ESRF	**Experiment title:** Study of the uptake and distribution of heavy metal in the different organs of plant species suitable for phytoremediation	**Experiment number**: 08-01-1008
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Report:

Scientific Background

Phytoremediation has been suggested as an inexpensive, sustainable, *in situ* biotechnology to help rehabilitate soils contaminated by heavy metals without destructive effects on soil properties[1]. The complexity of the interaction between soil, pollutant and plant characteristics, make these techniques highly site-specific and emphasize the need to understand the mechanisms and processes involved by means of controlled laboratory experiments[2]. Most studies reveal that tolerance, metal uptake capacity, and compartmentalization are highly variable among different plant species[3-5]. High biomass productive species such as poplar[4,5] and willow[6,7], are particularly suitable for phytoextraction because of their capacity of absorb heavy metals and translocate them to their aerial parts.

XAS techniques have been widely applied to the phytoremediation field in order to obtain information on metal localization and binding in plants[8]. However, because of the strong variability in the plant response connected to the species and the metal, it is necessary to investigate the specific metal-plant interaction. To this purpose, in this experiment we decided to assess the compartimentalization and coordination of Zn, Cu and Mn in poplar and willow. These metals have been investigated in different plant species with different techniques[8] but, to our knowledge, no previous studies exists on the two selected plant species.

Experimental details, measurement strategy

XAS (XANES and EXAFS) spectra at the Zn (9659 eV), Cu (8979 eV) and Mn (6539 eV) K-edge were collected at the European Synchrotron Radiation Facility (ESRF) of Grenoble, France, at the LISA CRG beamline (BM08). The spectra have been recorded at room temperature in low vacuum conditions in fluorescence mode, and a reference transmission spectrum of a metallic (Cu or Mn) foil was also acquired in order to provide internal energy calibration. Because of the low concentration of metals in plant tissues high integration time was necessary and several spectra per sample had to be averaged in order to improve the signal-to-noise ratio. However, the comparison between the first and the last spectrum show no beam damage on samples even after 6-8 hours of measurements.

Samples details

Populus and *Salix* plants were grown in pots under natural conditions and watered with aqueous solutions of the toxic metals of interest (Cu, Zn or Mn soluble salts). After one-month exposure to the contaminant, the different plant organs (roots, stems and leaves) were harvested and weighted. The samples were then oven-dried at 75°C for 72 h and the dehydrated plant materials were ground using a stainless steel mill.
For XAS analysis, the obtained powder was pressed in pellets using anhydrous pure cellulose binder, according to XafsMass calculations for fluorescence-suitable pellets.

Results

During the experimental session at the CRG-LISA beamline we were able to measure 16 selected samples: 6 samples for Zn (3 plant organs x 2 species), 6 for Cu, and 4 samples (only roots and leaves) for Mn.
Preliminary fluorescence analysis allowed us to estimate metal concentrations in samples from the relationship between transmission and fluorescence edge jump: Zn ranged between about 40 and 330 µg/g, while Cu concentrations were between 3 and 12 µg/g. For both metals, the concentration in stems was the lowest between the plant organs, and this led to exclude stem samples from further analysis. Mn samples were too diluted and we weren't able to estimate the fluorescence edge jump from the spectra.
XANES analysis, consisting of comparing the edge position of samples to that of reference compounds, was performed for Cu and Mn that resulted to be both in the +2 oxidation state.
The high dilution of Cu and Mn samples led to noisy spectra, that could be analysed in EXAFS only for the first shell. As regards Zn, however, we were able to work on 1st shell fit residues and to identify a metal-metal interaction in the 2nd shell.
Our analysis (performed with specific codes[9]) show that Zn and Cu are bound to low-Z ligands. We found local order differences between plant organs and species for Cu and Mn whereas no differences were found for Zn.

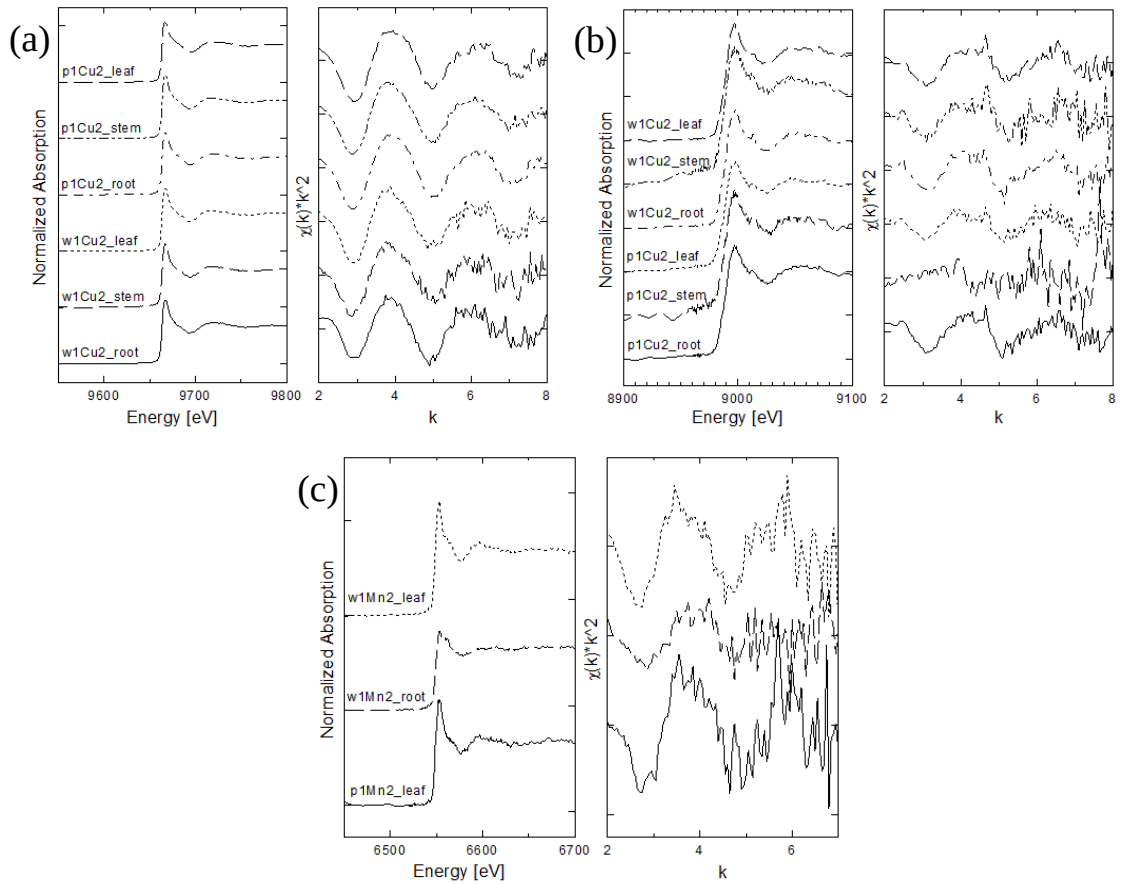

Figure 1 – XANES and EXAFS spectra of samples for Zn (a), Cu (b), and Mn (c). EXAFS spectra are reported as $\chi(k)$ weighted for k^2

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