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Experiment Report Form

	Experiment title: Cu speciation in local pseudo-metallophytes from a vineyard soil to go toward phytoextraction	**Experiment number**: EV-526
Beamline: BM30-Fame	**Date of experiment**: from: 20/06/2023 to: 26/06/2026	**Date of report**: September 11 2023
Shifts: 18	**Local contact(s)**: O. Proux & A. Aguilar	*Received at ESRF:*

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Report:

Introduction

Vineyard soils are frequently contaminated with copper due to the application of copper-based fungicides, such as the Bordeaux mixture composed of $CuSO_4$ and $Cu(OH)_2$, even in organic viticulture. Phytoremediation can serve as an alternative to extract a portion of Cu from these soils, and indigenous plants naturally growing in vineyards might be valuable for this purpose (Cornu et al., 2021). Two indigeneous plant species, *Amaranthus retroflexus* and *Chenopodium album*, were selected and grown in pot experiments in the native soil (containing 40 ppm of Cu) and in the native soil doped with 250 ppm, and 400 ppm of Cu from Bordeaux mixture. The objective of this study was to identify the speciation of copper in the various organs of the plants and in the vineyard soils to establish the mechanisms of Cu transformation and sequestration in the plants. For that, we used Cu K-edge EXAFS spectroscopy.

Material and methods

Cu concentrations were measured by ICP MS in plant organs (roots, leaves, stems, and fruits), and they were below 20 $\mu g.g^{-1}$ (Dry Weight) in all the aerial parts (leaves, stems and fruits) whatever the Cu exposure (40, 250 or 400 ppm). In contrast, roots from both species showed higher copper concentrations, ranged from 20 to 50 $\mu g\ Cu.g^{-1}$ DW, 350-380 $\mu g\ Cu.g^{-1}$ DW and 430-580 $\mu g\ Cu.g^{-1}$ DW for 40 ppm, 250 ppm and 400 ppm exposure, respectively. These concentrations suggested a strategy of metal exclusion and we focused on roots and soils samples to go deeper in the understanding of this strategy.

A. retroflexus and *C. album* roots were carefully washed, frozen, ground using liquid nitrogen, and prepared as frozen pressed pellet. Due to limited beamtime, we analyzed only the 40 and 450 ppm Cu exposures (4 root samples). Soil samples were air-dried, sieved at 2 mm, finely ground, and subsequently pelletized. We analyzed the 40 and 450 ppm soils before and after the culture including a control with no plant, thus accounting to 8 soil samples.

Fifteen Cu references 7 including mineral (Cu(0), $CuSO_4$, $CuCO_3$, Bordeaux mixture, $Cu(OH)_2$, Cu_2S, Cu-phosphate) and 9 organic compounds (Cu-acetate, Cu-humic acid, Cu-fulvic acid, Cu-pectine, Cu-cellulose, Cu-histidine, Cu-citrate, Cu-malate and Cu(I)-cysteine) were prepared as powdered pellets or aqueous solutions and analyzed. Plants, soils and references samples were mounted on a Al holder and Cu K-edge EXAFS spectra were collected in fluorescence mode using a 13-element detector and a Si(220) monochromator. All measurements were done using a helium cryostat at 10 K. EXAFS spectra for soils and roots were then compared to Cu reference spectra and treated by Linear Fitting Combination (LCF) of reference spectra. Data treatment is still in progress and shell simulation will be performed.

Results

Cu in roots:

EXAFS spectra showed that spectra from the two plant species for the 40 ppm exposure were similar and differed from the spectra for the 400 ppm exposure (Fig. 1). Both root spectra from the 400 ppm exposure were also similar indicating that Cu speciation was similar in the two plant species. Comparison with Cu references showed that spectra from the 400 ppm exposure have a frequency and spectral features ressembling those of organic Cu compound with carboxyl/hydroxyl ligands (e.g. cellulose) or amine ligands (e.g. histidin). In contrast, spectra of the 40 ppm exposure were more similar to Cu(0), questionning the origin of this metal copper. LCF indicated that COOH/OH ligand (potentially provided by Cu-cellulose ranged between 61 and 66 ± 10 % and Cu-histidine between 34 and 39 ± 10 % for the high exposure. For the native soil, best fits were found for a mixture of carboxyl group (63- 79 ± 10 %) and Cu(0) (21-37% ± 10 %)(Fig 3.).

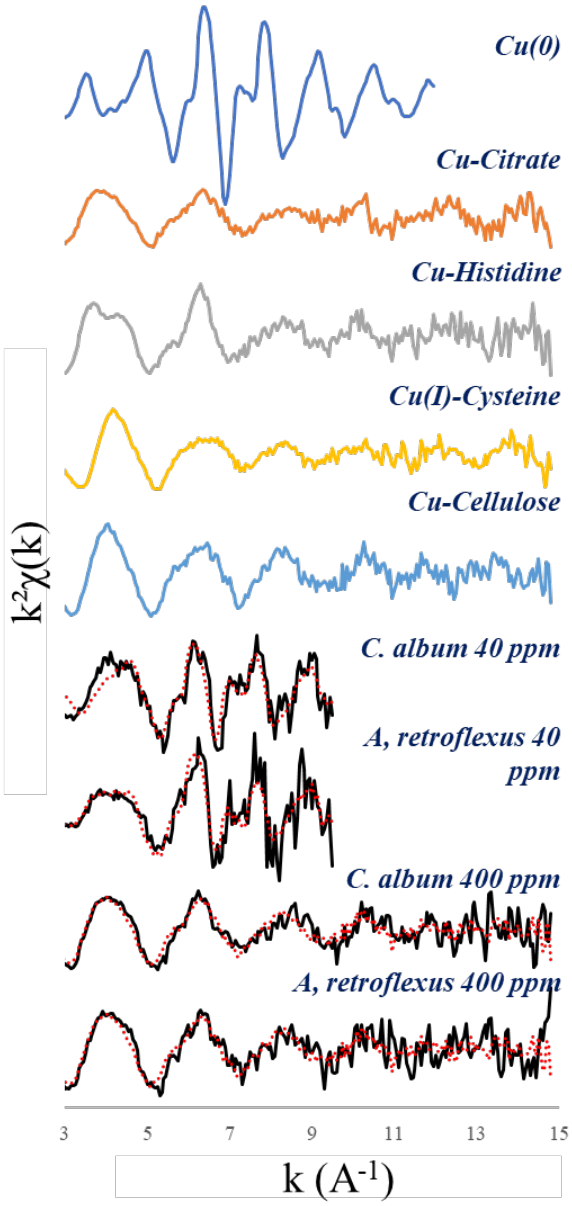

Fig. 1: Cu K-edge EXAFS spectra of A. retroflexus and C. album roots exposed to 40 and 400 ppm of Cu, compared to Cu-reference spectra. The dotted red lines represent the fits obtained by linear combination of reference spectra.

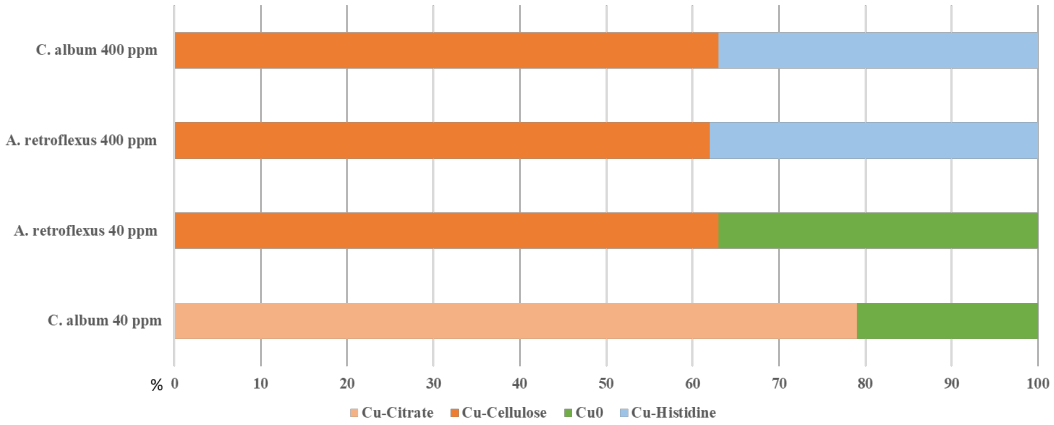

Fig.2: Proportion of Cu-species determined by LCF for A. retroflexus and C. album roots.

Cu in soils:

We measured EXAFS of soils doped with 400 ppm Cu (Bordeaux mixture), before culture (*S Pre-cult 400*) and after culture of *A. retroflexus* (*S TF A.retroflexus* 400) and *C. album* (*S TF C. album* 400), along with a control soil that underwent the watering process in the greenhouse without cultivation (*S Control 400*).

Similarly, we examined the same soils at a lower Cu concentration (40 ppm) before culture (*S Pre-cult 40*) and after culture (*STF A.retroflexus* 40, *STF C. album* 40, *S Control 40*).

The spectrum of soil at 400 ppm before the pot experiment showed a first oscillation that is asymetric. This asymetry was not clearly observed in the control at the end of the experiment, suggesting that the watering process and maturation has slighlty changed Cu speciation in the soil. The initial spectrum was also different from the one of Bordeaux mixture indicating that Cu has been probably dissolved and evolved into other chemical species. The spectra from roots at 400 ppm slightly differed from the soil at the end of the experiment (arrows), suggesting that the pattern of Cu species has evolved. LCF is in progress to identify and quantify the Cu species in the soil.

The 40 ppm soils spectra are quite noisy but seem to be different than the 400 ppm soils. As for roots, we suspect the occurrence of Cu(0) in the signature, and work is in progress to chek this point.

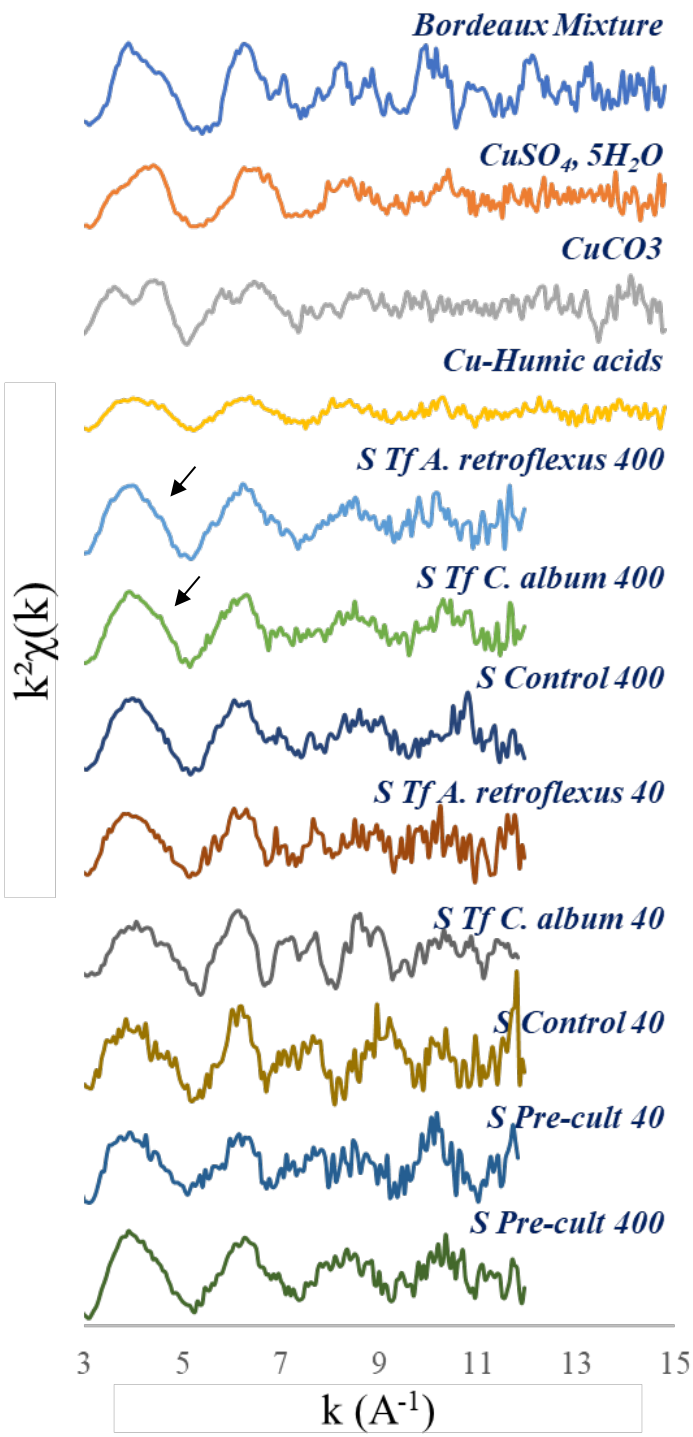

Fig. 3: Cu K-edge EXAFS spectra of initial native soil, (T0 40), soil doped with 400 ppm of Cu (T0 400), final soils with no plants (Tf control 40 and Tf control 400), soils grown with A. retroflexus at 40 (S Tf A retroflexus 40) and 400 (S Tf A retroflexus 400) ppm and C. album at 40 (S Tf C. album 40) and 400 ppm (S Tf C. album 400) and both soils before the cultivation (S Pre-Cult 40 & S Pre-Cult 400).

Conclusion

Our results showed that both plant species have similar Cu-speciation in the roots suggesting a commun mechanism of Cu sequestration, resulting from complexation by O from carboxyl and hydroxyl groups as well as N ligands. The occurrence of Cu(0) in roots for the low Cu exposure is questioning and needs to be confirmed. In soils, Cu is not present as Bordeaux mixture and data treatment is still in progress to clarify the Cu speciation.

Bibliography

Cornu, J.-Y., Waterlot, C., Lebeau, T., 2021. Advantages and limits to copper phytoextraction in vineyards. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-021-13450-3