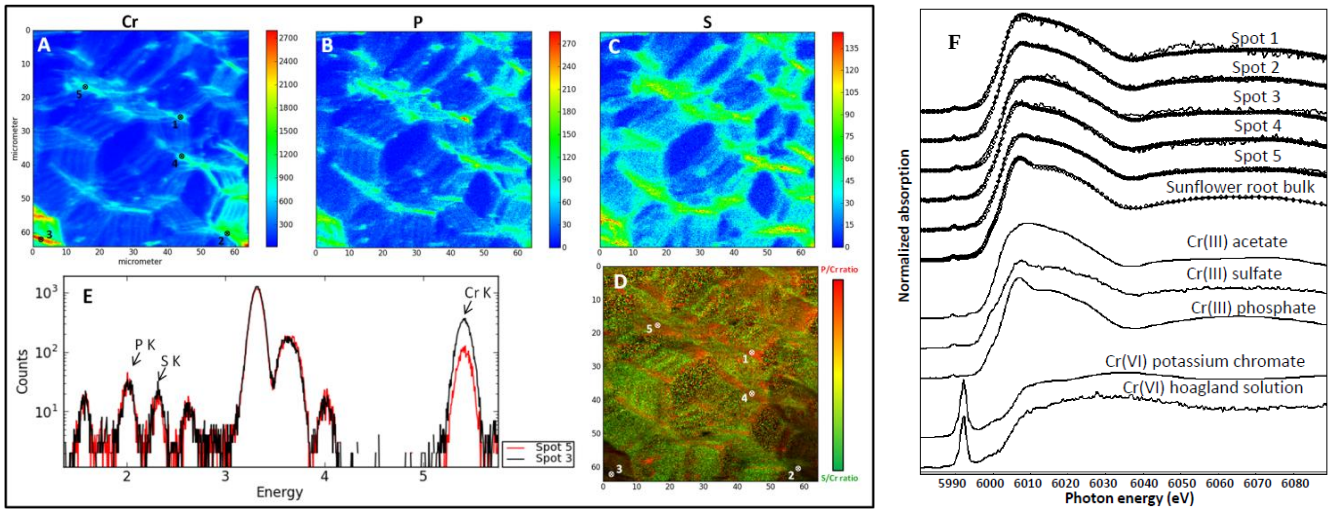

Figure 2. *Cr distribution in the root vascular region of seedlings germinated and grown for 15 days in 0.19 mM Cr(VI) and 1 mM sulfate.* ***A)*** *Cr K-edge,* ***B)*** *P K-edge, and* ***C)*** *S K-edge X-ray fluorescence images acquired at 6.1KeV (0.5µm² pixel), color bar scale in raw counts.* ***D)*** *Red Green Blue (RGB) image of the P/Cr and S/Cr ratios.* ***E)*** *X-ray fluorescence sum spectrum of 4 pixels (2µm² pixel) located at spot 5 and spot 3.* ***F)*** *Spots 1-5 and H. annuus L. bulk sample were fitted using linear combination of Cr(III) model compounds, the fit is overlaying the raw data in solid line.*

This beamtime provided important information about the localization of Cr in the roots tissues of sunflower plants. The results confirm full reduction of Cr(VI) to Cr(III) in the tissues and the presence of Cr(III) in the xylem vessels suggests low molecular organic acids and proteins should be participating in the translocation of Cr to aerial tissues. Future experiments are being prepared to study further the chemical forms that are responsible for translocation and to correlate the tolerance of sunflower to Cr to the cell wall composition (content of ligning, cellulose, etc).

Scientific production related to this experiment

De la Rosa, G., Castillo-Michel, H., Cruz-Jimenez, G., Bernal-Alvarado, J., Cordova-Fraga, T., Lopez-Moreno, L., Cotte, M., 2012. Cr Microlocalization and pseciation in roots of chromate fed sunflower seedlilngs using synchrotron techniques. International Journal of Phytoremediation. (Submitted in second revision).