Localization and speciation of Zn in mycorrhized roots by μSXRF and μEXAFS

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Abstract. Mycorrhizae are symbiotic associations between soil fungi and plant roots, which enhance mineral nutrition for the plant, and might play an important role in metals acquisition and accumulation. The processes allowing metals mobilization in the soil, absorption by the root and/or the fungus, transfer or bioaccumulation are still poorly understood. However, the properties of mycorrhizal fungi could be used for phytoremediation, a soft technique using plants for the clean-up of metal polluted soils. In this work, mycorrhized roots of tomato plants grown in a Zn-contaminated soil were investigated. The distribution of metals and the speciation of Zn were studied at the micron scale using micro synchrotron-based X-ray fluorescence (μSXRF) and micro X-ray absorption spectroscopy (μEXAFS). Zn associated to the root was Zn malate and/or Zn citrate, and Zn associated to the fungus was Zn phyllosilicate. This study illustrates the great potential of X-ray microbeams for the study of biological samples containing various amounts of metals.

1. INTRODUCTION

Mycorrhizae are symbiotic associations between soil fungi and plant roots, which concern the majority of both wild and cultured plants. The fungal symbiont generally receives carbohydrates from the plant, and supplies mineral nutrients such as phosphorus, nitrogen, and sulfur whose bioavailability in the soil is very low. Mycorrhizal fungi increase nutrient and metals acquisition by the plant in several ways. First, they strongly increase the soil volume explored and exploited. Second, they can increase the bioavailability of heavy metals by solubilizing metal-bearing minerals. This action is mainly attributed to the acidification of their environment and to the exudation of organic acids in the rhizosphere. Third, they have a high metal uptake capacity.

In the perspective of phytoremediation, mycorrhizae could be used to improve the accumulation of metals by crop species, which present the advantages of a rapid growth and a high biomass, unlike hyperaccumulating species. However, a better understanding of the mechanisms of metal absorption by mycorrhizae and transfer to the roots is still required.

The purpose of this study was to localize metals in a root-mycorrhizae system, and to compare the chemical form of Zn in both symbionts. Physical techniques including micro-synchrotron-based X-ray fluorescence (μSXRF) and micro X-ray absorption spectroscopy (μEXAFS) were used. These tools are particularly adapted to the study of heterogeneous natural samples, containing various amounts of metals [1,2]. They were previously tested for the study of metal-containing mycorrhizae [3, 4].

2. MATERIALS AND METHODS

2.1 Plant culture

Tomato plants (*Lyocpersicon esculentum*) were grown in a heavy metal polluted soil inoculated by *glomus interradices*. After harvesting, the roots were shock frozen in liquid propane and freeze dried (temperature of -80°C, vacuum of 10^{-7} torr) over 3 days. Soil particles were removed from freeze dried roots by microsurgery.

2.2 µSXRF analyses and µEXAFS spectroscopy

The distribution of metals in the whole root was investigated on micrometer-scale using µSXRF. The µSXRF maps were recorded on the 10.3.2 beamline at the Advanced Light Source (ALS, Berkeley, USA) operating at 1.9 GeV and 200-400 mA. The samples were scanned with a 10×5 µm step through a 10×7 µm sized X-ray beam at an energy of 9.7 KeV. The fluorescence-yield was measured using a 7-element Ge solid-state detector, and normalized by the incident intensity and the dwell time (200 ms/point).

The speciation of Zn in the root itself and in the fungal filaments was determined by extended µEXAFS spectroscopy on the same beamline. Zn K-edge µEXAFS spectra were recorded in fluorescence mode using a 10×5 µm sized beam. EXAFS data reduction was carried out following a standard procedure [5]. EXAFS spectra were compared to reference spectra, and their likeness was evaluated by the criterion $C = \Sigma \ (k^3 \ \chi(k)_{exp.} - k^3 \ \chi(k)_{reference})^2$.

3. RESULTS

Figure 1 shows the elemental distribution of Zn, Fe, Mn and Cu in the mycorrhized root of tomato plant. Zn is highly concentrated both in the root itself and in the mycorrhizal filaments. Fe and Mn are more concentrated at the surface of the root and in the fungal filaments, and Cu is almost equally distributed between the two organisms.
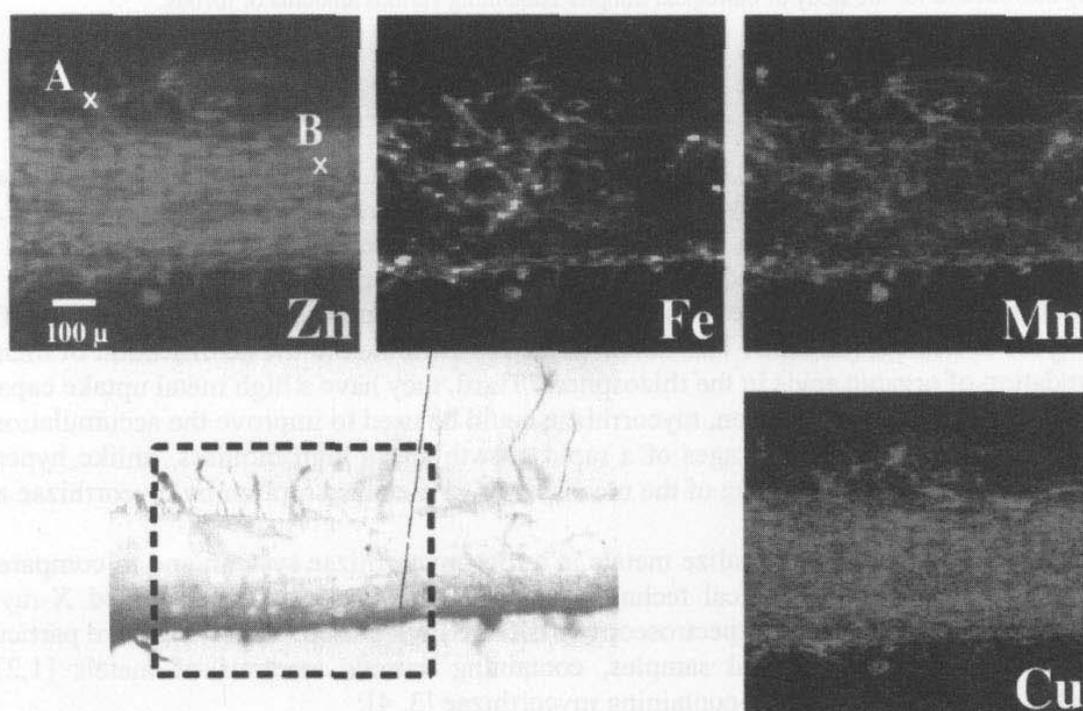
Figure 1. Photograph and μSXRF elemental maps of the mycorrhized root of tomato plant for Zn, Fe, Mn and Cu (Zn, Kα : 0 to 132500 cnts/s, Fe, Kβ: 0 to 25150 cnts/s, Mn, Kα: 0 to 42500 cnts/s, Cu, Kα: 0 to 4200 cnts/s). On Zn elemental map, A and B represent the points where μEXAFS spectra were recorded.

The speciation of Zn associated to the root and to the mycorrhiza was studied by μEXAFS. Several μEXAFS spectra were recorded in regions of the root where Zn concentration was diffuse and homogeneous (such as point B on the Zn map, Fig. 2). The spectra were very similar, suggesting that Zn is present as one major form in the root. In order to identify the Zn species, the root spectrum was compared to a large database of Zn-containing mineral species, including pure minerals (carbonates, sulfides, oxides, phosphates, etc.) various Zn-substituted and Zn-sorbed references (phyllosilicates, Fe and Mn (oxyhydr)oxides, etc.; [3]), and organic compounds [5]. Zn malate provided the best match, with a criterion C = 80 (Fig. 2A). Zn citrate was the second best match (C = 120), but the third oscillation was slightly phase-shifted. Other organic (Zn oxalate, Zn histidine, …) and inorganic (Zn phosphate, Zn carbonate, …) references did not provide satisfactory fits (C > 260). In Zn malate and Zn citrate, the first coordination shell for Zn is octahedral, and the second shell is composed of C and O atoms. On the Fourier transform, they differ mainly by the imaginary part at $R + \Delta R = 2.2$ Å (Arrows on Fig. 2A). These comparisons suggest that Zn malate is the major species in the root. However, owing to the great similarity of the two reference spectra, the occurrence of Zn citrate as well cannot be excluded. These results are in agreement with those obtained by powder EXAFS spectroscopy on the roots of the Zn hyperaccumulating species *Arabidopsis halleri* [6].
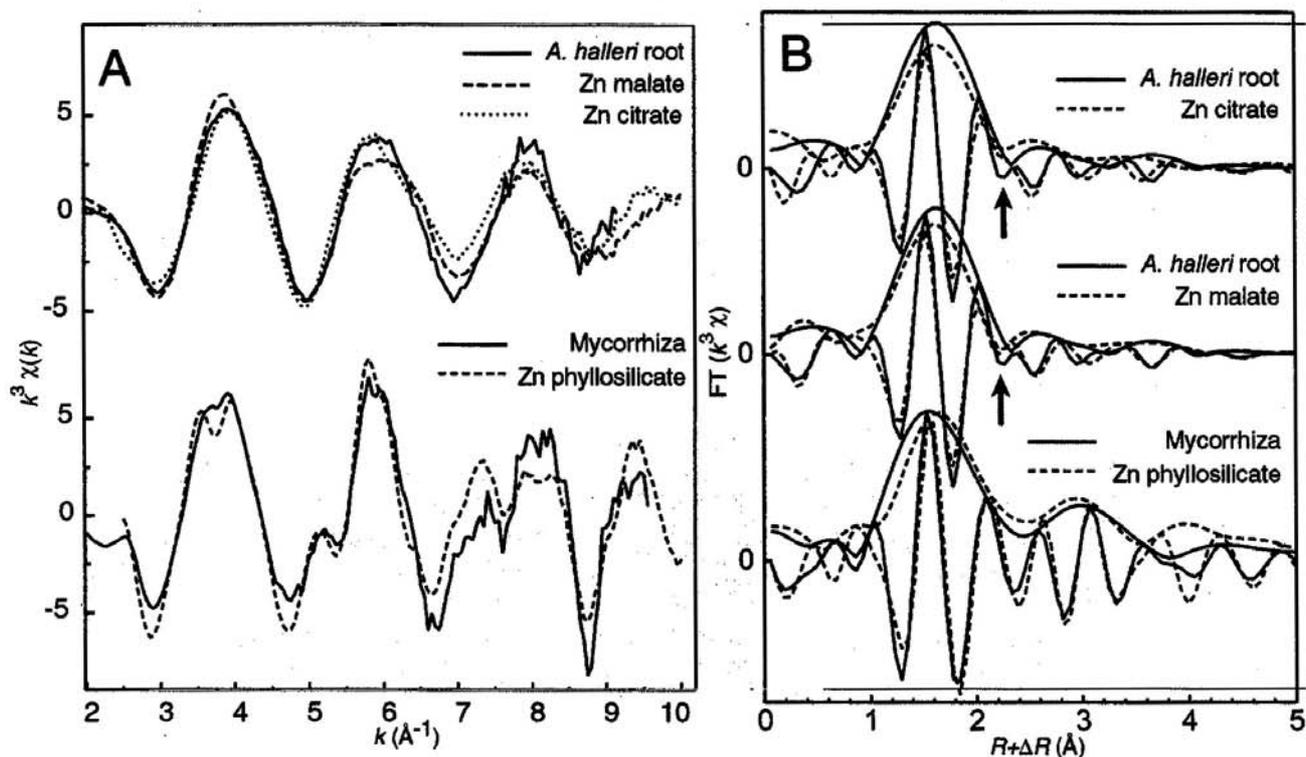
Figure 2. Comparison of k^3-weighted μEXAFS spectra (A) and Fourier transforms (moduli and imaginary parts, B) for the root and for the mycorrhiza. Each spectrum is the sum of two μEXAFS spectra recorded in different zones of the sample (points A and B on Fig. 1 and on other regions of the root, not shown).

The EXAFS spectrum for the mycorrhiza strongly differs from the root spectrum. The sharp shoulders on the oscillations (at about 5.5 and 7.5 Å⁻¹) denote a well-ordered Zn structural environment. The best spectral match was obtained with kerolite ($Zn_{1.35} Mg_{1.65} Si_4O_{10} [OH]_2$), a (Mg, Zn) phyllosilicate [7]. In this trioctahedral smectite, Zn is located in an octahedral sheet, and is surrounded by four Si neighbors of the tetrahedral sheet, and 2.7 Zn + 3.3 Mg cations of the octahedral sheet. [7]. Thus, the Fourier transform for this compound is identical to Zn malate for the first peak, whereas the second peak is more intense and shifted to a higher

distance (Fig. 2B). Owing to the small size of the filaments compared to the lateral resolution of μEXAFS used in this experiment (10 × 5 μm), it was not possible to determine whether Zn phyllosilicate is intracellular or bound to the fungal cell wall. The spontaneous formation of Zn phyllosilicates has been observed in acidic soils contaminated with Zn. Indeed, in these conditions, solutions are supersaturated or near saturation with respect to Zn-phyllosilicate, and this mineral can readily precipitate [1, 2]. Thus, the precipitation of Zn phyllosilicates at the surface of the fungus is possible. No Zn-organic complexes were detected in the mycorrhiza. These results do not imply that other forms were absent, but that in the zones probed by the beam, Zn phyllosilicate was amply the major species.

μEXAFS spectra were recorded on a limited number of zones of the fungus, and further investigations are planned to determine whether Zn phyllosilicates are evenly present on the filaments or form local precipitates. In this latter case, the μEXAFS investigation of precipitates-free zones will allow the investigation of other Zn possible forms, intracellular or complexed to the cell wall. In the species *Cortinarius*, Zn associated to the fungus was precipitated as Zn oxalate [4].

4. CONCLUSION

The chemical form of Zn was found to be Zn malate and/or Zn citrate in the root itself, and a Zn phyllosilicate in/on the fungus. This study illustrates the potential of μEXAFS for studying the chemical status of metals in complex biological systems such as mycorrhized roots. This type of information is particularly relevant for the understanding of metals bioaccumulation by plants.

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