ESRF	**Experiment title:** Copper speciation and tolerance of bamboos	**Experiment number**: EC-751
Beamline: BM30B	**Date of experiment**: from: 07/07/2010 to 12/07/2010	**Date of report**: 26/08/2010
Shifts: 12	**Local contact(s)**: Jean-Louis HAZEMANN	*Received at ESRF:*

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Report:

Introduction

From a waste management standpoint, bio-recycling is a way of making effective use of liquid organic waste of different origins (animal manure, wastewater, etc.). In this promising gap, phytoremediation can be an interesting alternative. The selection of bamboos permits to optimize the phyto-recycling process. Indeed, bamboo is a quick-growing plant whose rate of biomass generation is unsurpassed by any other plants (10-30 % annual increase in biomass versus 2-5 % for trees). Such a biomass production, combined with a high absorption capacity of water and mineral elements, allows installing bamboo on very restricted areas compared to conventional agricultural wastes recycling. But some organic wastes such as pig slurry contain high concentration of copper as a result of their use as growth promoter in animal feeds. And the information about copper tolerance of bamboo is still very limited. Therefore, we proposed to study the speciation of copper into various part of bamboo (root, stem and leaf) and to describe the effects of Si on the distribution of Cu in bamboos and the strategy by which Si affects heavy metals resistance in bamboos. Indeed, it is well documented that Si has beneficial effects on plants and alleviate various stresses such as heavy metal phytotoxicity.

Experimental details

Hydroponic experiment were conducted in order to cultivate bamboo (*Semiarundinaria fastuosa*) with different Cu and Si concentrations to understand the effects of Si on the distribution of Cu in bamboos and the strategy by which Si affects heavy metals resistance in bamboos. Only results for samples obtained with excess of Cu in hydroponic solution, and excess of Cu in hydroponic solution with Si samples will be presented in this report.

Frozen root, stem, sap and leaf 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 K-edge XANES measurements on plant soil 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).

Results

Further insight into the symmetry and oxidation state of Cu was obtained by XANES. XANES spectra analysis is based on comparisons with several reference compounds of well-known crystal structure. Figure 1 presents the normalized Cu K-edge XANES spectra of two reference compounds. Differences were noted among the near-edge spectra (Figure 1). Inflections in the absorption edge provided information on the Cu oxidation state. Indeed, Feature A, from 8977 to 8978 eV, was the pre-edge feature and corresponded to dipole-forbidden electronic transitions 1s → 3d (but hybridized by p orbitals of the ligands). As Cu(0) and Cu(I) did not have empty 3d initial states, the pre-edge was a signature of Cu(II). The intensity of this feature was weak because there was only one empty 3d orbital (out of 10 available) to accept the excited 1s electron. Feature B, at 8982 eV, corresponds to the 1s → 4p transitions for Cu(I) compounds. No Cu(II) reference had a maximum pre-edge between 8980 and 8985 eV. Consequently, the presence of a shoulder between 8980 and 8985 eV in the Cu absorption edge spectrum and the absence of a pre-edge (feature A) from 8977 to 8978 eV indicated the presence of Cu(I) in the sample.

Some other inflections provided information on the 3D geometry and coordination environment of Cu. Indeed, Features C (8988 eV) and D (8995 eV) correspond respectively to the 1s → 4p and 1s → continuum transitions for Cu(II) compounds in octahedral symmetry.

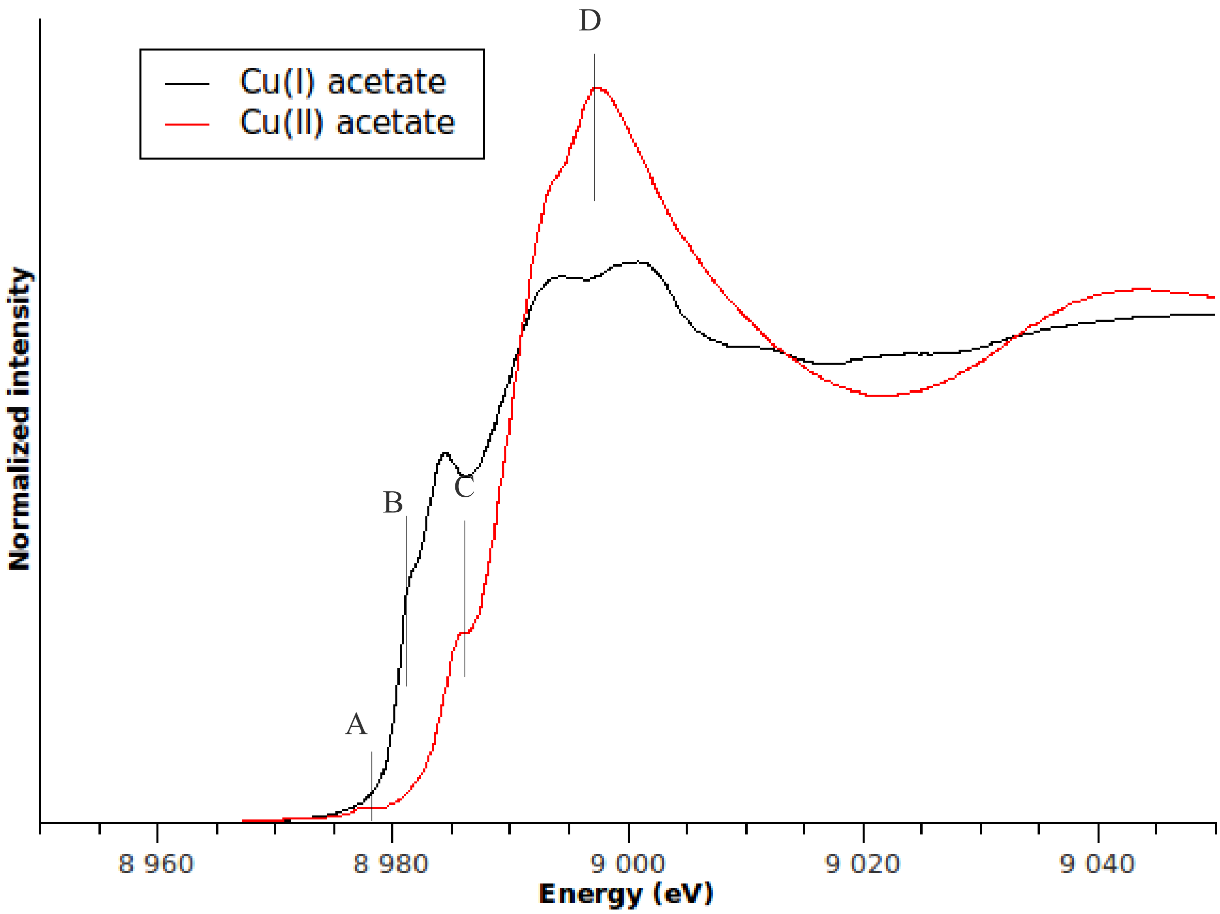
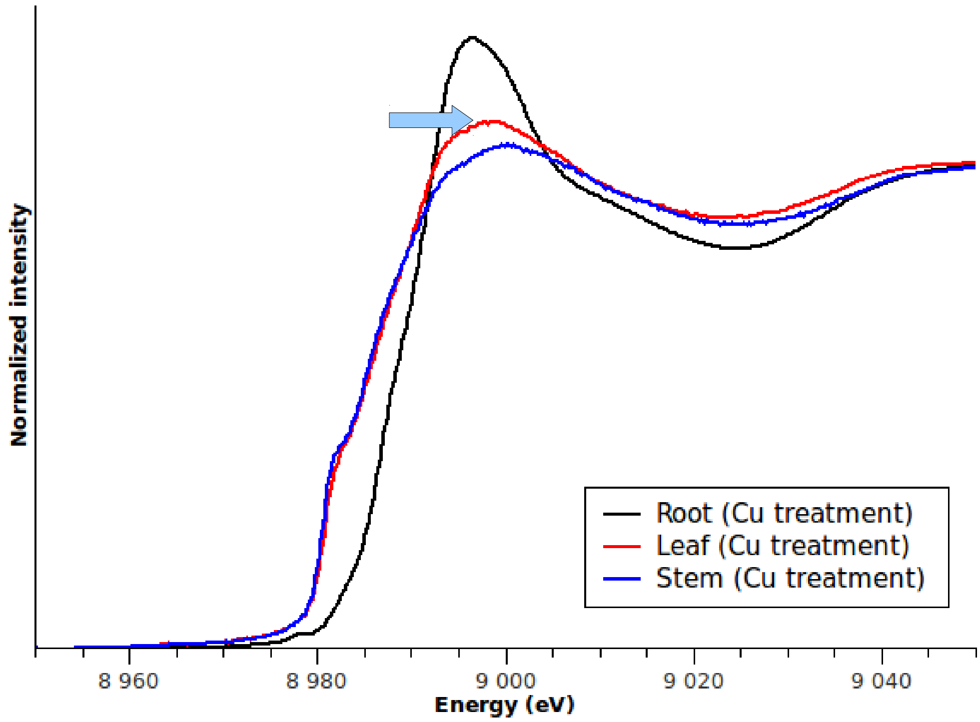


Figure 1. Normalized Cu K-edge XANES spectra of reference compounds (Cu(I) and Cu(II) acetate)



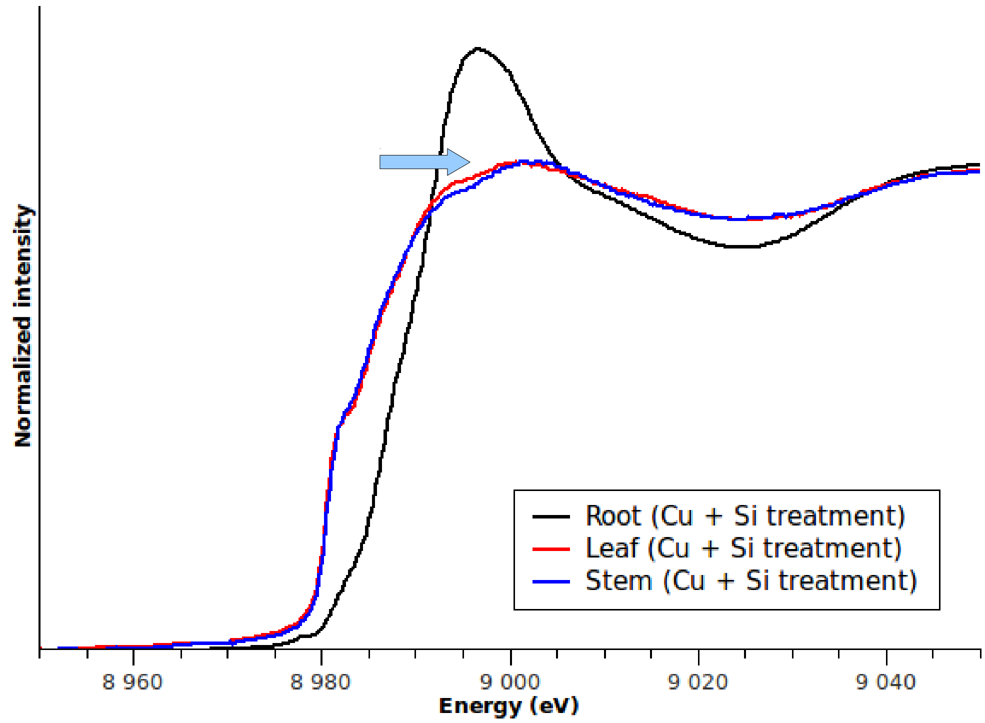


Figure 2. Normalized Cu K-edge XANES spectra of various parts of bamboo obtained with excess of Cu in hydroponic solution (top) and with excess of Cu and Si in hydroponic solution (bottom)

Cu K-edge XANES 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. W can identify different inflections in the absorption edge similar to those reported for reference compounds. Feature A (1s → 4p transition for Cu(II)) is only present for Root sample whatever the composition of hydroponic solutions (Cu or Cu+Si). Feature D (1s → 4p and 1s → 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 → 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 adsobs 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 hydoponic solutions (Cu or Cu+Si).