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| ESRF | **Experiment title:**<br>Zn and Cd speciation in the symbiotic association Anthyllis vulneraria/ Mesorhizobium | **Experiment number**:<br>30-02-1029 |
| **Beamline**:<br>BM30<br>FAME | **Date of experiment**:<br>from: 7/06/2012 to: 12/06/2012 | **Date of report**:<br>14/09/2012<br>(submitted to Soleil) |
| **Shifts:**<br>15 | **Local contact(s)**: Proux Olivier | *Received at ESRF:* |


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**Objective and expected results**

The legume plant *Anthyllis vulneraria* has been revealed as a pionner plant to revegetalize mining sites, and the aim of our project is to understand the mechanisms developped by the plant to cope with Zn and Cd toxicity in a context of phytoremediation. The aim of this proposal was to determine the mechanisms developed by the symbiotic association *Anthyllis vulneraria-Mesorhizobium metallidurans* to tolerate Zn and Cd, and specifically to elucidate the role of rhizobium (metallicolous strain MET, non metallicolous strain N-MET or no inoculation) in the storage of metals. We intended 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 (non inoculated, MET and N-MET strains) using EXAFS (Extended X-ray Absorption Fine Structure).

**Results and conclusions of the study**

Due to limited beamtime and consuming time related to the change of edges, we could only investigate Zn in this experiment.

*Anthyllis vulneraria* plants were grown in hydroponics, inoculated with the MET or N-MET rhizobium, or not inoculated at all (nitrogen is provided by nitrates in that case), and exposed to 1000 µM Zn during four weeks. Plants were also collected on the site and leaves were investigated for comparison.

Plants were rinsed in $CaCl_2$ and deionized water, and leaves, roots and nodules were separated, ground in liquid nitrogen and prepared as frozen pressed pellets. The chemical species of Zn in the three compartments were investigated using EXAFS at Zn K-edge. 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.

Figure 1 displays EXAFS spectra collected on leaves, roots and nodules depending on the inoculation in comparison to leaves from the field and Zn model compounds. Results show that the spectra collected on leaves differ than those collected on roots and nodules. For leaves the EXAFS oscillations are shifted to lower k values, with frequency ressembling Zn-malate and Zn-nicotianamine. We also see that the non inoculated leaves slightly differ than the inoculated ones, suggesting that the rhizobium plays a role in the form of Zn stored in the leaves. Spectra from leaves from the field seem to have features ressembling those of the non inoculated plants. Roots spectra roughly match with Zn-phosphate/ Zn-silica spectra and no significant variation is observed depending on the inoculation. Both rhizobium nodules are similar but the first oscillation is different than the one from roots spectra suggesting that the Zn forms in roots and rhizobium are not exactly the same. Work is in progress to determine more precisely the Zn speciation in the various organs using a linear combination fitting approach and simulation of the first coordination spheres.

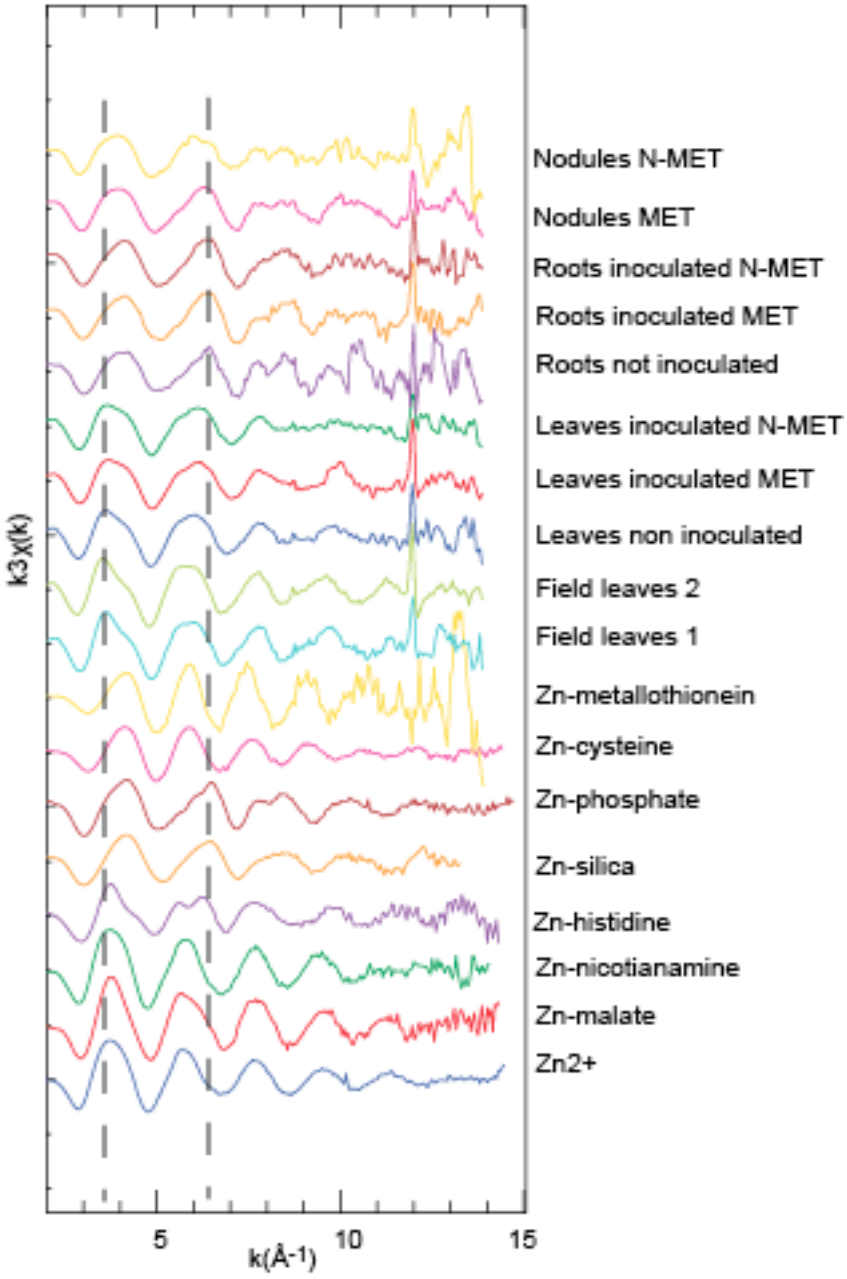


Fig.1 : Zn K-edge EXAFS spectra of leaves, roots and rhizobium nodules in A. vulneraria inoculated with MET, N-MET rhizobium and not inoculated compared to leaves collected on the field and to Zn model coumpounds.

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

Beamtime was dedicated to the analysis of the various organs depending on the inoculation and some Zn model compounds were also collected (15% of the beamtime). The beamline worked very well and no technical issues occurred.

**Publications :**

Data treatment is still in progress.

NB : Because we obtained CRG beamtime, this report was submitted to Soleil Sunset in september 2012.