	Experiment title: Tracing cadmium in cacao: Cd uptake, translocation and accumulation in a native cacao tree	**Experiment number**: EV-476
Beamline: BM30	**Date of experiment**: from: 6 July 2022 to: 11 July 2022, and sept 3rd 2022	**Date of report**: 13 12 2022
Shifts: 15	**Local contact(s)**: Remi Tucoulou Tachoueres, Madeleine Han	*Received at ESRF:*

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Context and Objectives

New Cd regulations on cadmium (Cd) concentration in cacao-derived products affects the cacao market worldwide. Genetics and breeding research to reduce Cd in cacao beans is currently limited by a lack of understanding of how Cd is loaded, stored and detoxified into the developing cocoa fruit of this cauliflorous tree. We wanted to study the localization of Cd in cacao organs to elucidate detoxification strategies of the tree on cellular scale.

Experimental procedure

We studied sections of branches, leaves, and roots of a native cacao tree (NA 312) from a conservatory of cacao cultivars, containing between 3 and 6 mg kg-1 DW. Sample preparation was performed on ID21, equipped with a cryomicrotome. Intact fresh pieces of the plant tissues were put in an OCT-embedding, and flash frozen in isopentane to produce frozen blocks for cryosectioning. After cryosectoning at 20 µm, the samples were freeze-dried. We recorded nanoXRF maps at ID16b in monochromatic mode for the regions of interest. Prior to the experiment, it was decided to not use the He mini-cryostat as proposed in the proposal, as the cooling after sample change (5h) was limiting the number of samples that could be measured. Tests were done to record Cd K-edge nanoXANES spectra in regions enriched in Cd in several parts of the branch; i.e. the bark, cambium and medulla. However, repeated measurements showed that the radiation damage in non-cryo conditions was too

large. Consequently, we could not accumulate several spectra to retrieve valuable spectra. Therefore, it was decided to focus on elemental mapping only.The experiment in July was in monochromatic mode (Flux It 4.8 10^{10} Ph/s), and 3 additional shifts in PINK beam mode were granted in September (Flux It 4.0 10^{11} Ph/s). The beam size was 71 nm horiz x 65 nm vertical. Maps of 100x 100 µm and smaller, with a step size of 1 x 1 µm (coarse maps) to 0.1 x 0.1 µm (fine maps), and exposure time per pixel of 50 to 200 ms were recorded, and treated by batch fitting using pyMCA and associated macros.

Results

For the branches, we recorded maps in the different regions (cork, bark or phloem region, cambium, wood or xylem region, and medulla containing secretory vessels). For the leaves, maps were recorded in the region in the epidermis, veins, and leaf blade. The roots were also tested, but Cd was not detected, which is consistent with the lower total Cd content. Figure 1 shows two examples of elemental maps obtained, and a representative XRF spectrum in the Cd-rich regions. Thanks to the high incident energy, we could record maps of a many elements of interest, like Zn, Mn, Ni, Co, Ca, Sr, etc… With this large set of data, we could make some hypothesis on the management of metals by this plant species. Different detoxification mechanisms for Cd, depending on the type of tissue, could be observed.

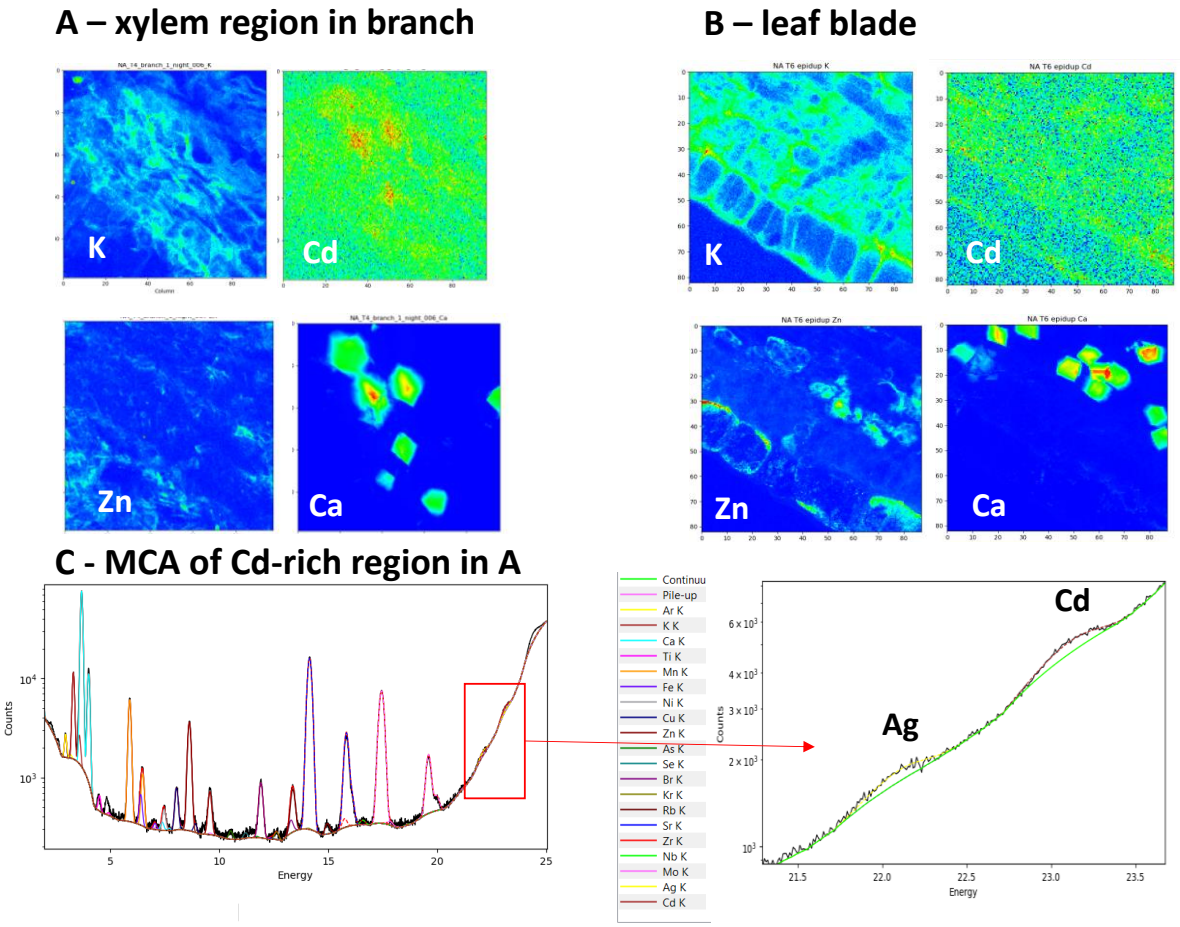

Figure 1: Example of nanoXRF maps obtained on a section of wood (A) and leave (B). C XRF spectrum extracted from the Cd-rich region and its fit.

Overall, we obtained unprecedented data on Cd localization in plant samples at background concentration. For XANES data, it is however more advise to measure in cryoconditions, as we did on ID21 at the Cd L-edge in a parallel experiment. We could fully use the beamtime available, and we thank the beamline manager for granting additional beamtime in pink mode.