	Experiment title: Deciphering chromium oxidation state in staple food	**Experiment number**: EV 430
Beamline: BM16	**Date of experiment**: from: 18 to: 23/11/2021	**Date of report**:
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Report:

1. Introduction

Organic wastes produced on the island of Réunion exceed the French total chromium (Cr) standard values for their sales as soil amendment. Accordingly, the French Agency for Food, Environmental and Occupational Health & Safety (Anses) explicitly required additional information about the distinction between CrIII and CrVI in the edible parts of market gardening and forage crops grown on soils amended with organic wastes in La Réunion.

Therefore this project aimed at measuring quantatively Cr speciation in organic waste, soil, and edible parts of market gardening and forage crops. To acheive this goal, four challenges had to be overcome.

- The identification of a low CrVI signal in complexe samples, where CrIII is expected to be majoritary
- The evolution of Cr speciation
- The low signal/noise ratio in these diluted samples.
- A possible Cr0 contamination

2. Overcome the challenge of identifying low CrVI signal in complexe CrIII rich samples.

Fame UHD allow to perform High Energy Resolution Fluorescence Detected X-ray Absorption Spectroscopy (HERFD-XAS). We used 4 analytical cristals. We selected the energy at which the fluorescence spectra were recorded to maximize the signal of CrVI. To do so we performed RIXS analysis.
RIXS analysis consist of analysing the fluorescence intensity signal at different emitted energy along the incoming energy.

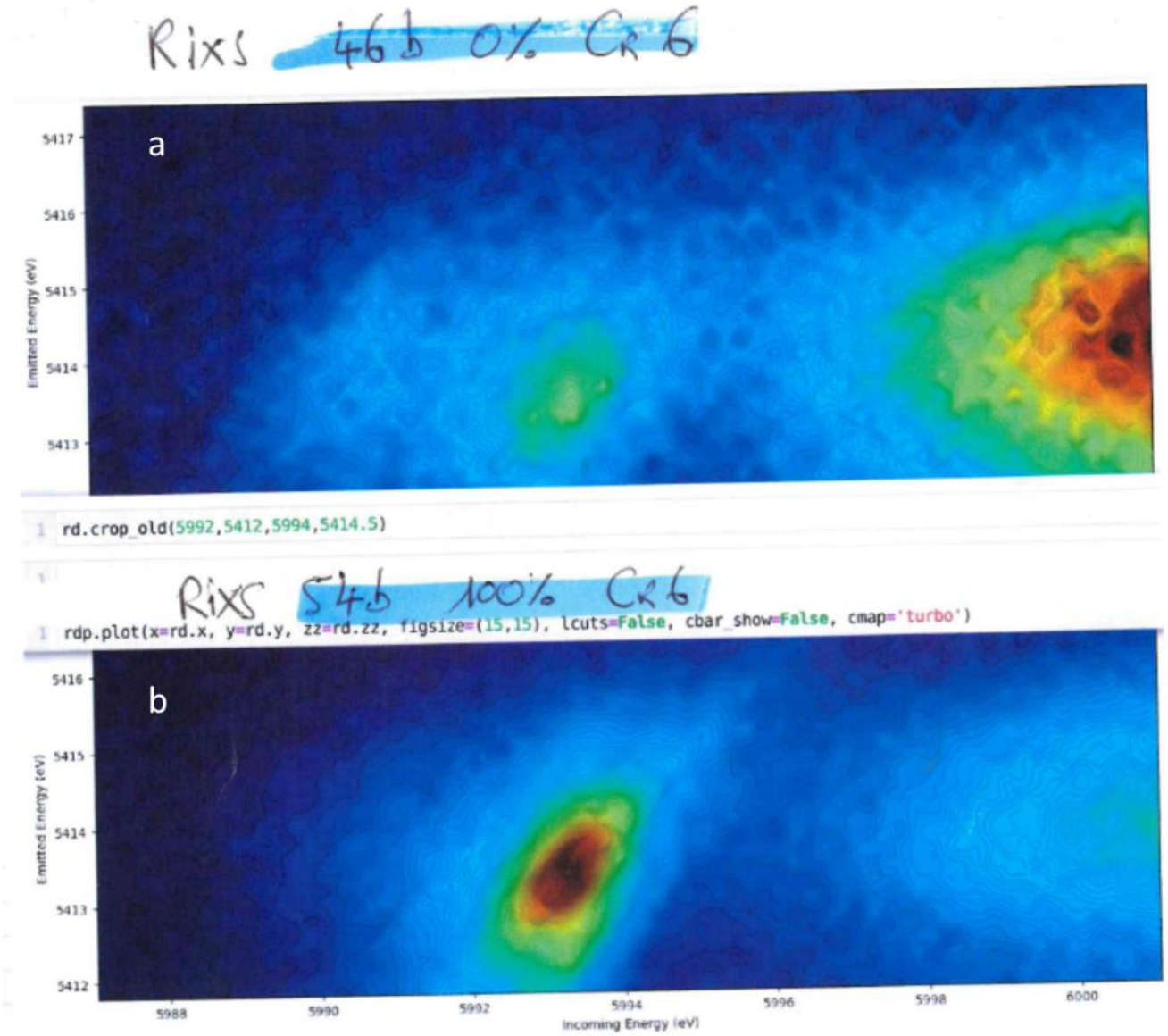

Figure 1 : RIXS images

We perform this analysis for a reference sample containing only CrVI (figure 1b). The results showed a maximum fluorescence intensity signal in the range of incoming energy between 5992 and 5994 eV, corresponding to typical energy of the CrVI pre-edge peak. The corresponding emitted energy was in the range of 5412.5 and 5414.5 eV.
We also perform this analysis for a reference sample containing only CrIII (figure 1a). The results showed that the incoming energy between 5992 and 5994 eV, there was also a contribution of CrIII signal. the corresponding emitted energy was in the range of 5413 and 5414 eV.
Therefore we selected an emitted energy at 5413.4 eV at which the signal for CrVI is maximal and the signal for CrIII is a low as possible.

3. Overcome the evolution of Cr speciation challenge.

a) No evolution of Cr speciation in the plant samples

Many precautions have been taken to avoid any modification of the Cr speciation in the samples during sample preparation and analysis.
The plant samples have been plunged in liquid nitrogen immediately after the harvest and stored frozen. Then two procedure were compared : (i) Frozen samples were ground and compacted into pressed pellets in liquid nitrogen, with special care to keep the pellets frozen in liquid nitrogen prior to the analysis ; (ii) Frozen samples were freezedried, ground and compacted into pressed pellets.
Figure 2 shows the comparison of the spectrum of Raygrass sample frozen and Raygrass sample freezedried. No difference were observed. Therefore, the freezdrying procedure was selected afterwards as it is far easier to handle on the beamline.

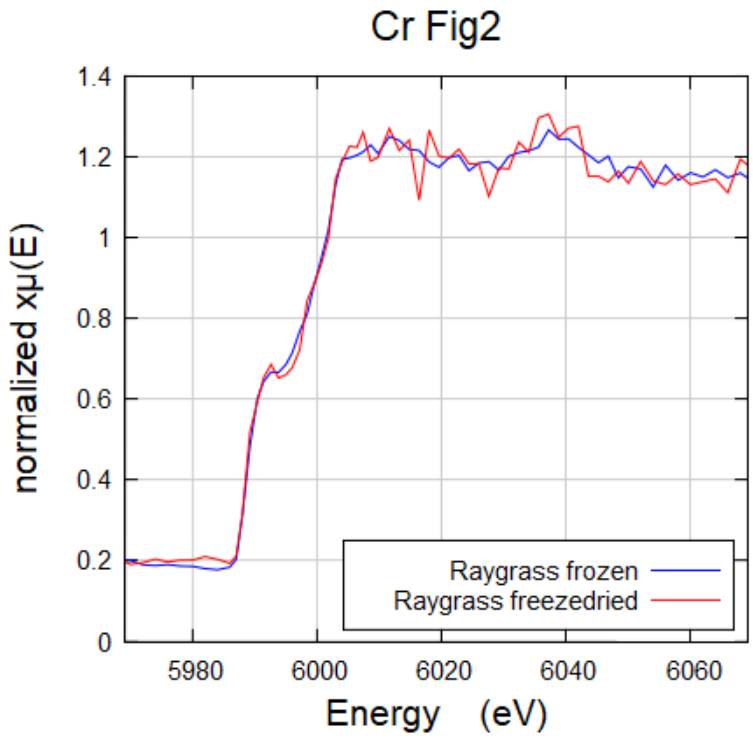

Figure 2 : comparison sample preparation freezedrying vs freezing

The Fame UHD beamline has been chosen, among other properties, because of the fact that it is equiped with a liquid He Cryostat, that greatly decreases beam damage. During the expermiment we checked for any evolution of the Cr speciation by recording the intensity of the fluorescence signal obtained at an incoming energy of 5993 eV (this energy correspond the CrVI pre-edge peak) during 600 s. No evolution were observed for the frozen leaves solid sample (Table 1).

b) Evolution of Cr speciation in the calibration references sets

The ambition of the experiment was to measure quantitatively the CrVI proportion in our samples. To do so, we had prepared several calibration references set. The first calibration set contain different proportion of CrVI and CrIII in a cellulose matrix, and the second set was in a Al_2O_3 matrix.
During the expermiment we also checked for any evolution of the Cr speciation in this calibration sets as we did for the vegetal sample (Table 1).
For the Al2O3 matrix we checked 2 samples out of our calibration set. They both contained 500 mg/kg of Cr but one was 100% CrVI and the other 100% CrIII. For the 100% CrVI we observed no evolution of the fluoresence intensity, but for the 100% CrIII we observed an increase of the fluorescence intensity that mean an oxydation of the CrIII in CrVI.

For the celulose matrix we tested 500 mg/kg Cr with 100% CrVI. We observed a decrease of the fluorescence intensity that mean a reduction of the CrVI in CrIII. Therefore, those two matrices seemed insuitable for the calibration.

Table : Cr speciation evolution in different matrices

	Fluorescence T0	Flurescence T 600s	Conclusion
[Cr]tot = 500 mg/kg 100% Cr6 + $Al_2O_{3\ solid}$	72	73	No Evolution
[Cr]tot = 500 mg/kg 100% Cr3 + $Al_2O_{3\ solid}$	3.0	5.0	Oxydation
[Cr]tot = 500 mg/kg 100% Cr6 + cellulose solid	87	45	Reduction
[Cr]tot = 500 mg/kg 100% Cr3 + BN solid	4.0	5.5	Oxydation
[Cr]tot = 100 mg/kg 100% Cr3 frozen solution	0.5	0.5	No Evolution
[Cr]tot = 3 mg/kg Frozen leaves solid	0.5	0.5	No Evolution

We tested a BN solid matrix with 100% CrIII and we observed an oxydation of the CrIII in CrVI.
Finally we tested and 100 mg/kg Cr in a frozen water matrix with 100% CrIII. We observed no evolution of the fluorescence intensity. This results showed that the calibration reference set has to be done in a frozen water matrix.

4. Overcome the low signal/noise ratio challenge in these diluted samples

Fame UHD is also well adapted to diluted samples. Figure 3 shows HERFD-XANES spectra for 3 of our samples.

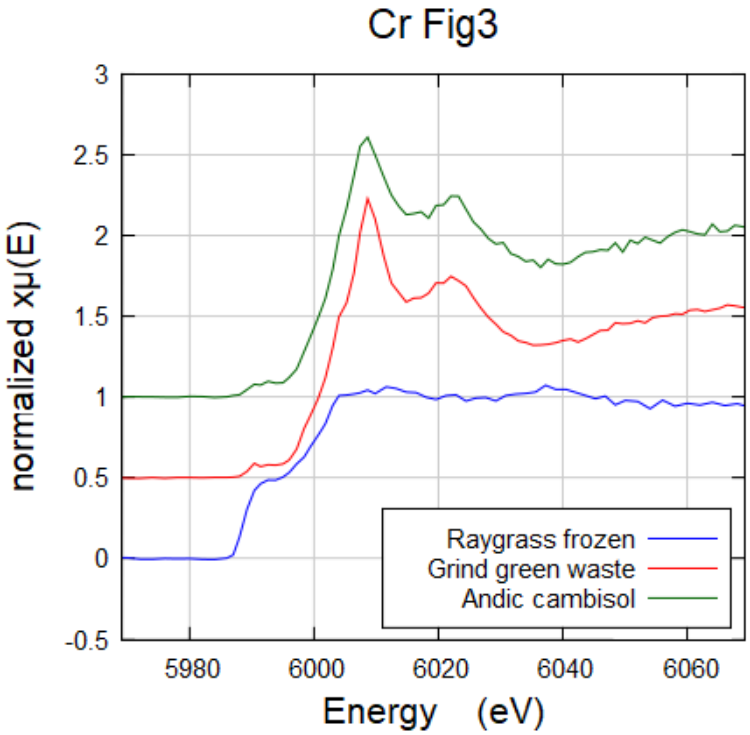

Figure 3 : examples of spectra of diluted samples

The andic cambisol sample contained 88 mg/kg of Cr, the signal to noise ratio was satisfactory with 17 spectra of 11 min. The grind green waste sample contained 44 mg/kg of Cr, and we add to ncrease the number of spectra to 23 for the signal to noise ratio to be satisfactory. The raygrass sample contained only 0.4 mg/kg of Cr. We had to do 48 spectra of 11 min each (~ 9 hours of acquisition) to reach an acceptable signal to noise ratio.

5. A possible Cr0 contamination

A preliminary data treatment of the spectra obtained for all vegetal samples showed the presence of Cr0 (Figure 3). This result was unexpected and unprobable, therefore we checked for contamination. The Cr0 presence was observed only in the plant sample when the total Cr concentration was very low, around few mg/kg. No such problem was observed when analysing samples with Cr concentration > 40 mg/kg.

a) The pellet mold hyopthesis

We hypothezed that this Cr0 contamination could be due to the procedure of pelletisation. Indeed the mold in which the pellet are done is in stainless steel that contain metallic Cr. In order to evaluate this hypothesis we analyzed a plante sample whitout pelletisation. This sample was a chloris plant containing 2.7 mg/kg of Cr. The HERFD-XANES spectrum (that is a merge of 56 scans) still showed a Cr0 pattern (Figure 4). We fitted by LCF this « Chloris merge 56 scans » spectrum with 84% of Cr metal (Cr0) and 16% of a CrIII reference.

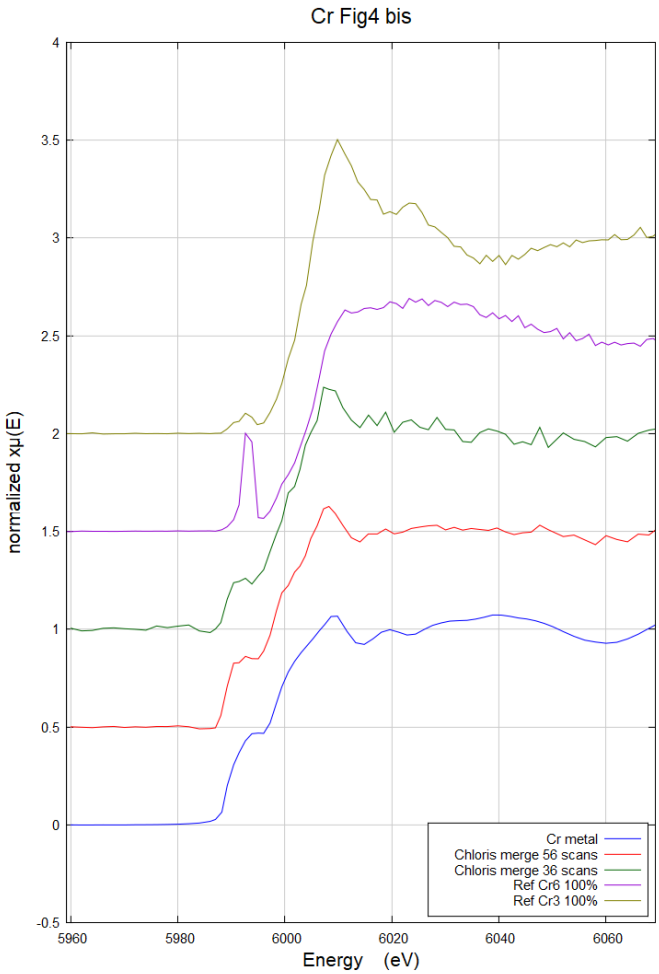

Figure 4 : Cr0 contamination

b) Post-treatment attempt

We tried a mathematical post-treatment on the data based on PCA. PCA permitted to indentify among the 56 scans recorded the ones that are very close to a Cr0 spectrum. 20 scans were identified as Cr0 like and elimineted from the merge. Nonetheless, the resulting « Chloris merge 36 scans » spectrum still showed a dominting Cr0

pattern. We fitted by LCF this spectrum with 62% of Cr metal (Cr0) and 38% of grind green waste (CrIII). Eventhough the Cr0 signal contamination could be decrease with this post-treatment, it is still dominating the sample signal. Note that the CrVI reference was considered in the LCF fits and gave far less good fits.

6. Conclusion

This experiment permited to demonstrate the possibility to measure Cr speciation in natural sample with Cr concentration down to 40 mg/kg on FAME-UHD. We expect that spectra at even lower concentrations could be measured with more analytical cristals. At this level of concentration CrVI can be decipher from CrIII in complexe samples. We could also overcome the problem of Cr speciation evolution by modifying the reference matrices for frozen water.

For plant sample with Cr concentration in the range of 0.4 to 4 mg/kg, we could overcome the low signal to noise ratio problem by accumulating a very high number of scans. However, at this level of concentration we discovered a Cr0 signal.

We hypothesis that this Cr0 contamination could come from a signal very low pollution coming from the metal parts of the beamline. However this hypothsis was discarded, since on FAME-UHD, the beam is very focused on the sample and the signal is filtered by the analytical crsitals.

We hypothesis, then, that this Cr0 contamination could come from the sample sampling on the filed trials. The plante samples were cut with stainless steel tools. The only plant samples that could be analysed in this experiment were field trial samples. We could not analyse the laboratory experiment samples that we believe do not have this problem.