ESRF	**Experiment title:** Cd speciation in the symbiotic association *Anthyllis vulneraria/Mesorhizobium metallidurans* candidate for the phytostabilisation of mine tailings.	**Experiment number**: 30-02-1049
Beamline: BM30 FAME	**Date of experiment**: from: 7/05/2013 to: 14/07/2013	**Date of report**: 26/08/2013
Shifts: 15	**Local contact(s)**: Proux Olivier	*Received at ESRF:*

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Objective and expected results

The legume plant *Anthyllis vulneraria* with its symbiotic rhizobium has been revealed as a pionner plant to revegetalize mining sites, and the general aim of our project is to understand the mechanisms developped by the plant to cope with Zn and Cd toxicity in a context of phytostabilization. One of our specific objectives is to determine the mechanisms developped by the symbiotic association *Anthyllis vulneraria – Mezorhizobium metallidurans* to tolerate Zn and Cd and specially to elucidate the role of *rhizobium* in the storage of metals. We particularly intend to determine the chemical forms of Zn and Cd in the leaves, roots and nodules of *A. vulneraria* grown in various conditions of bacterial inoculation *i.e.* non inoculated (NI), inoculated with a metallicolous rhizobium strain (MET) and inoculated with a non-metallicolous rhizobium strain (N-MET). For that, EXAFS (Extended X-ray Absorption Fine Structure) spectroscopy was used, and in a first experiment we focused on Zn speciation (report 20110931). This present experiment was dedicated to Cd speciation, and we expected to identify and quantify the forms of Cd in nodules, roots and leaves of NI, MET and N-MET plants to clarify the connection between the nodules and the capacity of the plant for Cd storage in shoots and roots.

Results and conclusions of the study

Anthyllis vulneraria plants were grown in hydroponics, inoculated with MET or N-MET *rhizobium* or not inoculated (NI) and exposed to 0, 10 and 70µM Cd during four weeks. Plants were washed with $CaCl_2$ and deionized water, and nodules, roots and leaves were collected, ground in liquid nitrogen and prepared as frozen pressed pellets. Cd chemical forms were studied using Cd K-edge EXAFS. Measurements were performed at 10K using a He cryostat and the fluorescence signal was collected with the 30 element Germanium detector. EXAFS signal was extracted using Athena software. The k^3-weighted EXAFS spectra recorded on the plants were least-squares fitted over a wave vector (k) range of 3.0–11.5 Å using a combination of standards spectra from a library of Cd model compounds. Cd complexes with carboxyl and-or hydroxyl groups were grouped as a generic family referred to as "Cd-O ligand. Cd-S complexes, e.g. Cd-cysteine, Cd-Phytochelatin or Cd-glutathione were grouped as "Cd-S ligand".

Figure 1 displays EXAFS spectra collected on leaves, roots and nodules depending on the inoculation and Cd exposure. Results show that spectra collected on leaves differ than those collected on roots and nodules, indicating that Cd occurs as different chemical forms in these three organs. The same observation was done for Zn speciation in *A. vulneraria* (cf report 20110931).

In leaves exposed to 70µM, Cd is predominantly bound to S ligands and thiol is even the only binding group for NI plants. Around 30% of Cd is associated to O atoms in inoculated plants. Although not very significant, the proportion of S ligands is slightly higher for the N-MET leaves than for the MET leaves. For the low Cd treatment, only XANES was collected : no difference was observed between leaves from MET and N-MET plants while in NI leaves the proportion of thiol ligands seems higher (not shown).

In both MET and N-MET nodules, Cd is associated to both S ligands and O ligands, and the proportion of thiol increases with Cd concentration. For the low Cd treatment, the speciation is similar for both strains with 45% of Cd bound to O atoms and 55% to S atoms, while for the high Cd treatment, N-MET rhizobium has more thiol groups involved in Cd binding (70%) that the MET one (60%).

In roots, NI plants also show higher Cd-S binding than inoculated ones whatever the Cd concentration is. For the 10 µM Cd treament, Cd speciation is dominated by O ligands in roots inoculated with the two strains (50-55%). For the 70 µM Cd treatment, this proportion decreases to 40% for in N-MET while the speciation does not change much in MET roots.

As a conclusion, these EXAFS results showed that Cd speciation was impacted by the rhizobium inoculation: the proportion of thiol ligands was higher in roots and leaves from the non inoculated plants than from the inoculated ones whatever the Cd treatment was (10 µM or 70 µM Cd). These results are completely new. We also showed that Cd speciation was not very different in leaves, roots and nodules when *A. vulneraria* was inoculated with a MET or N-MET rhizobium strain for the low Cd treatment. In contrast, when Cd amount increased, the proportion of S ligands was higher in N-MET roots, nodules and to a lesser extent roots than in MET organs. Also, the proportion of thiols groups increased with Cd exposure although this was not so clear for MET roots.

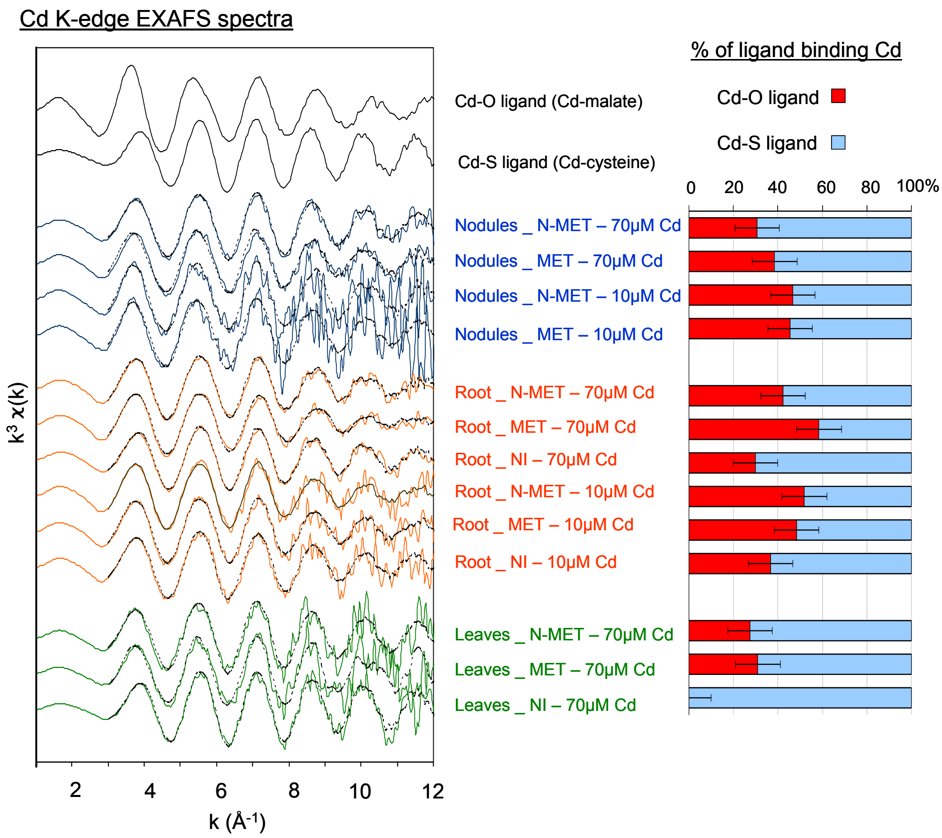

Figure 1: Cd K-edge EXAFS spectra for plant samples and for two references: Cd-malate as representative of Cd-COOH/OH group with Cd-O/N bonds and Cd-cysteine as representative of Cd-thiols composed of Cd-S ligands. Each spectrum (colored lines) is shown with its linear combination fit (black lines). Distribution of Cd species are presented for the samples after normalization of the percentages to 100%.

Justification and comments about the use of beam time (5 lines max) :

Beamtine was dedicated to the measurement of various organs (leaves, roots and nodules) depending on the inoculation and for two Cd concentrations. Some Cd model compounds were also collected. The beamline worked very well and no technical issues occurred. One shift total was dedicated to beam aligment and cryostat cooling.

Publications :

Data treatment is still in progress (the structural parameters for Cd in plant samples are being determined by shell simulations using ARTEMIS software).

Huguet S., Soussou S., Clayet-Marel J.C., Proux O., Castillo-Michel H., Trcera N. and Isaure M.P. **Poster** at the 4th International Symposiulm of Metallomics – Oviedo (Spain) – July 8-11 2013.