ESRF	**Experiment title:** **Micro X-ray fluorescence and micro-XANES spectroscopy of trace elements (Zn, Fe) in the soil-root system**	**Experiment number**: LS1865
Beamline: ID22	**Date of experiment**: from: ***May, 30*** to: ***June, 2, 2001***	**Date of report**: Sept, 1st, 2001
Shifts: 9	**Local contact(s)**: Alexandre Simionovici, Sylvain Bohic	*Received at ESRF:*

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Abstract

The purpose of this project was to study (1) the modifications of Zn and Fe localization and speciation induced by the root activity in a model soil and (2) the chemical form of the metals inside the roots by µ X-ray fluorescence (µXRF) and µXANES. We studied *in vivo* two soil-plant systems composed of a genetically modified tobacco growing in two Zn-containing substrates. µXRF allowed the mapping of Zn and Fe in the substrates and in the roots. No gradient of metals was observed around the roots, possibly because Zn diffusion in the substrate compensated the depletion of Zn due to the root absorption. The chemical form of Zn and Fe in the substrates and in the roots was studied by µXANES. Results showed that Zn was in tetrahedral coordination in both substrates and in the roots, and that Fe was present in its oxidized form (ferric ion) in the substrates and in the roots. This study shows the interest of µXRF and µXANES for the *in vivo* study of dynamic phenomenons such as metals mobilization, transport and absorption by plants.

The model plant chosen was a genetically modified tobacco that over-expresses ferritin gene (Van Wuytswinkel, 1999). This over-expression enhances the acidifying and reducing capabilities of the roots, therefore increasing the accumulation of metals in the plant (Vansuyt et al., 2000). The growing substratum chosen was an agarose gel medium containing either Zn adsorbed on ferrihydrite ($Fe(OH)_3$) (medium 1), or Zn nitrate (medium 2). The plants were grown in hydroponic solution for 4 weeks, and were placed in the gel 24h before the experiment. A special sample holder was designed for this experiment. The soil-plant container was composed of a polycarbonate frame and 8µ–thick kapton windows (Fig. 1). The soil-root system was studied in hydrated state, *in vivo* and at room temperature.

µ X-ray fluorescence

The X-ray fluorescence experiments were performed using a monochromatic beam (14 KeV). We used compound refractive lenses (CRL) for the focalization, and the beam size on the sample was 1*8 microns. The flux was 10^8 to 10^9 photons/s on the sample. The incident intensity was measured with a pindiode, and the fluorescence was recorded with a Si-Li fluorescence detector. We realized a 250 µ * 60 µ mesh for the root in the medium 1, and a 90 µ * 60 µ mesh for the root in the medium 2 (Fig. 2). In this case, Zn was much more concentrated in the root than in the medium, except in very localized spots. Similar spots were observed far from the root, so they probably do not result from the root activity. These spots are more likely Zn precipitates, as the chemical conditions (pH 6, 100 mg/L Zn) are close to the limit of solubility of Zn oxides.

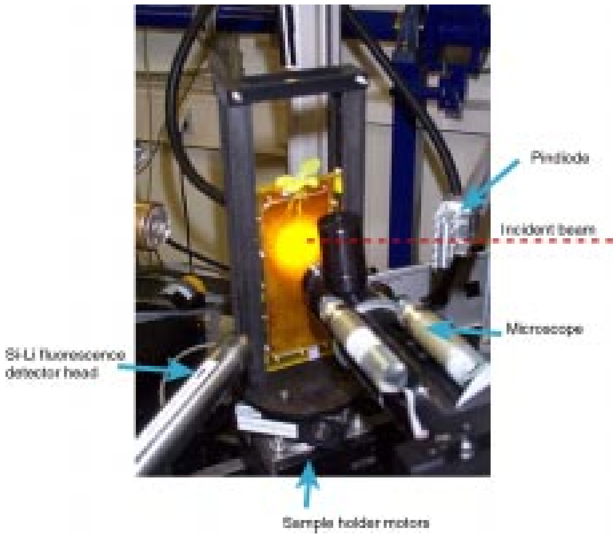

Figure 1: View of the sample during the measurement.

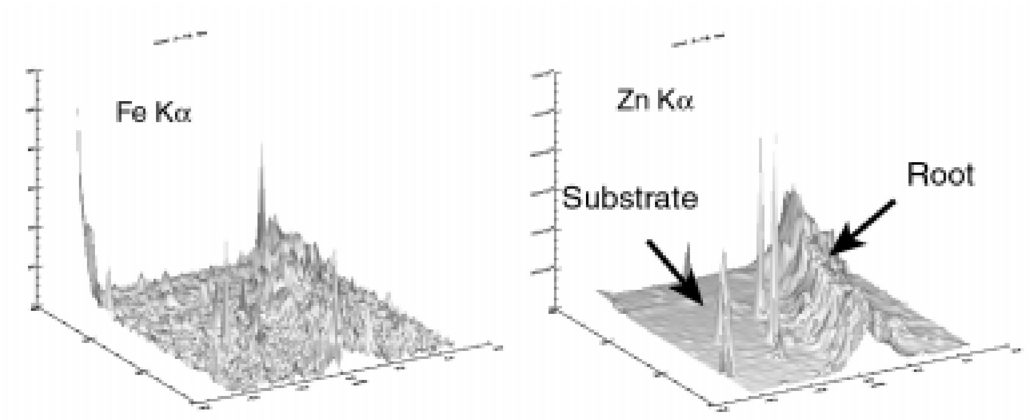

Figure 2: Elemental maps for the root in medium 2.

The chemical analyses showed that in both media, 1/6 of Zn present in the gel was absorbed by the plant. However, we did not observe a gradient of Zn in the growing medium. The presence of some Zn-rich grains in the medium may hide a weak gradient. Also, the diffusion of Zn in the medium may compensate the gradient created by the root. In our case, this diffusion was even enhanced by the flux of solution created by the root activity.

μXANES spectroscopy

The μXANES experiments were performed at Zn and Fe K-edge. We used a Freznel zone plate (FZP) with a pinhole for the focalization. The FZP was preferred to the CRLs because of the strong absorbance of the CRLs at low energy (<10 KeV). The beam size on the sample was 1*8 μ. The incident intensity was measured with a ionization chamber placed before the pinhole, and the fluorescence yield was recorded with a Si-Li fluorescence detector.

Figure 3A shows the Zn K-edge μXANES spectra for the reference compounds. They differ on the position of the edge, and on the amplitude and shape of the white line. For Zn in octahedral coordination (Zn^{2+} in solution, Zn oxalate dihydrate), the edge is shifted to the right compared to the tetrahedral coordination (Zn phosphate). The spectra for the media in the presence and in the absence of root are identical, which suggests that the root did not modify Zn speciation in the substrate (Fig. 3B). Based on the position of the edges, we can conclude that in these two substrates, Zn is in tetrahedral coordination. The XANES signal of the apex differs from the one of the hairy part of the root, suggesting a different form of Zn in the absorbing and in the growing part of the root (Fig. 3C). Fe K-edge μXANES spectra showed that Fe was predominantly in the Fe^{3+}

form in the growing medium 1 and in the roots. Ferrous iron, transiently present at the surface of the root and inside the root, was not detected. In order to clearly identify the chemical form(s) of Zn and Fe in the root, complementary experiments would be necessary to increase the signal/noise ratio of the spectra.

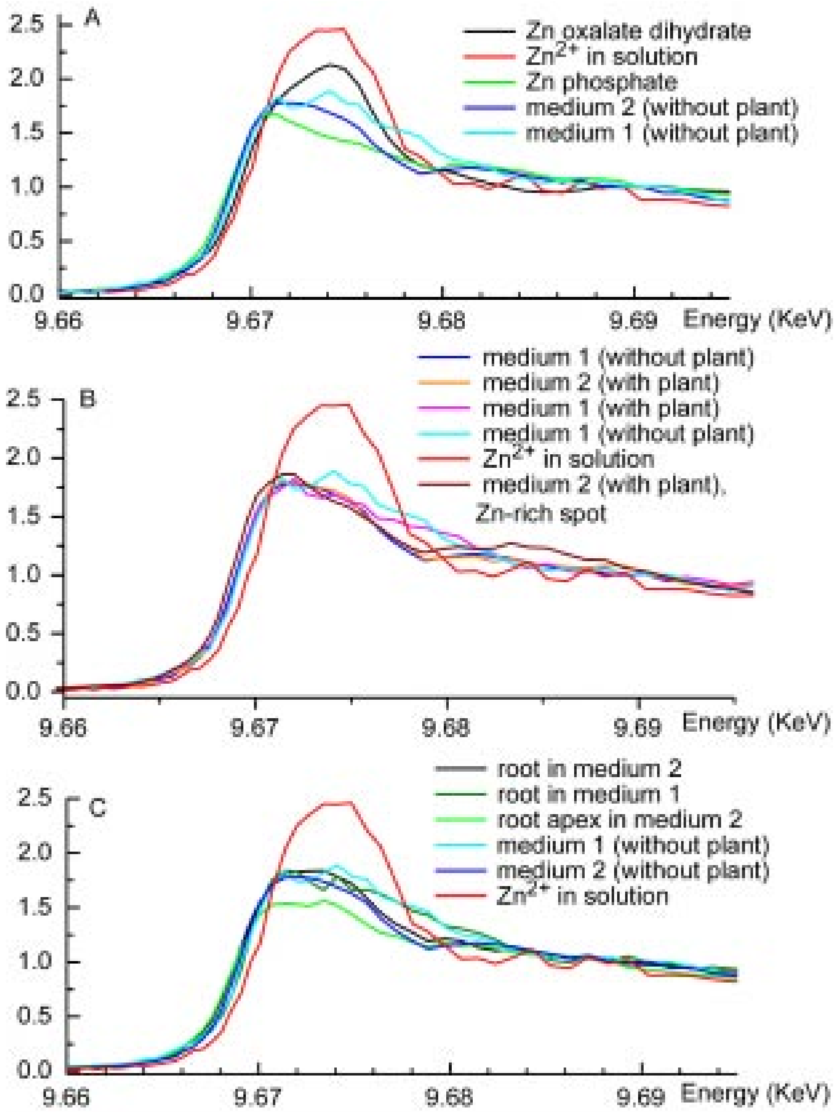

Figure 3: Zn K-edge µXANES spectra for some reference compounds (A), the growing media (B) and the roots (C).

Conclusions

The characteristics of the beamline and the sample environment were perfectly adapted to the system studied. Thanks to the high intensity of the beam, the exposure time of the root was limited (1s/point), and the root was not damaged by the beam. More statistics are necessary to determine the exact chemical form of Fe and Zn in the roots. This experiment shows the interest of µXRF and µXANES for the *in vivo* study of trace elements mobilization by plants.

References

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