| ESRF | **Experiment title: Towards phytoextraction of Cu from vineyard soils with local pseudo-metallophyte plants: insights from µXRF and µXANES** | **Experiment number:** **EV533** |
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| **Beamline:** **ID21** | **Date of experiment:** from: 24/02/2023 to: 28/02/2023 | **Date of report:** March 22nd 2023 |
| **Shifts:** 12 | **Local contact(s):** Castillo Michel, Hiram | **Received at ESRF:** |

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# Report:

## 1. Introduction

Vineyard soils are often contaminated with copper due to the use of copper-based fungicides, such as Bordeaux mixture- based on $CuSO_4$ and $Cu(OH)_2$ - even in organic viticulture. Phytoremediation can represent an alternative to extract part of Cu from these soils and local plants that grow naturally in the vineyard can be of interest for that. After a screening of local plants, we have selected two plant species *Amaranthus retroflexus* and *Chenopodium album*, that were grown in pot experiments and exposed to three concentrations of Bordeaux mixture (natural level of 40 ppm, 200 ppm and 400 ppm). The objective of this proposal was to identify the compartments of Cu accumulation in the various organs of the plants by micro X-ray fluorescence (µXRF), and the Cu speciation by micro X-ray Absorption Near Edge Structure spectroscopy (µXANES). The results may provide insights into the mechanisms of copper accumulation and resistance in these plants and potentially have implications for the development of a copper phytomanagement and recovery strategy, contributing to environmental safety assessment and sustainable viticulture practices.

## 2. Materials and Methods

Based on ICP MS quantification results on plant tissues (roots, leaves, stems, and fruits), it was observed that the copper concentration in the aerial parts of the two species was below 20 $\mu g.g^{-1}$whatever the Cu exposure of the pot experiment was. In contrast, *C. album* and *A. retroflexus* roots showed high copper concentrations, surpassing 300 µg.g-1 for both species. Consequently, due to the time constraints of the analysis, the focus was shifted to the investigation of roots and soils.

Roots of the two species were carefully washed, embedded in OCT, frozen and stored in liquid nitrogen and cryo-sectionned as 10 µm thick-cross sections just before the experiment. They were deposited between two ultralene films on an aluminium holder and analyzed using a liquid nitrogen cryostat available on ID21. Soils samples were crushed between ultralene films.

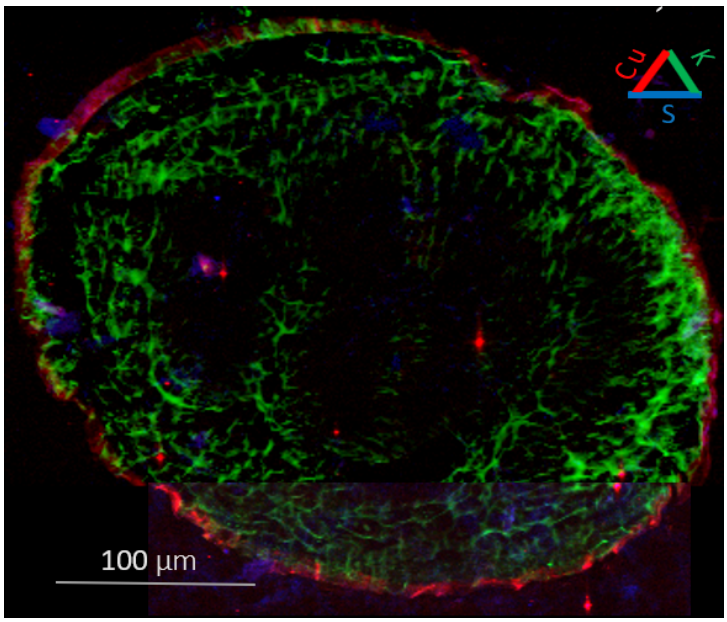
Elemental µXRF maps were collected using a Si(111) monochromator at 9.2 kev and a beamsize on the sample of 0.8 µm(H) x 0.4 µm(V) while collecting the fluorescence signal with a photodiode detector. Coarse (10 µm step), fine (2 µm step) and high resolution (0.5 µm step) maps were collected in flyscan mode with a counting time of 100 ms. Fluorescence maps were then fitted using batch fitting to remove elemental fluorescence overlapping. Then Cu K-edge µXANES spectra were collected on regions of interest evidenced by the XRF maps in fluorescence mode from 8.95 to 9.2 keV, a step of 0.5 eV and a 100 ms couting time. Micro-Xanes spectra were collected in the same conditions on Cu reference compounds. Among these references $CuSO_4$, Cu-acetate, $CuCO_3$, Bordeaux mixture, $Cu(OH)_2$, $Cu_2S$, Cu-Ferrihydrite, Cu-Goethite, Cu-humic acid, Cu-Montmorillonite, and Cu-phosphate were analyzed as powders. Cu-histidine, Cu-citrate, and Cu-cysteine were prepared as aqueous species.

During the 11 shifts, we analyzed a total of 619 µXANES spectra (including standards) and 70 µ-XRF scans. One shift was dedicated to beam aligment.

## 3. Results

- **<u>Cu distribution in roots exposed to 400 ppm Cu and soils.</u>**

Micro-XRF maps showed that Cu was mainly located at the epidemal level in roots for the two plant species (Fig.1), and was not transferred to the internal root tissues or vessels, indicating the low translocation to the shoots in the pot experiment. Copper may originate from soil particles adhering to the root and/or be sequestered by the plant at the rhizodermal membrane level.



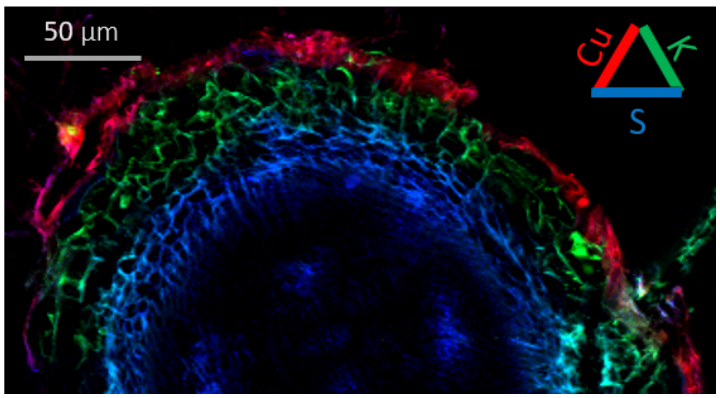




**Fig. 1.** µXRF elemental maps of half root cross-sections from **Amaranthus retroflexus (right)** and **Chenopodium album root (left)**, both exposed to vineyard soil spiked at 400 µg.g-1 in the Cu400 treatment. Amaranthus retroflexus map is sized 200x240 µm, while Chenopodium album map is sized 350x182 µm. Tricolor maps combine copper (Cu) in red, potassium (K) in green, and sulfur (S) in blue, collected from the intensity of the Cu(Kα), K(Kα), and S(Kα) emission lines.

Our results from the soil exposed to 400 ppm Cu and from the native soil (40 ppm Cu) (Fig. 2 and 3) revealed different patterns. Copper was heterogeneously distibuted in the 400 ppm soil and Cu nanoparticles were observed, potentially resulting from Bordeaux mixture residues, as evidenced by the Cu and S colocalisation

(Fig.2 maps 2 and 3). Cu also colocated with Fe in different areas (Fig. 2 map 4). In the native soil originating from the vineyard, Cu was mainly found with Ca (Fig. 3 maps 2 and 3) while colocation with Fe was also detected (Fig.3 map 4).

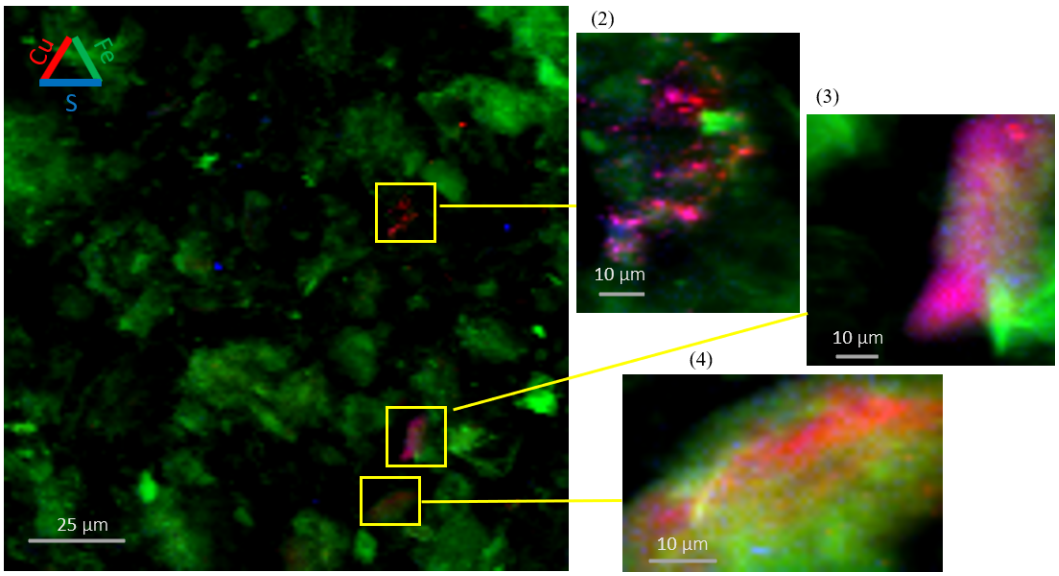



**Fig. 2.** µXRF elemental maps of vineyard soil spiked at 400 µg.g$^{-1}$[Cu], at varying resolutions: (1) 150x150 µm with 2 µm step size, and three finer maps with 0.5 µm step size at dimensions of (2) 52x52 µm, (3) 48x60 µm, and (4) 52x32 µm. Tricolor maps display copper (Cu) in red, iron (Fe) in green, and sulfur (S) in blue, derived from Cu(Kα), Fe (Kα), and S(Kα) emission lines.

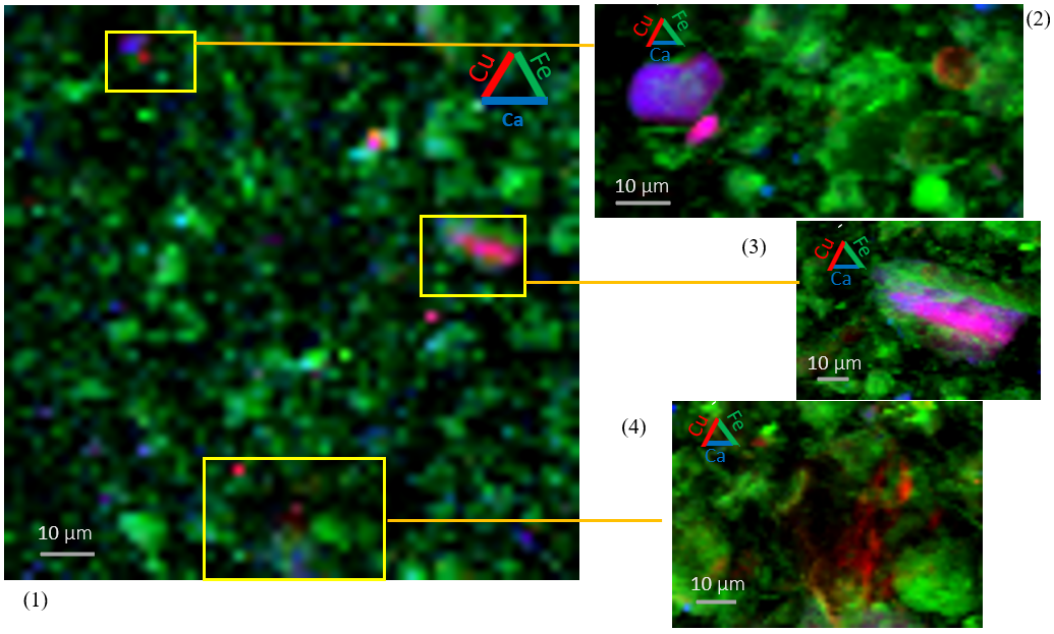



**Fig. 3.** µXRF elemental maps of vineyard soil natively enriched at 40 µg.g$^{-1}$[Cu] due to viticulture activity, at varying resolutions: (1) 150x150 µm with 2 µm step size, and three finer maps with 0.5 µm step size at dimensions of (2) 80x40 µm and (3)(4) of 75x55 µm. Tricolor images display copper (Cu) in red, iron (Fe) in green, and Cacium (Ca) in blue, derived from Cu(Kα), Fe(Kα), and Ca(Kα) emission lines.

- **<u>µXANES analysis of copper hotspot selected of roots and soil.</u>**

Cu K-edge µXANES spectra were collected on a Cu references and regions selected from root and soil maps. (Fig.4). Spectra from the native soil are different depending on the area attesting the heterogeneity of Cu species in this soil. Spectra from the soil exposed to 400 ppm Cu had also different features that also differed from the native soil. The occurrence of Bordeaux mixture residues seemed to be attested by the similarity between the Bordeaux mixture reference (Cu-BB) and some soil spectra. XANES spectra collected on the root epidermis of the two plant species showed similar features, especially at the edge level, suggesting a similar way of Cu

sequestration. No spectral similarity was found with soil spectra, thus suggesting that the accumulation of Cu at the epidermis level resulted from a physiological mechanism and not from accumulation of soil particles.

Our experiment was performed less than 1 month ago and data treatment is under progress. We collected more than 600 µXanes spectra and Principal Component Analysis (PCA) for spectral analysis is under process.

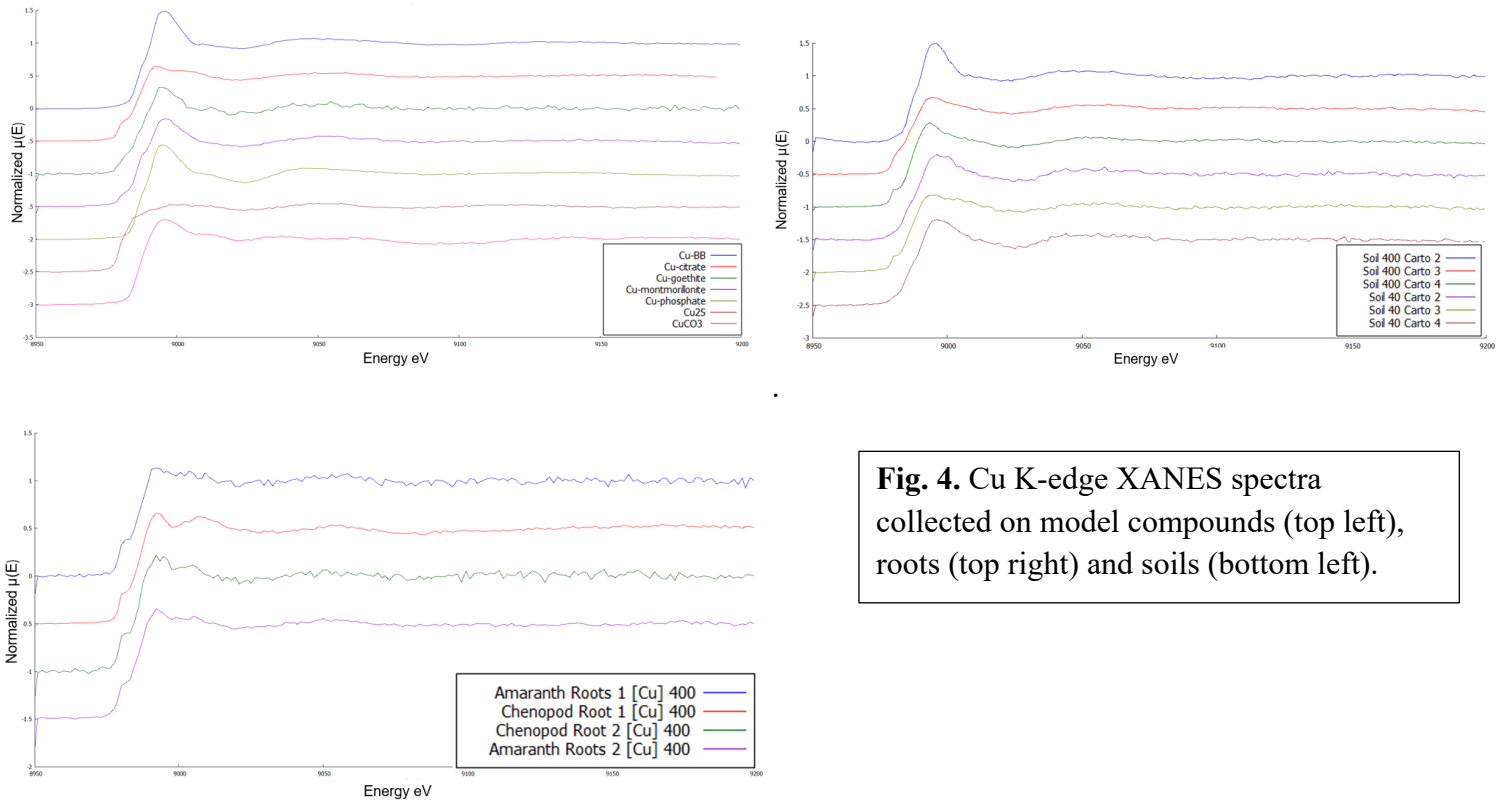



**Fig. 4.** Cu K-edge XANES spectra collected on model compounds (top left), roots (top right) and soils (bottom left).

## 4. Conclusion

This first experiment -still under data treatment – suggested the biotransformation of copper at the root interface of the examined wild plant species. This biotransformation may be responsible for the low translocation of Cu to shoots, that was observed in the pot experiment. In contrast, wild plants collected directly from the field, had intriguing patterns of high copper accumulation in their aerial parts. By extending our investigations in this direction, we hope to uncover additional details and complexities surrounding the role of these wild species in the biotransformation and distribution of copper within their environment.