



	Experiment title: High and low-affinity binding sites for Cu on the root apoplasts of plants	Experiment number: 30-02- 1035
Beamline: BM30B	Date of experiment: from: 05/12/2012 to 12/12/2012	Date of report: 19/02/2012 <i>Received at ESRF:</i>
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Report:

Introduction

Among soil contaminants, trace elements (TE) appear as a major concern for the contamination of soils worldwide. Among the potential target organisms for which TE contamination represent a potential risk, higher plants are of primary interest, because of their role in human food and animal feed, i.e. in terrestrial foodchains. Therefore, there is a need for reliable method to assess and to evaluate contaminant phytoavailability. When plant is the target organism, a range of chemical methods is suggested to assess phytoavailability, but none are per se designed to address the diversity of responses observed among different plant species or cultivars.

The main aim of this project is to determine the nature and the proportions of Cu and Zn binding sites present in the root apoplast as a function of plant types and how the coordination of these metal cations is affected by environmentally relevant variations of physico-chemical parameters (pH, ionic strength), i.e. determining the stability of the formed Cu and Zn species.

Root apoplast of mono- and di-cotyledonous selected plants were extracted by Triton X100 detergent (cryo-SEM was used to validate procedure of apoplast isolation). The characterization of functional groups in the root apoplast was assessed by coupling physical (FTIR; NMR ^{13}C) and chemical techniques (acid-base titrations). Carboxylic, hydroxyl, and amine groups were identified and the proportion of the potential TE binding sites could be assessed by spectroscopy techniques. FTIR spectroscopy allows to determine quickly difference between samples (e.g. different cultivars, addition of Cu, etc.), however unequivocal peak assignment and/or quantitative treatment of the data can be tricky. These limitations are fast less critical when using NMR, but the presence of paramagnetic element (Cu II) and the relatively low sensitivity of this spectroscopy preclude its use in the present project.

To overcome these difficulties, we proposed to use EXAFS spectroscopy measurements to examine the atomic environment of Cu and Zn in root apoplast of selected plants.

Experimental details

The samples have been studied to investigate the nature and the proportions of Cu and Zn binding sites present in the root apoplast of two selected plants: wheat and tomato. The measurements have been conducted as a function of metal:ligand ratio to better constrain the metal:site stoichiometry of the adsorption reactions than do standard pH edge adsorption experiments. Concentration-dependent reactions have been conducted at pH 4.9, over a wide range of Cu and Zn concentrations.

Frozen root, and apoplast samples were ground and compacted into pressed pellets in liquid nitrogen (77°K), with special care to keep the pellets frozen in liquid nitrogen until the XAS analyzes.

Cu and Zn K-edge EXAFS measurements on plant samples and reference compounds were performed between 5 and 10 K (helium cryostat), to avoid any possible dehydration or oxidation of samples. They were carried out on beamline BM30B/FAME with a Si(220) crystal monochromator operating at 6 GeV and 200 mA. The XAS spectra were all recorded in fluorescence mode, using a thirty-element solid-state germanium detector (Canberra, CT, USA).

In order to avoid artificial changes of speciation due to the drying of samples, the samples will be plunged in liquid nitrogen immediately after the adsorption reaction and stored frozen. Frozen root apoplasts will be ground and compacted into pressed pellets in liquid nitrogen (77 K), with special care to keep the pellets frozen in liquid nitrogen. Then, the samples will be characterized at the molecular-level using both Cu K-edge EXAFS.

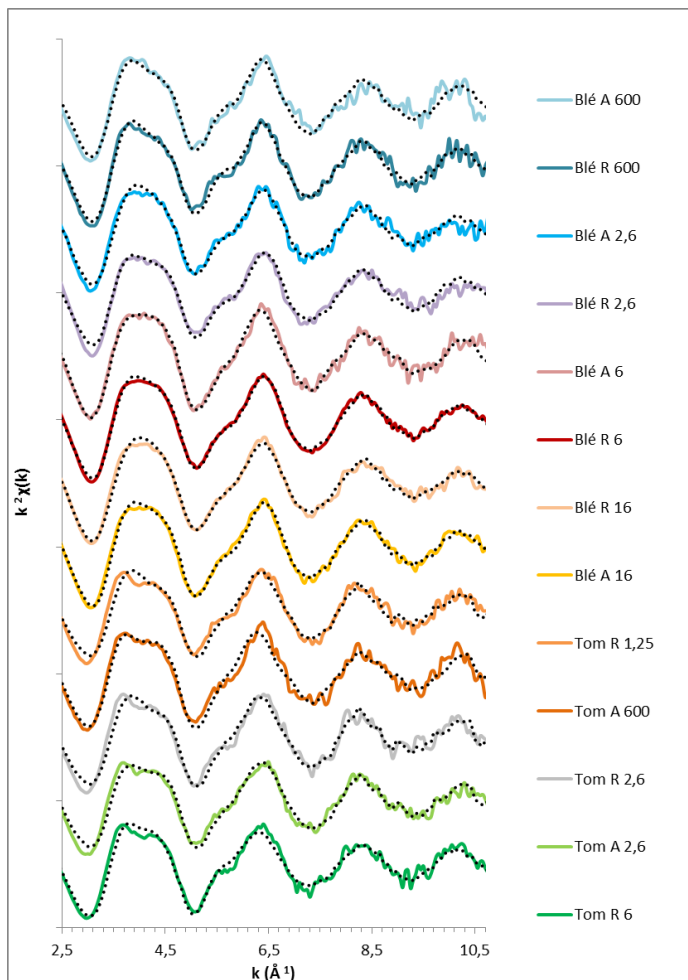
Results

Since the EXAFS spectrum of the unknown sample is a weighted sum of all species spectra present, the atomic fraction of each metal species was obtained by linear combination fits (LCF) of this spectrum to reference spectra. The minimum number of reference spectra needed to fit the unknown sample was determined by principal component analysis (PCA). Relevant reference compounds were identified via target transformation and the SPOIL function in a large collection of organic Cu or Zn minerals and species.

Results indicated that, in all samples, Cu and Zn oxidation state and chemical environment were observed as Cu or Zn(II) associated with organic matter(OM) mainly carboxylic functions (Figure 1 and 2).

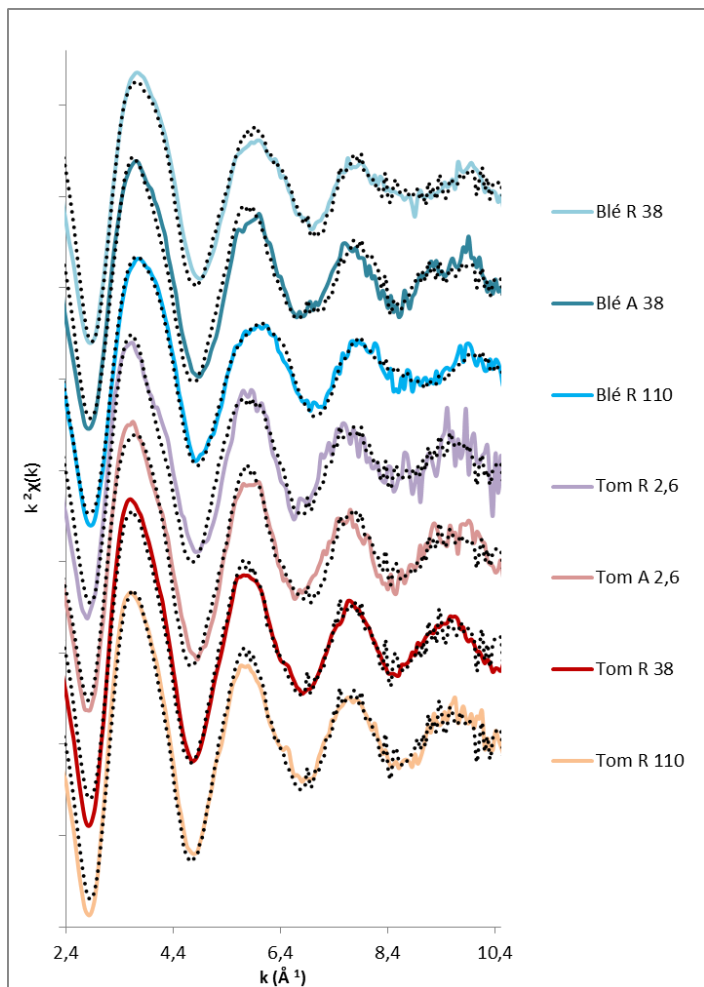
Unfortunately, the binding mechanisms didn't change as a function of metal:ligand concentration ratio, and were not able to detect high affinity metal binding sites on surfaces at low concentration.

XANES and EXAFS data analyses are done for all samples and publication with these results is currently in progress.



Samples	[Cu] ± std dev (mg.kg ⁻¹)
Blé R 0.6	113.4 ± 4.3
Blé A 0.6	127.5 ± 31.2
Blé A 2.6	210.2 ± 94.8
Blé R 2.6	296.4 ± 93.5
Blé A 6	302.9 ± 58.8
Blé R 6	485.2 ± 54.5
Blé A 16	651.2 ± 109.3
Blé R 16	890.6 ± 19.8
Tom R 1.25	79.8 ± 23.7
Tom A 0.6	125.9 ± 2.7
Tom R 2.6	270.1 ± 39.5
Tom A 2.6	463.4 ± 56.3
Tom R 6	657.5 ± 187.6

Figure1: Cu K-edge EXAFS of roots (R) and apoplast (A) samples and LCF with references components (dotted line), Cu concentrations of roots and apoplast samples (table).



Samples	[Zn] ± std dev (mg.kg ⁻¹)
Blé R 38	293.8 ± 5.0
Blé A 38	375.9 ± 46.4
Blé R 110	740.3 ± 59.0
Tom A 2.6	190.2 ± 7.1
Tom R 2.6	196.6 ± 17.4
Tom R 38	1435.4 ± 301.6
Tom R 110	2150.5 ± 633.2

Figure2: Zn K-edge EXAFS of roots (R) and apoplast (A) samples and LCF with references components (dotted line), Zn concentrations of roots and apoplast samples (table).

Results

Cu K-edge EXAFS spectra of samples cultivated in Cu hydroponic solution and Cu+Si hydroponic solution are reported in Figure 2. For both treatment, XANES spectra of Root and Leaf + Stem are clearly different.

We can identify different inflections in the absorption edge similar to those reported for reference compounds. Feature A ($1s \rightarrow 4p$ transition for Cu(II)) is only present for Root sample whatever the composition of hydroponic solutions (Cu or Cu+Si). Feature D ($1s \rightarrow 4p$ and $1s \rightarrow$ continuum transitions for Cu(II) compounds) is more intense for the Root sample. Therefore, Cu in the root was found as Cu(II).

In the stem and the leaf, the presence of Feature B, at 8982 eV ($1s \rightarrow 4p$ transitions for Cu(I) compounds) and D indicate that Cu was found as Cu(I) and Cu(II) whereas Cu oxidation state in the hydroponic solution is Cu(II).

Slight differences can be observed for Leaf and Stem XANES spectra (see arrows on figure 2). The intensity of the white line is not identical for the two hydroponic solutions. For excess of Cu in hydroponic solution, the white line is more intense for both samples.

Interesting results are apparent : bamboo takes up or adsorbs copper from the soil in the Cu(II) oxidation state and transports it to the leaves where copper is found as Cu(I) and Cu(II). We still ignore if copper reduction takes place during or after Cu incorporation into the roots, during Cu transport from the roots to the leaves or only into the leaves.

Data analysis is still in progress (principal component analysis and linear combination fits). In particular, we need to analyze the XANES signal of bamboos obtained with different hydroponic solutions (Cu or Cu+Si).