

Supplementary Information (SI) for the article: Characterizing the chemical composition of red coloring matter samples from the Altamira cave using synchrotron μ XRF imaging

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7 Synchrotron SOLEIL, Saint-Aubin, 91192 Gif-sur-Yvette, France

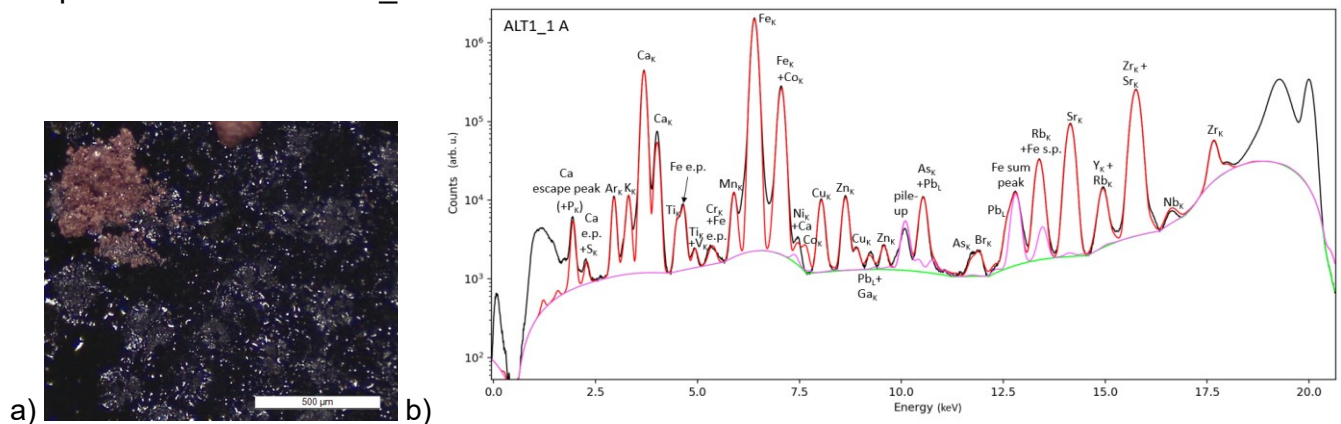
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For each of the thirteen samples will be provided: its provenance, an optical micrograph, the sum spectra of the each of the individual SR- μ XRF spectrum per analyzed pixel in the sample, the corresponding chemical maps of several elements, the Ca-K vs. Fe-K correlation of intensities, and the bi-plots representing the area under the peak of different elements against that of Fe-K and Ca-K.

Sample ALT1_1A

Representation: Claviform sign A, in ALT1 area (see Fig. 1c in the article)

Drop location name: ALT1_1



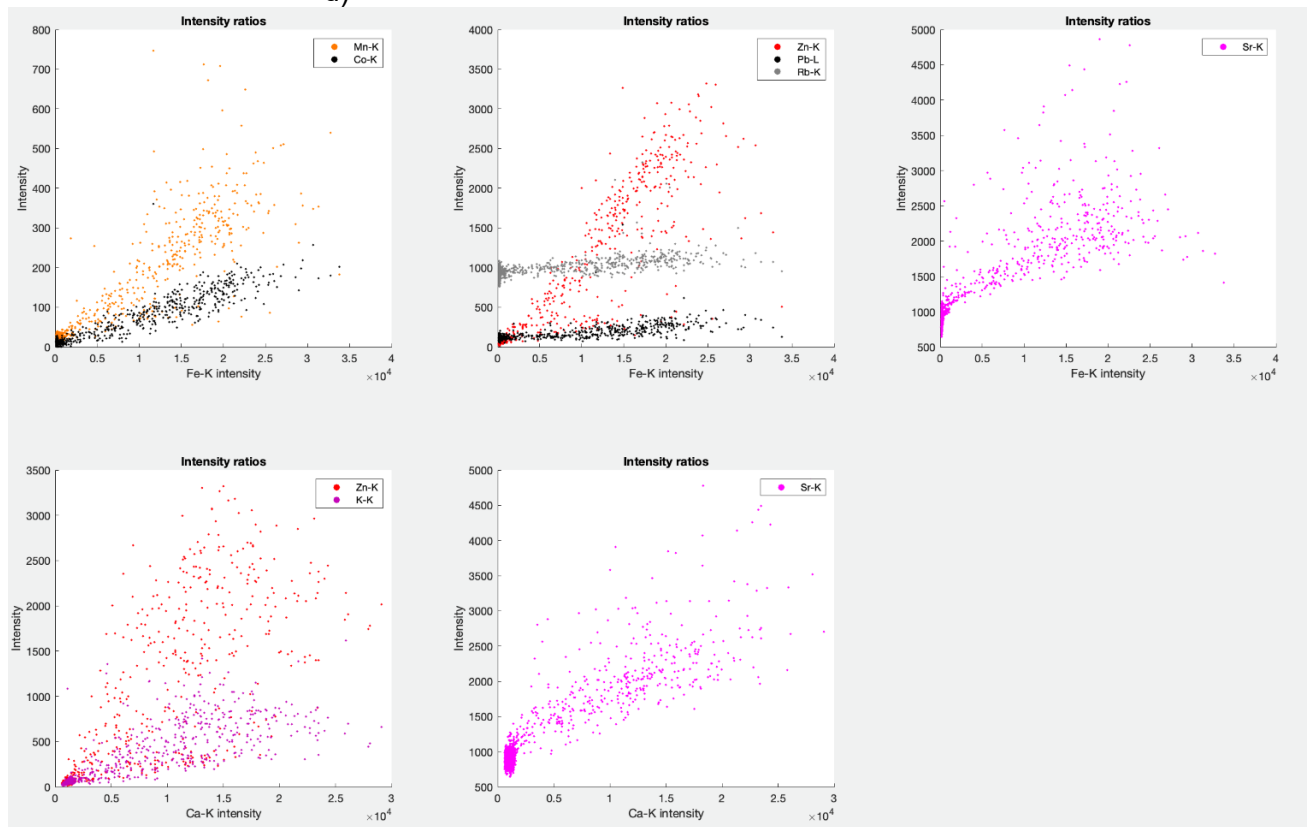
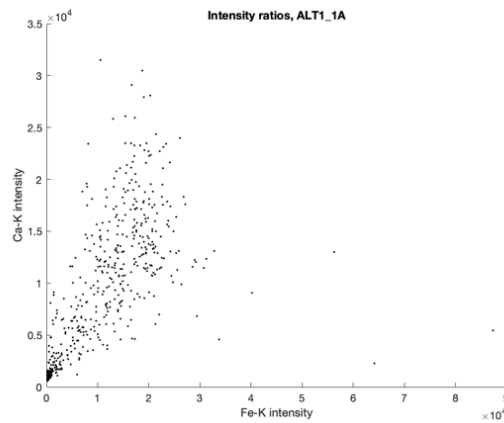
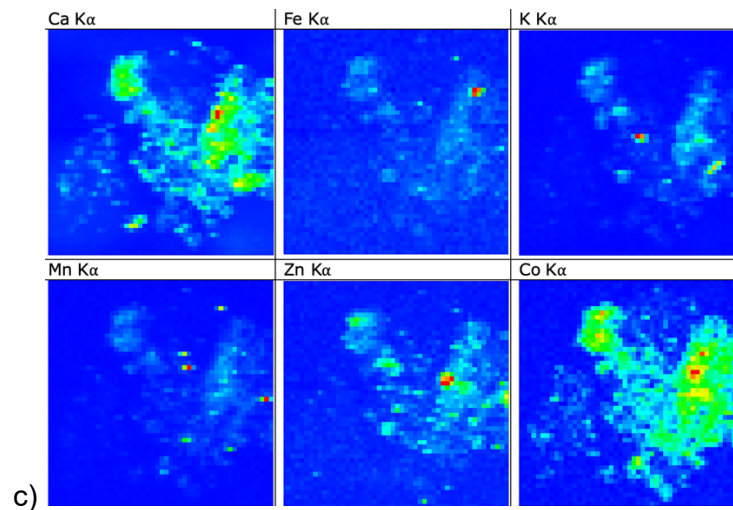
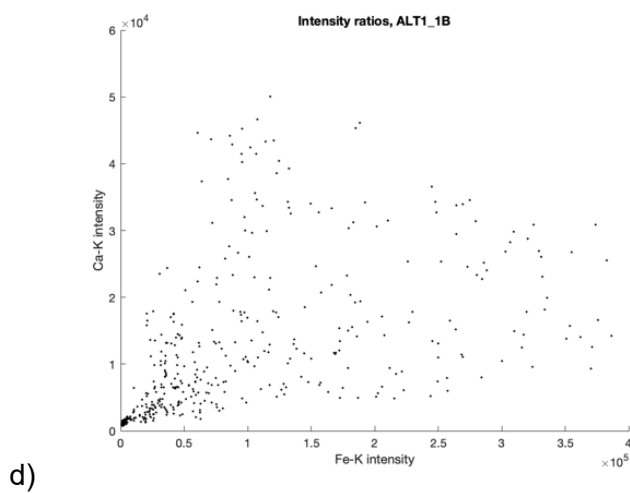
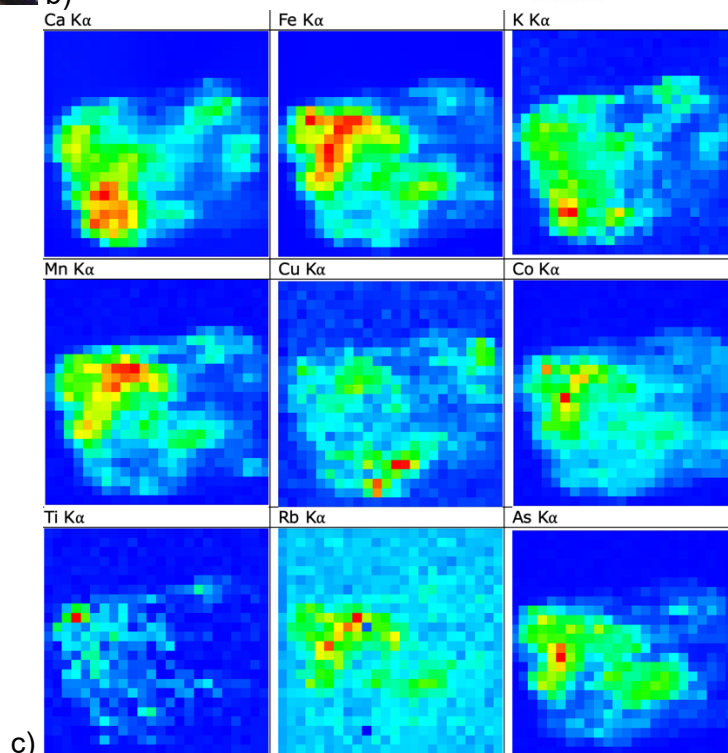
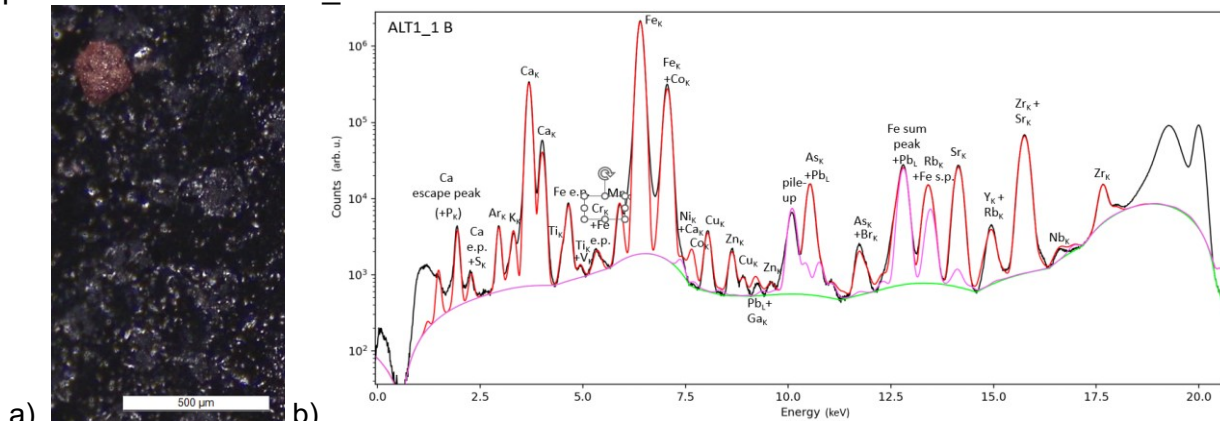


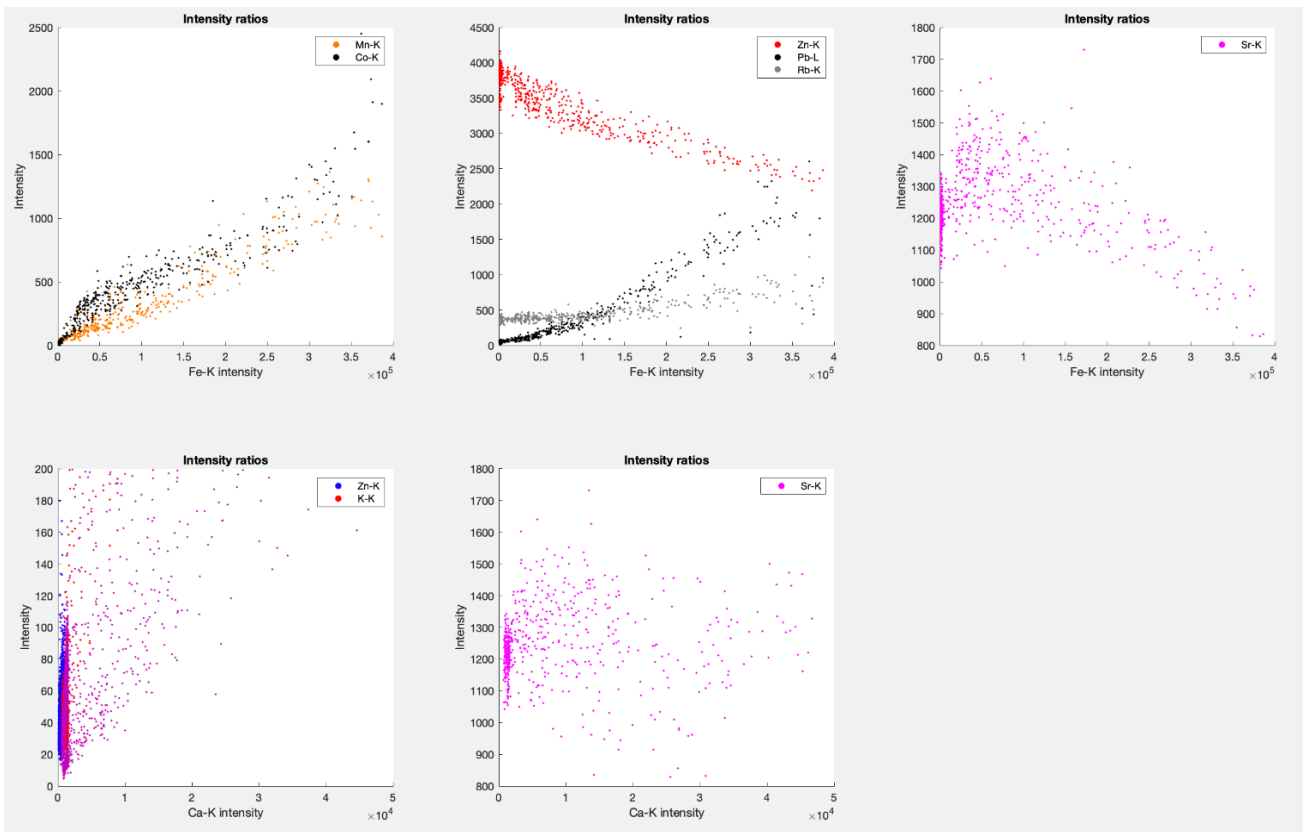
Fig. 1 For sample ALT1_1A: **a)** optical micrograph (x20 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

Sample ALT1_1B

Representation: Claviform sign A, in ALT1 area (see Fig. 1c in the article)

Drop location name: ALT1_1





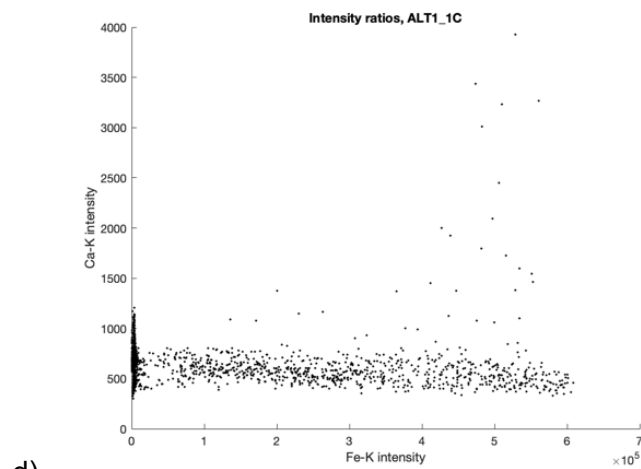
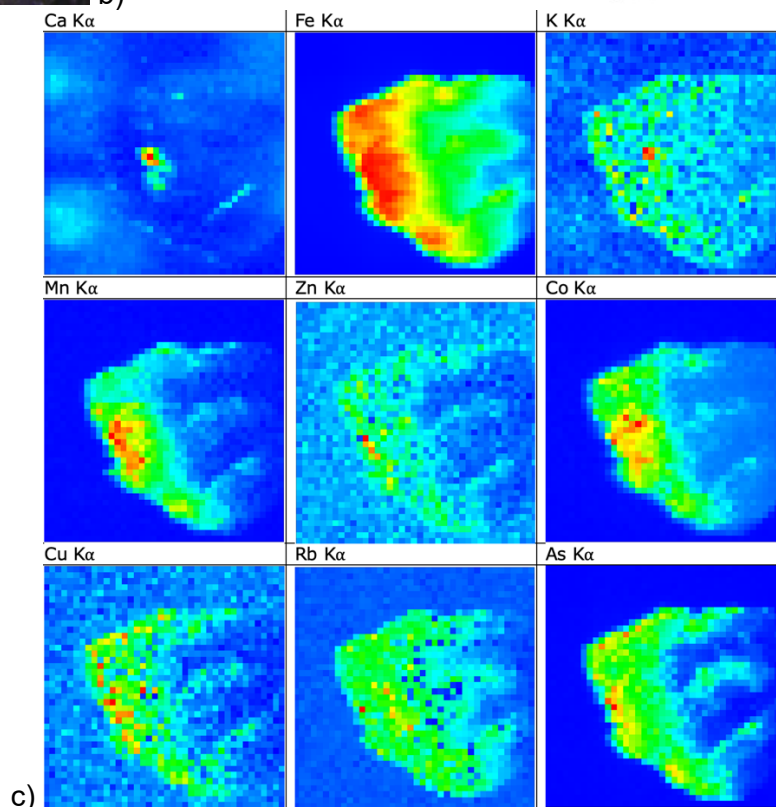
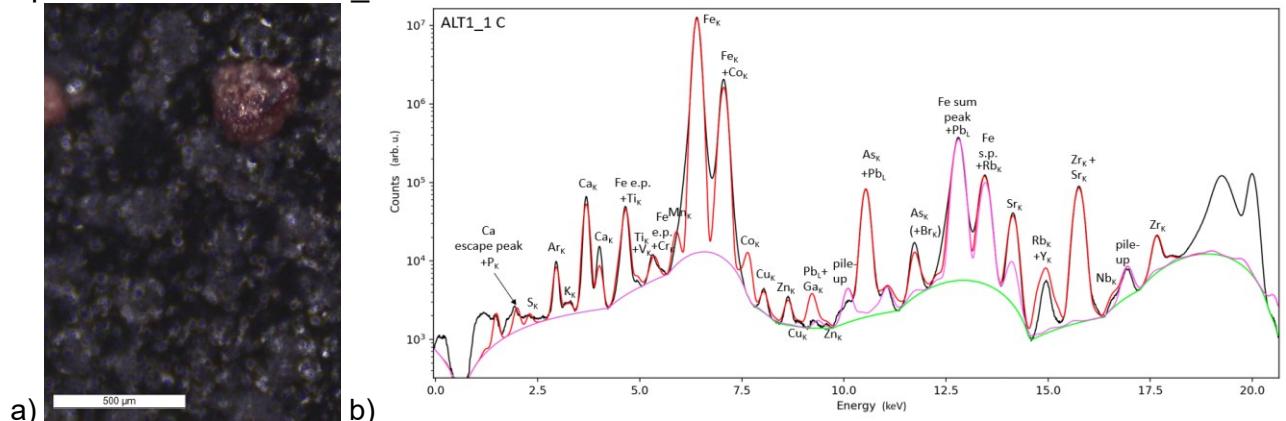
e)

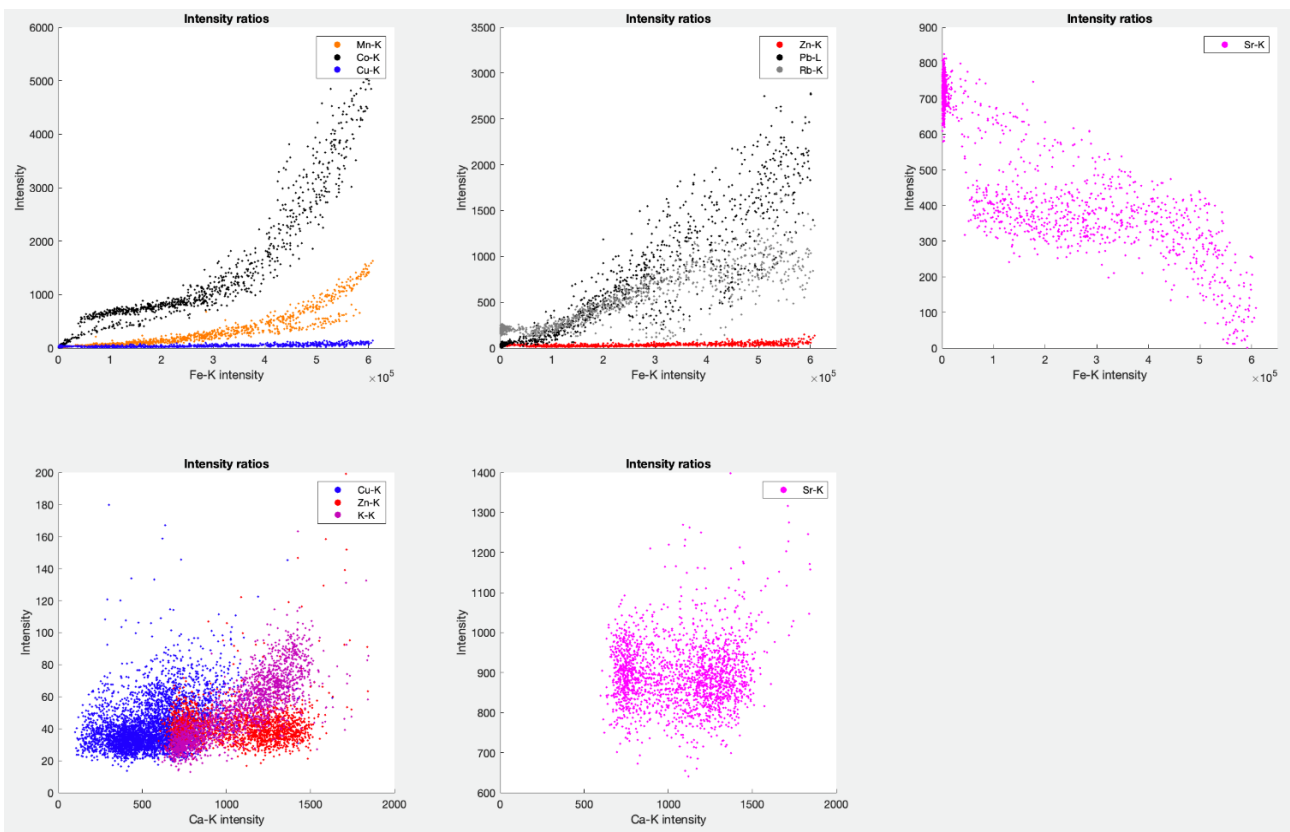
Fig. II For sample ALT1_1B: **a)** optical micrograph (x20 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

Sample ALT1_1C

Representation: Claviform sign A, in ALT1 area (see Fig. 1c in the article)

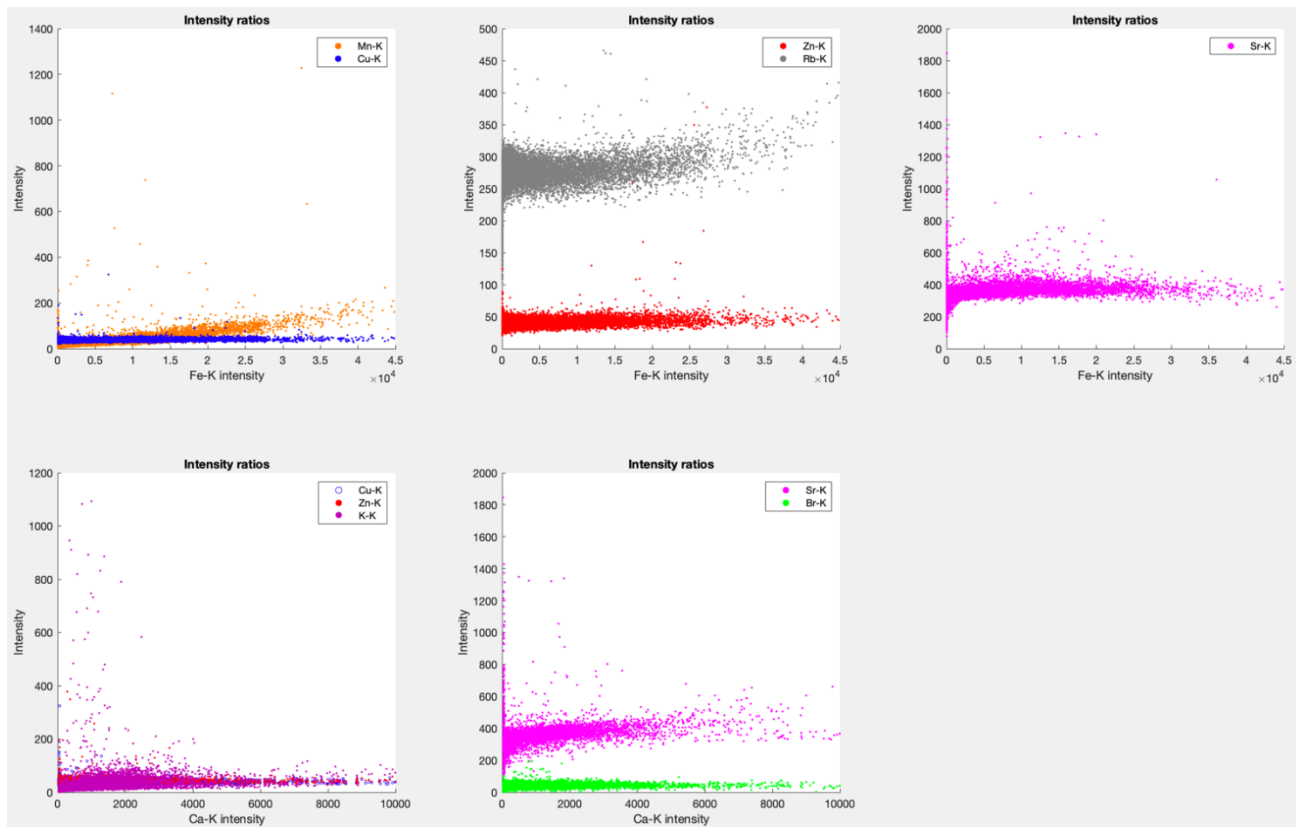
Drop location name: ALT1_1





d)

Fig. III For sample ALT1_1C: **a)** optical micrograph (x20 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.



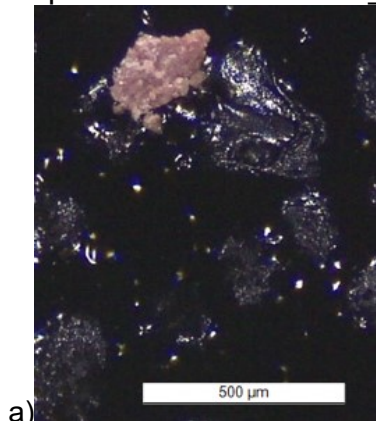
e)

Fig. IV For sample ALT1_1: **a**) optical micrograph (x10 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b**) sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c**) the corresponding chemical maps of several elements **d**) intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e**) intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

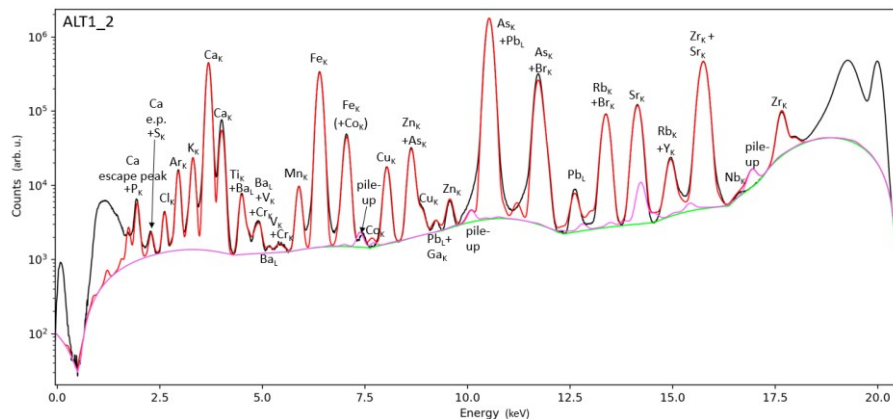
Sample ALT1_2

Representation: Claviform sign B?, in ALT1 area (see Fig. 1c in the article)

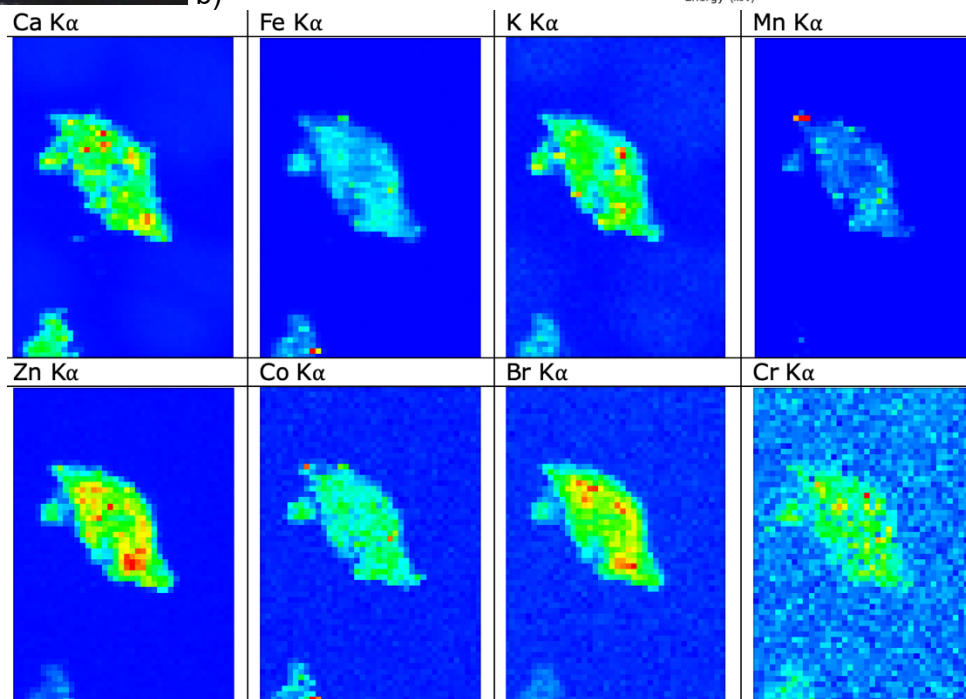
Drop location name: ALT1_2



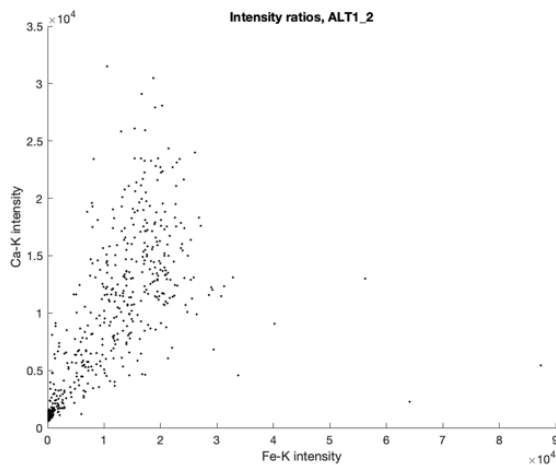
a)



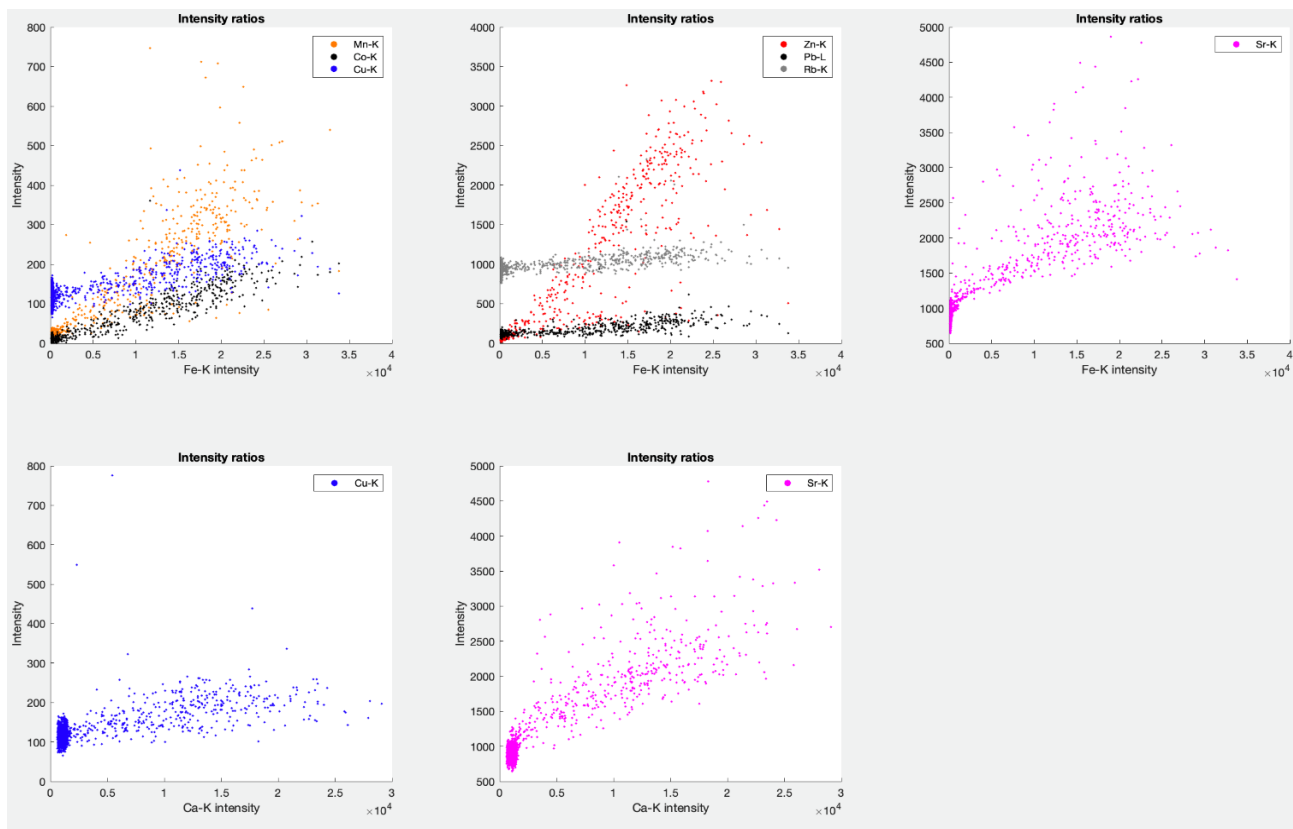
b)



c)



d)



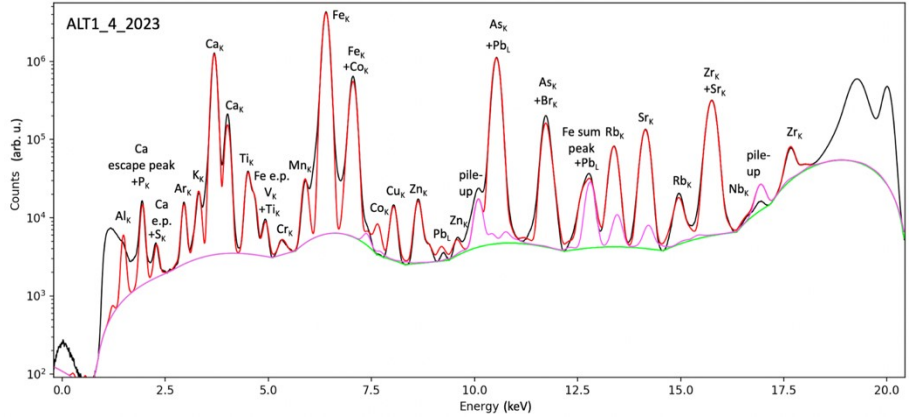
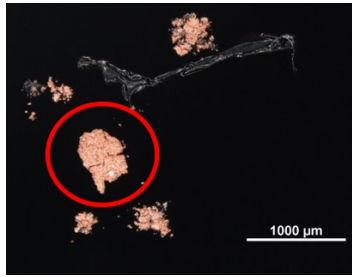
e)

Fig. V For sample ALT1_2: **a)** optical micrograph (x20 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

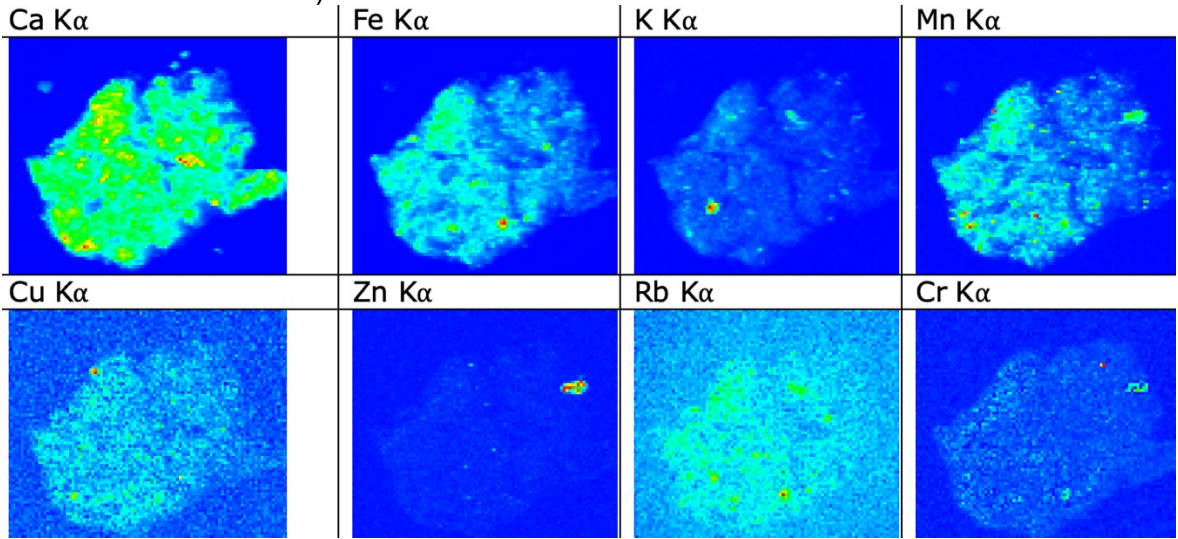
Sample ALT1_4

Representation: Superposition of claviform signs A and B, in ALT1 area (see Fig. 1c)

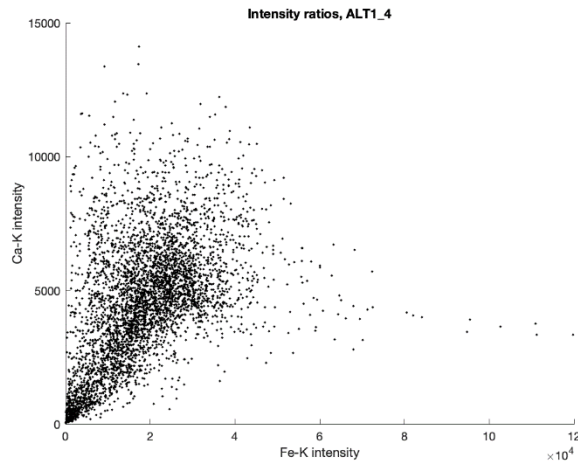
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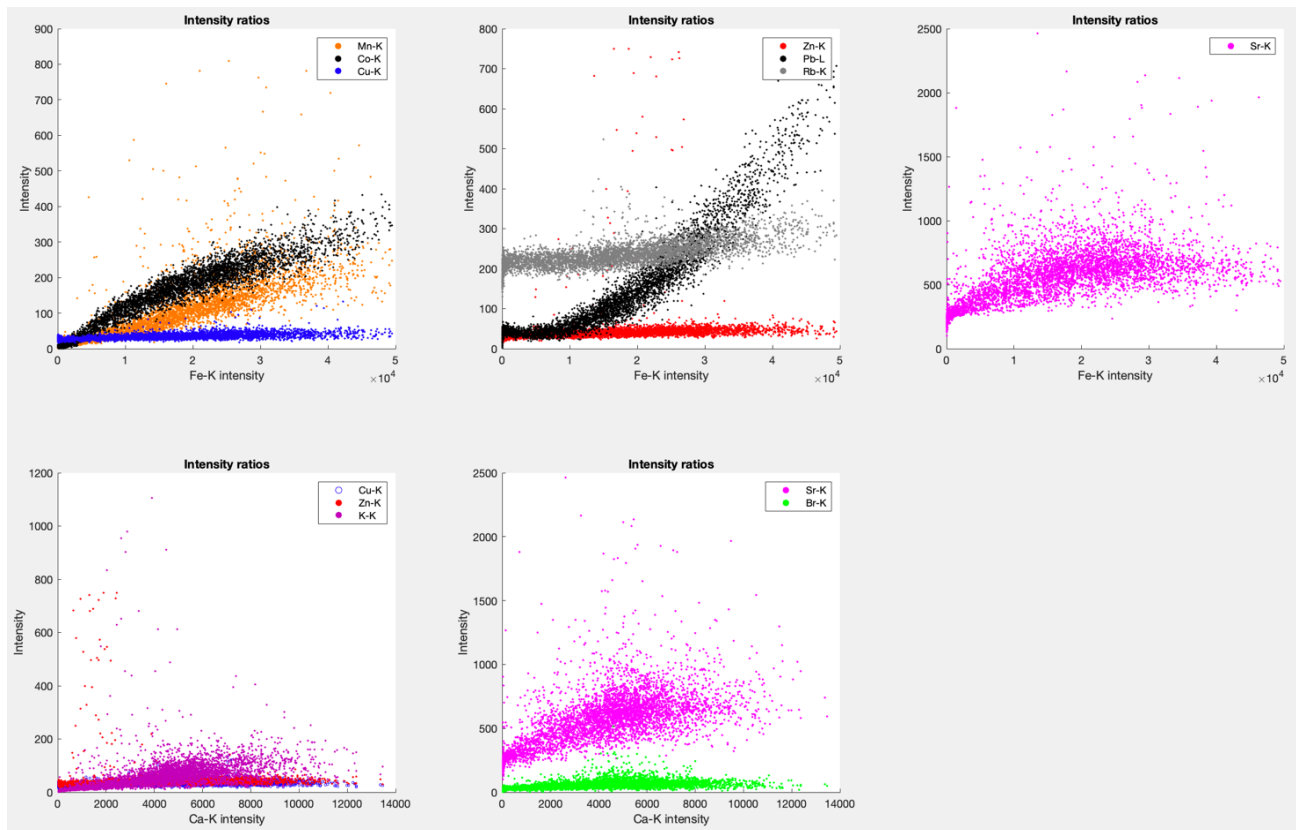
a)



c)



d)



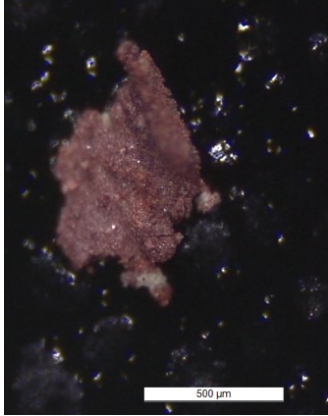
e)

Fig. VI For sample ALT1_4: **a)** optical micrograph (x3 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

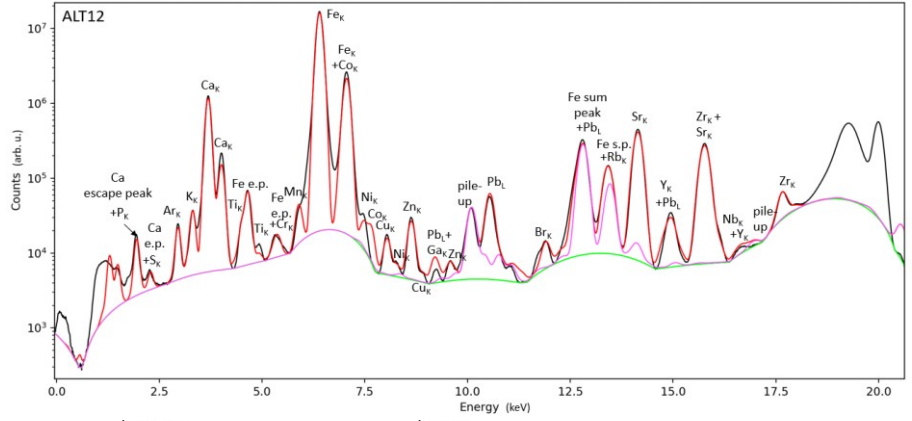
Sample ALT12

Representation: Claviform sign

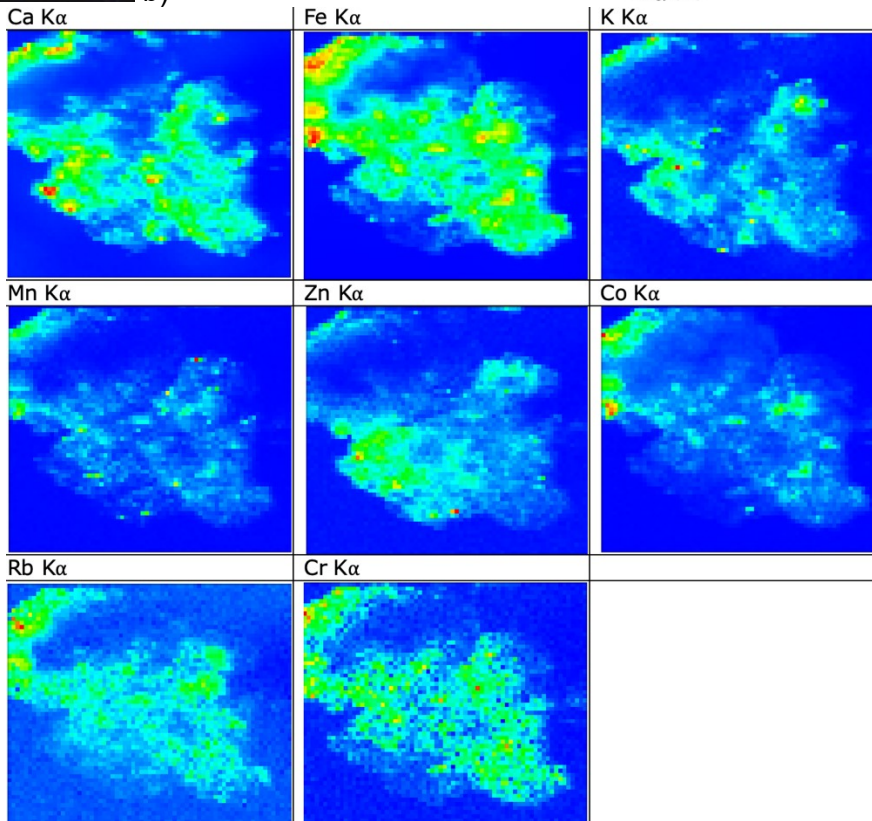
Drop location name: ALT12



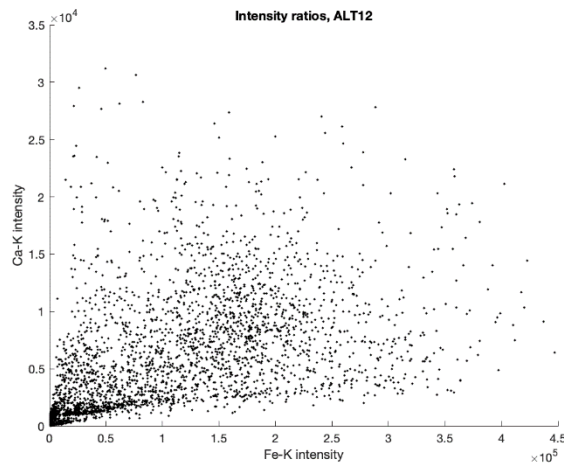
a)



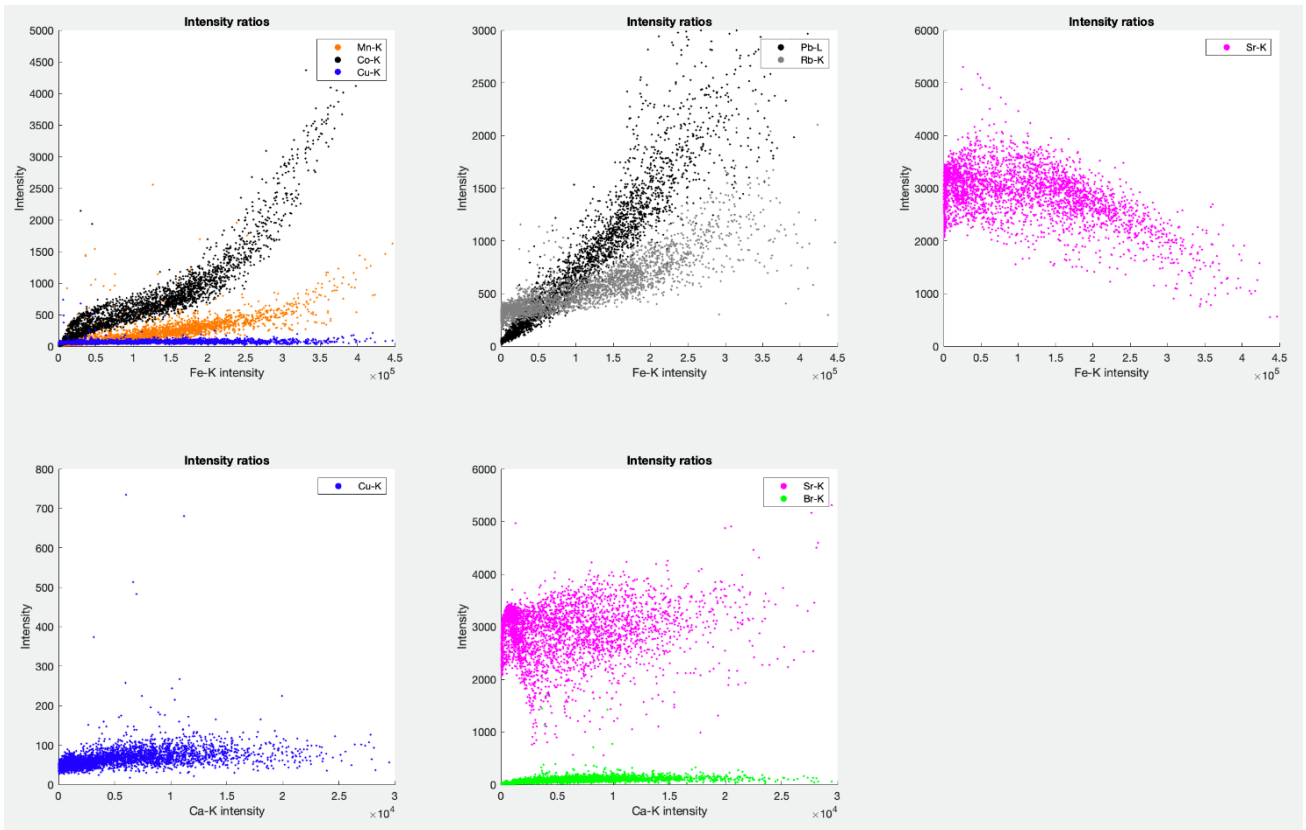
b)



c)



d)



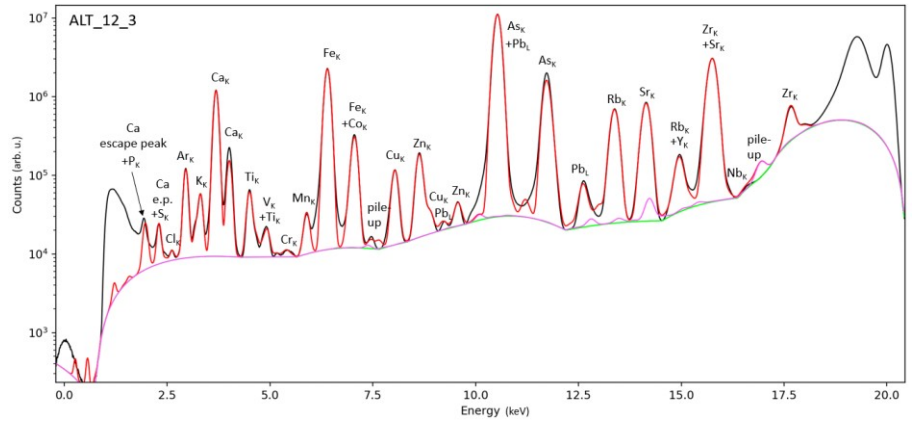
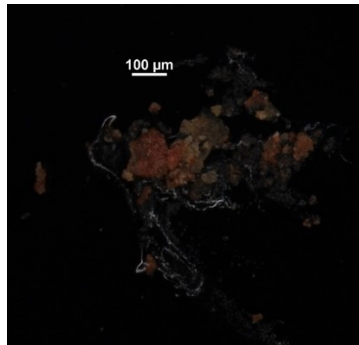
e)

Fig. VII For sample ALT12: **a)** optical micrograph (x20 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

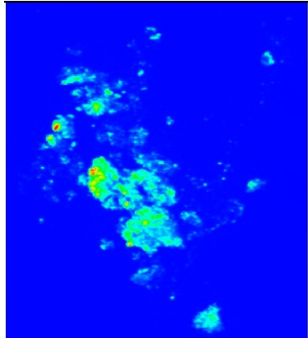
Sample ALT12_3

Representation: Claviform sign

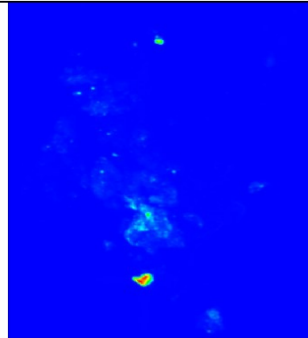
Drop location name: ALT12



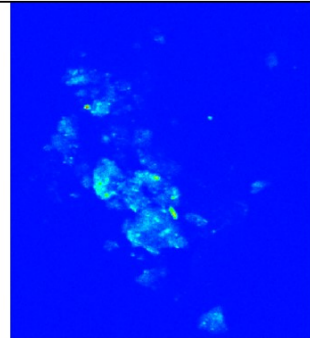
Ca K α



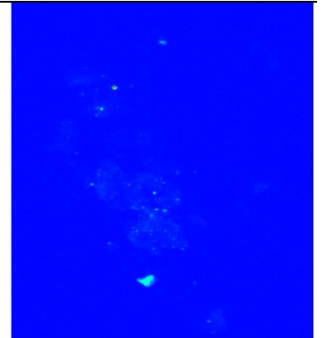
Fe K α



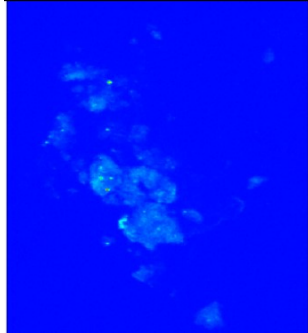
K K α



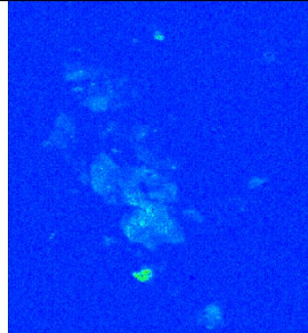
Mn K α



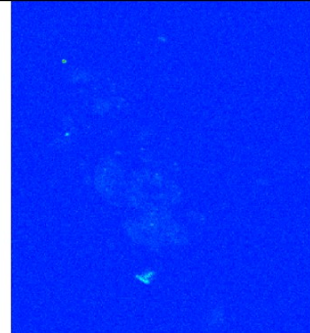
Zn K α



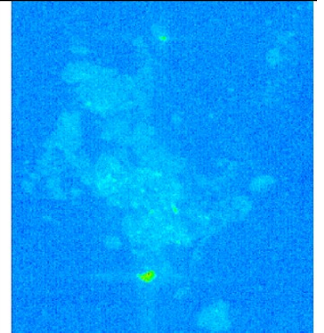
Cr K α



Cu K α

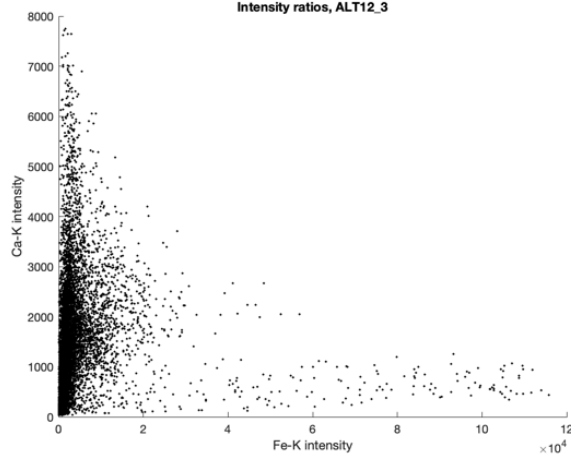


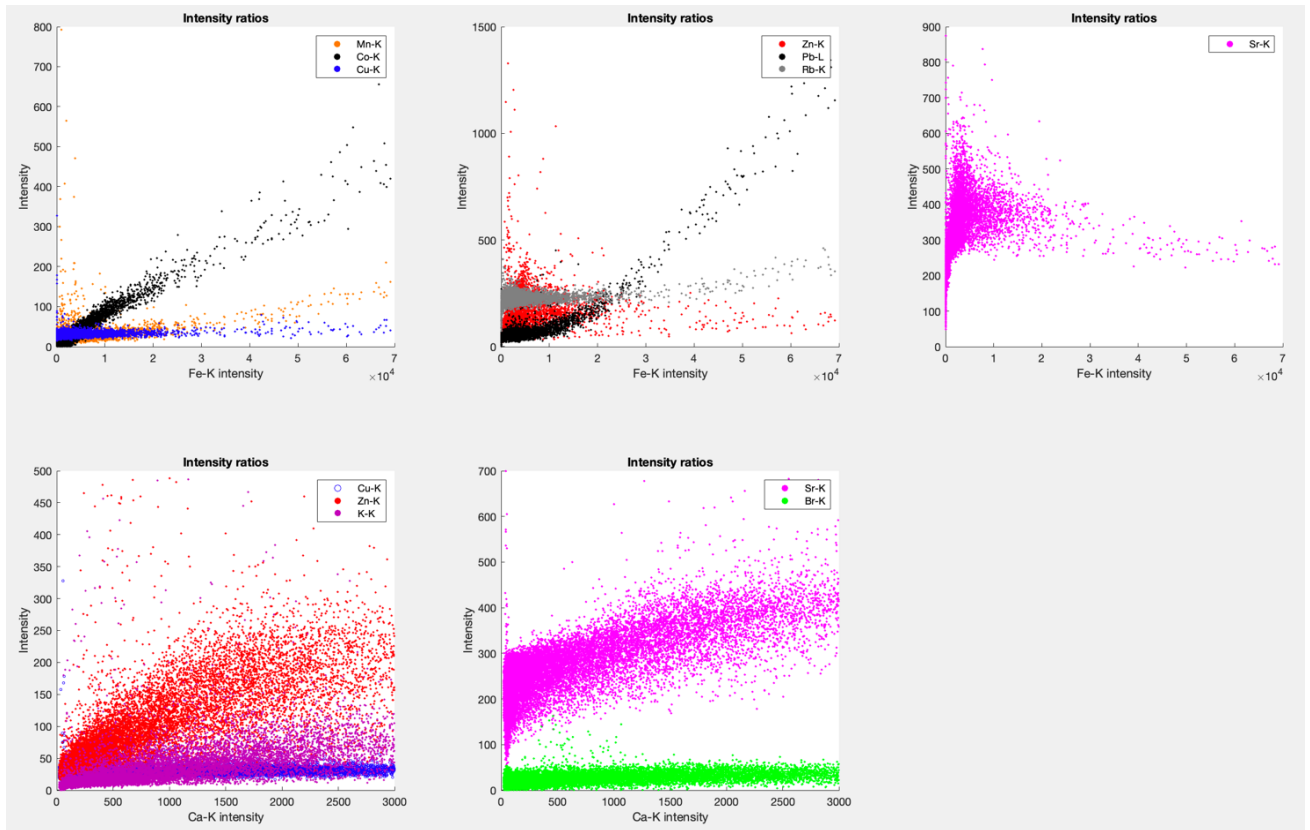
Rb K α



c)

Intensity ratios, ALT12_3

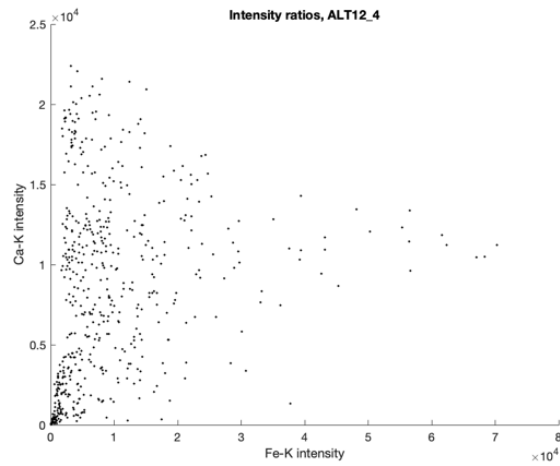
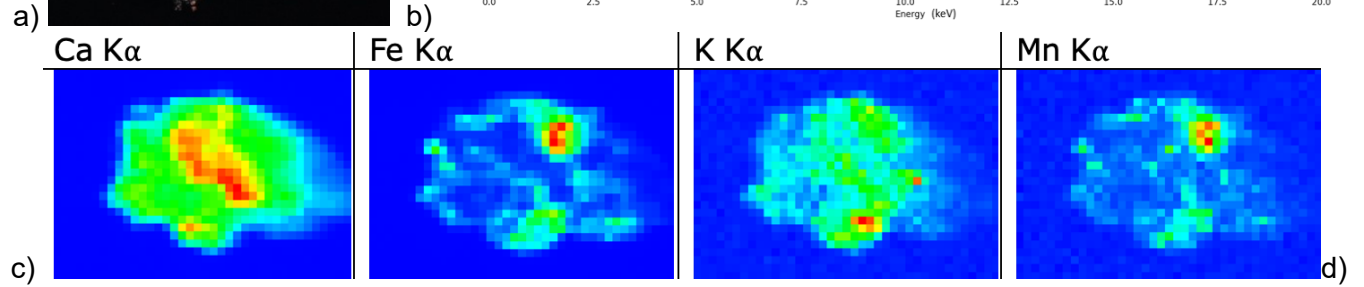
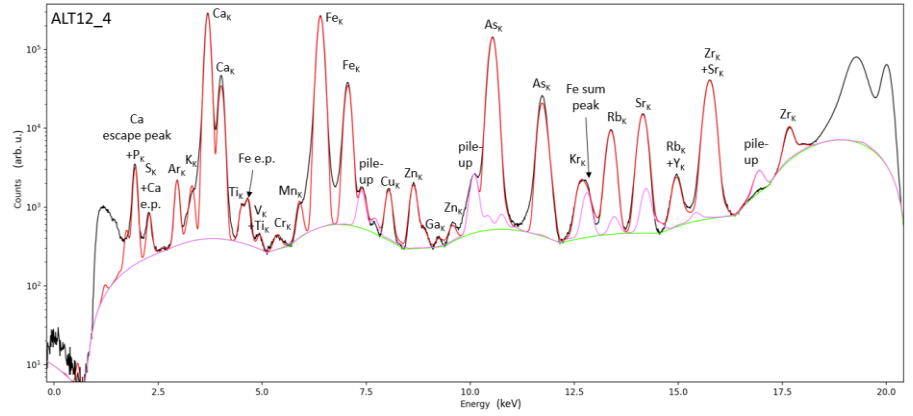
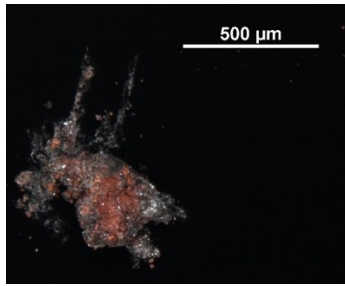


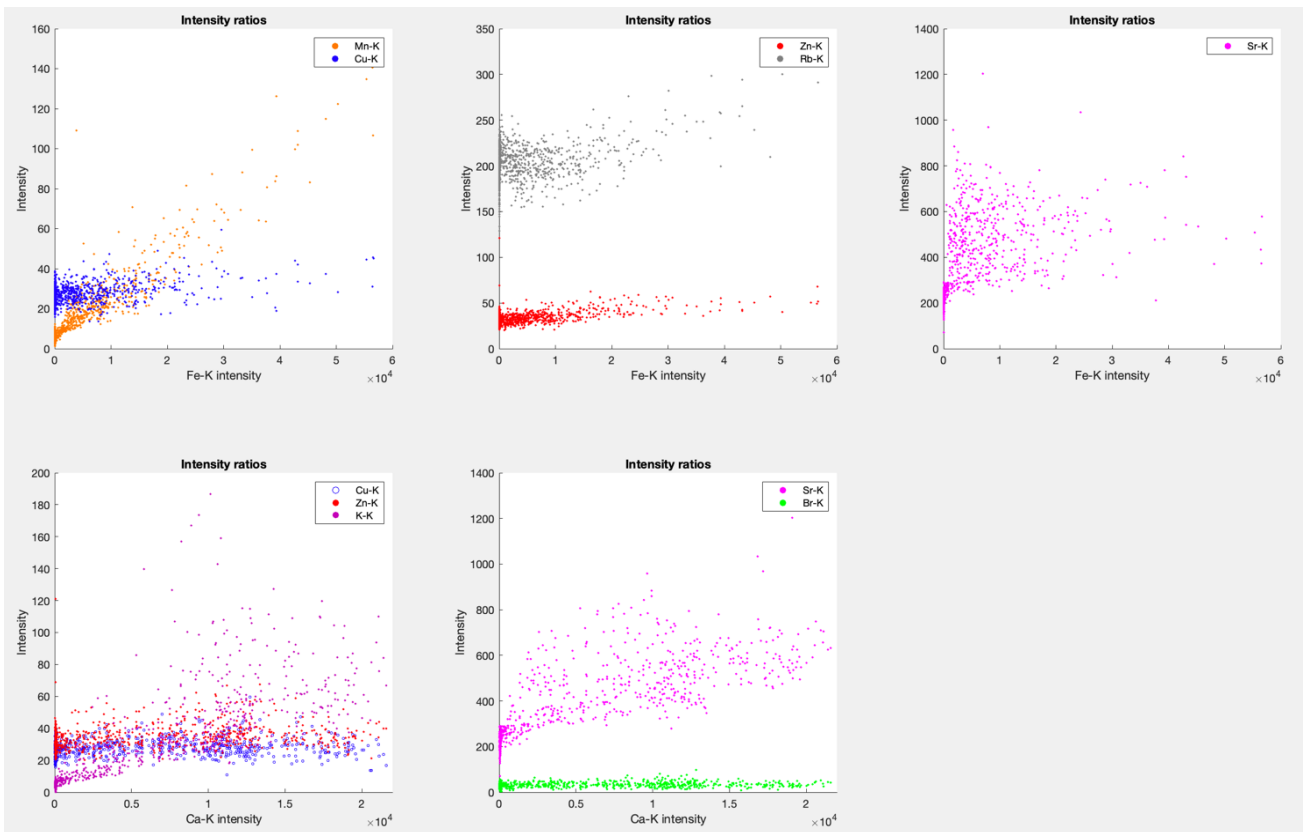


e)

Fig. VIII For sample ALT12_3: **a)** optical micrograph (x10 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

Sample ALT12_4
Representation: Claviform sign
Drop location name: ALT12





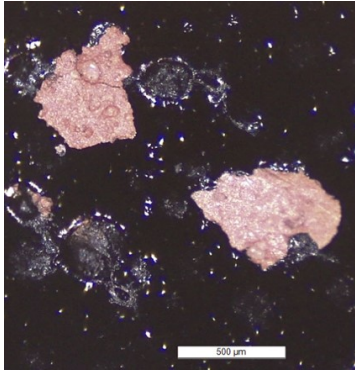
e)

Fig. IX For sample ALT12_4: **a)** optical micrograph (x6 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

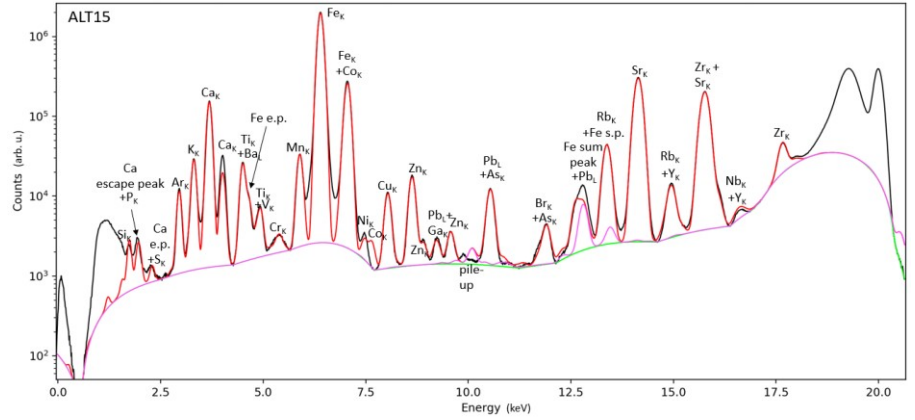
Sample ALT15

Representation: Claviform sign

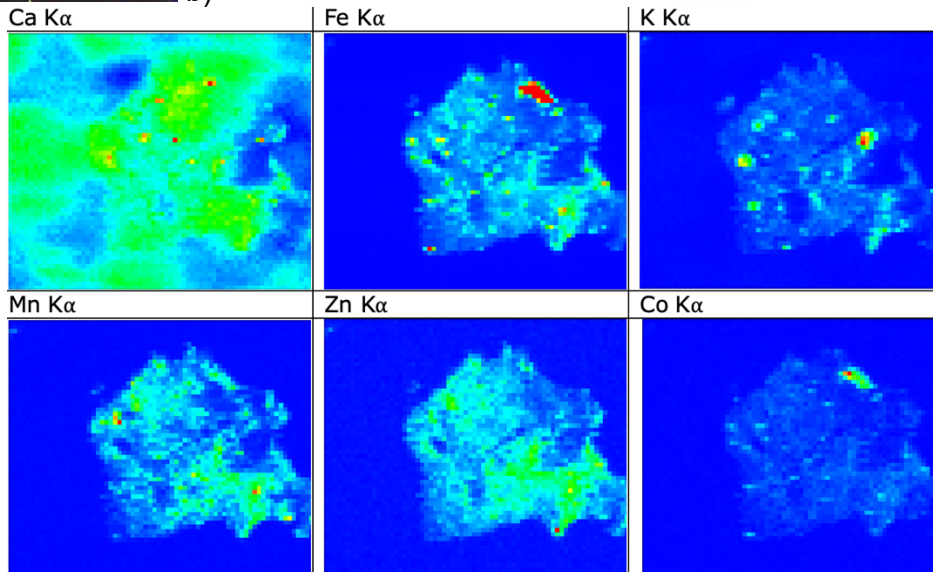
Drop location name: ALT15



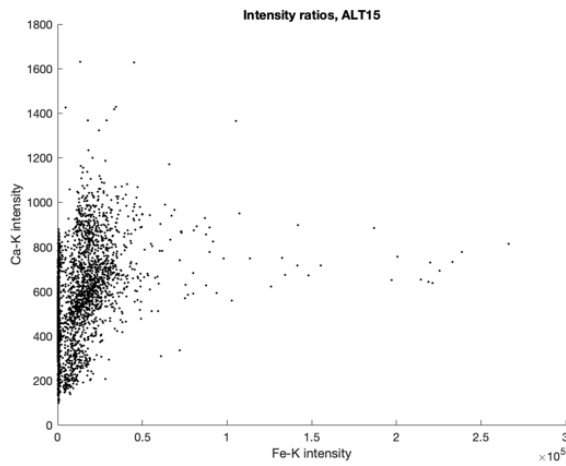
a)



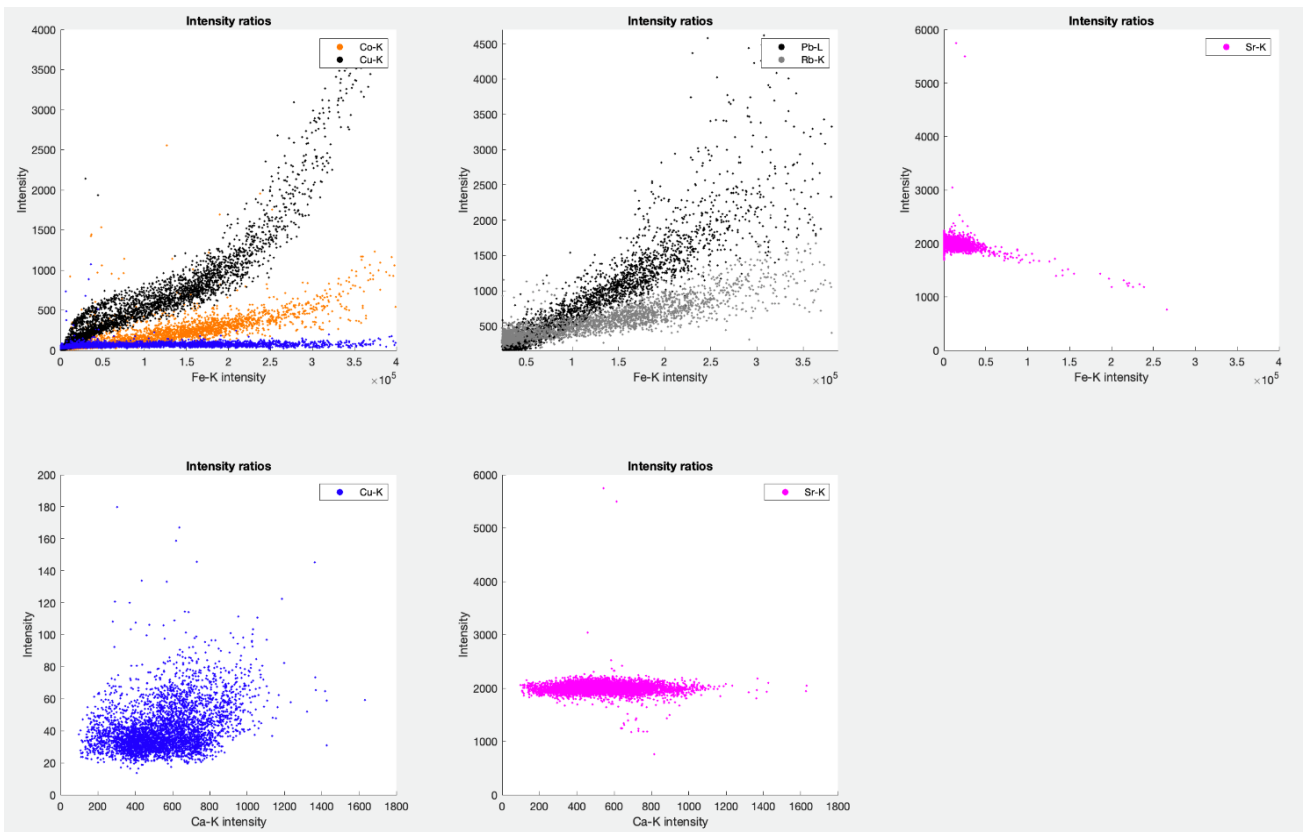
b)



c)



d)



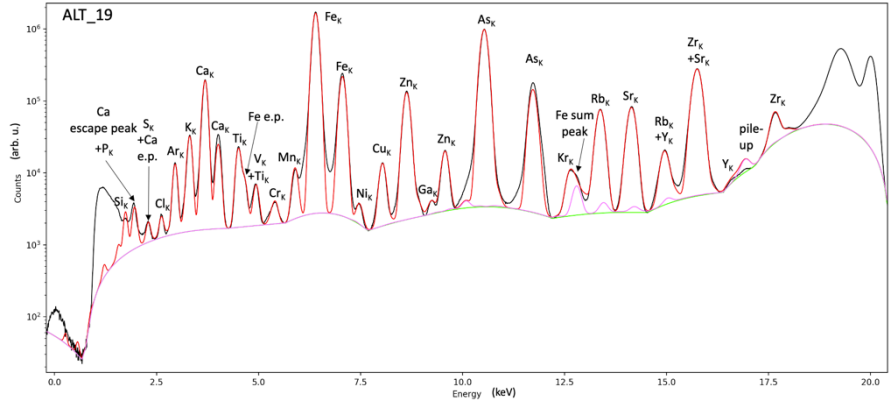
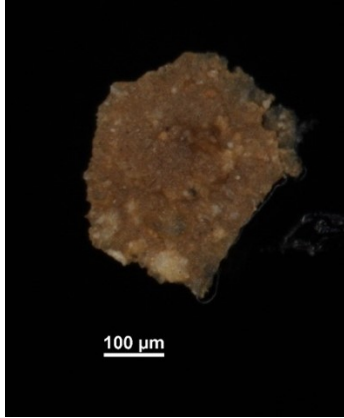
e)

Fig. X For sample ALT15: **a)** optical micrograph (x20 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

Sample ALT19

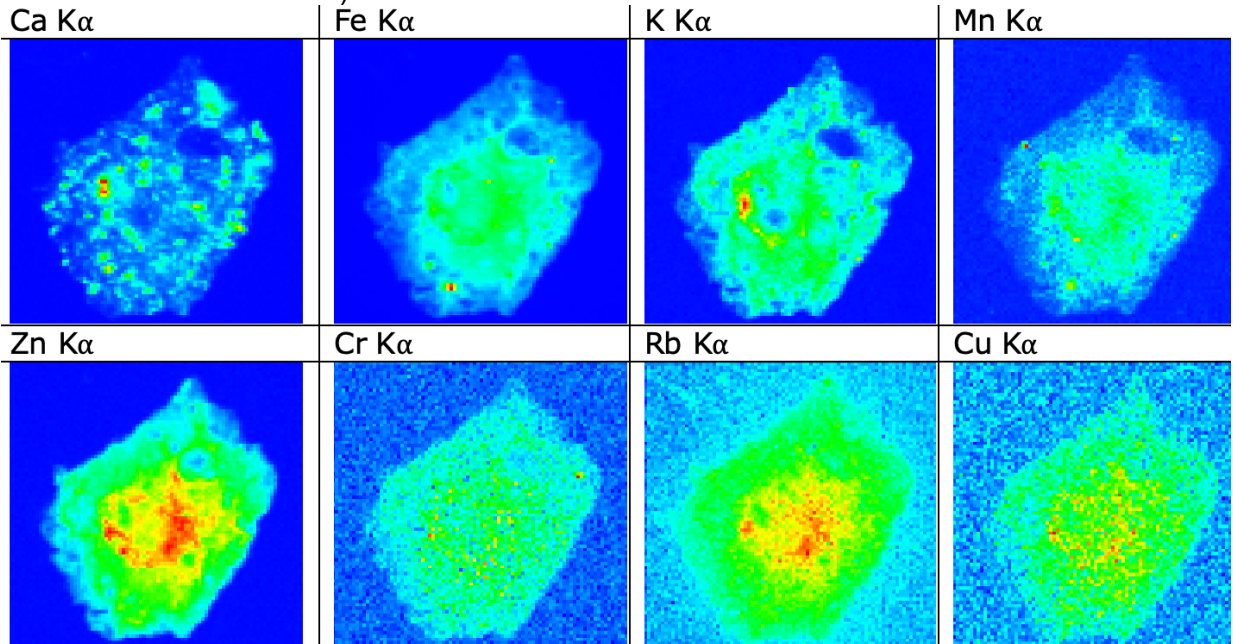
Representation: Polychrome bison

Drop location name: ALT19

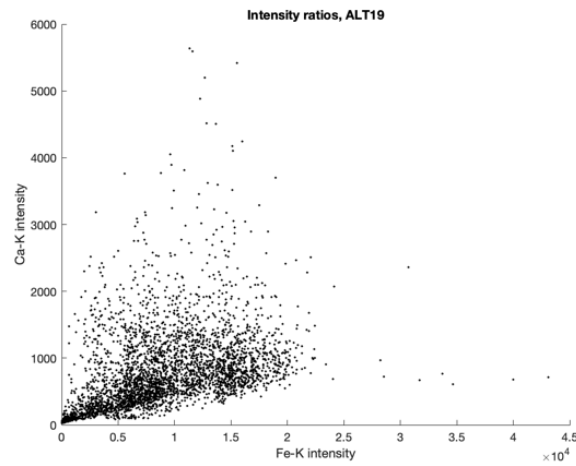


a)

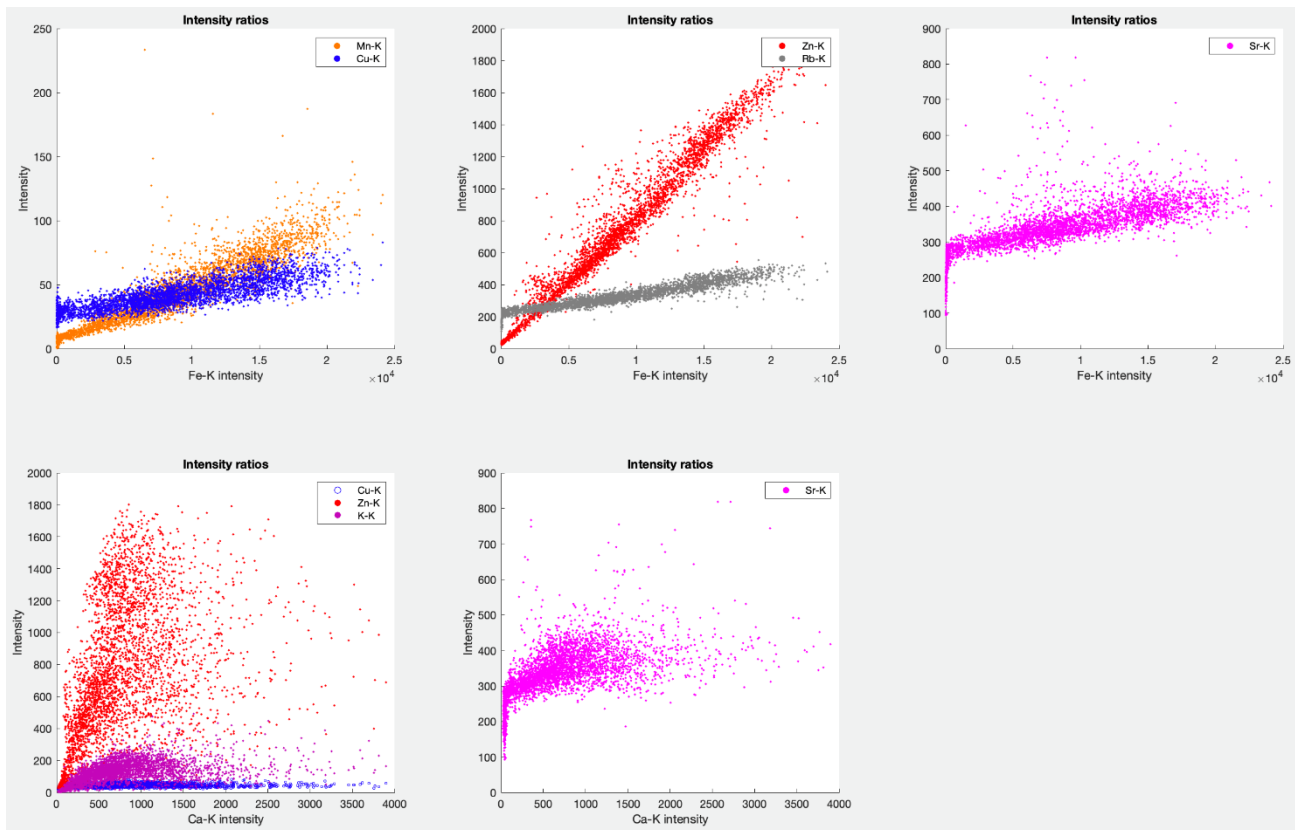
b)



c)



d)



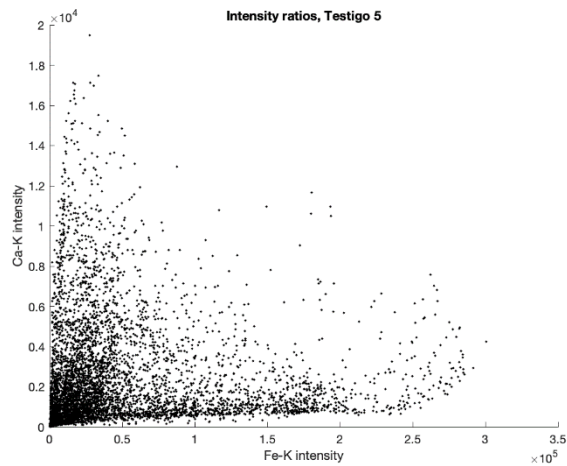
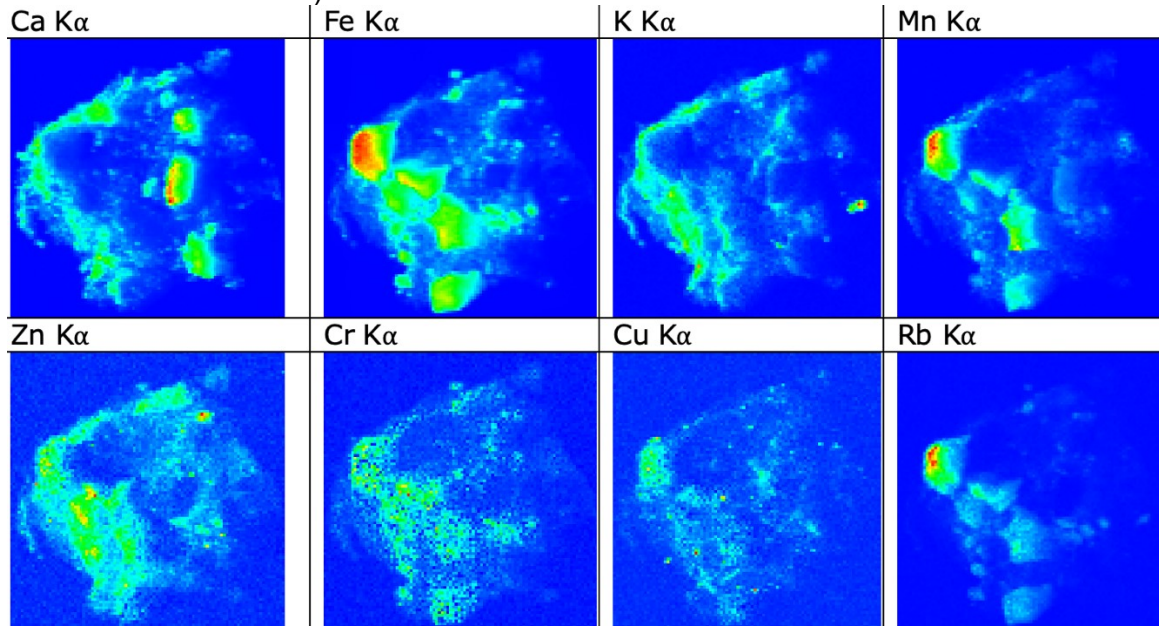
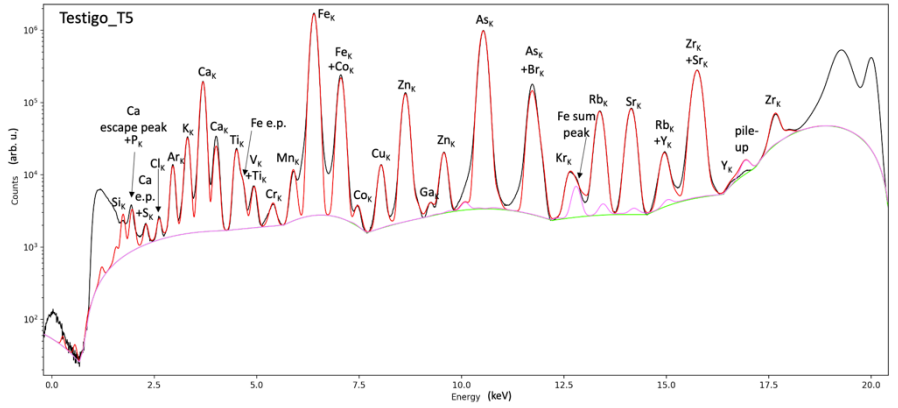
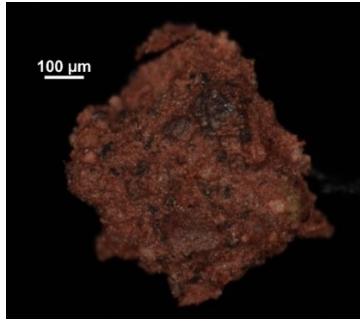
e)

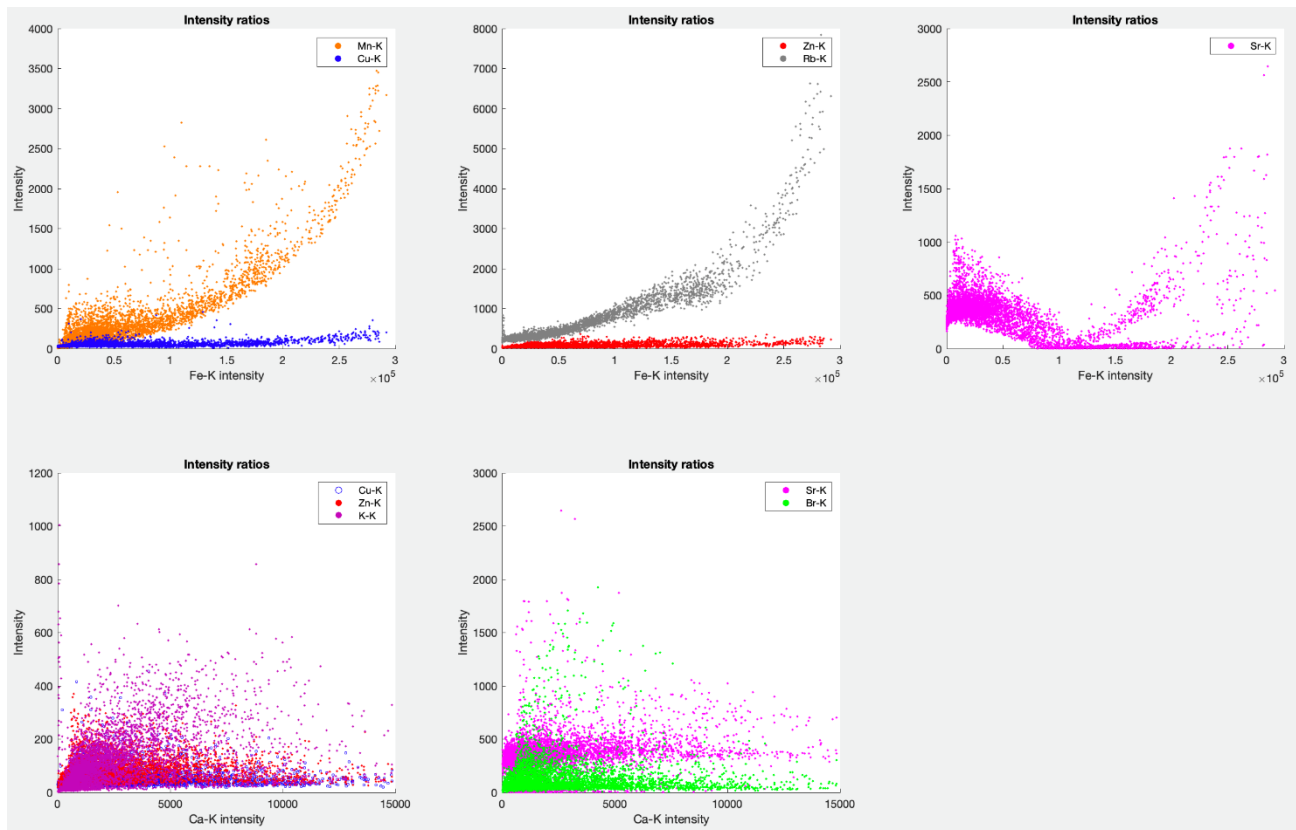
Fig. XI For sample ALT19: **a)** optical micrograph (x10 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

Sample Testigo 5

Representation: Claviform sign

Drop location name: (no name)





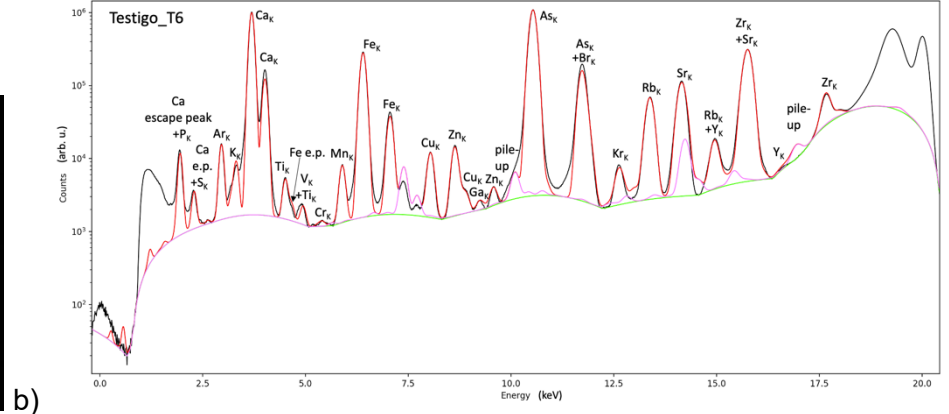
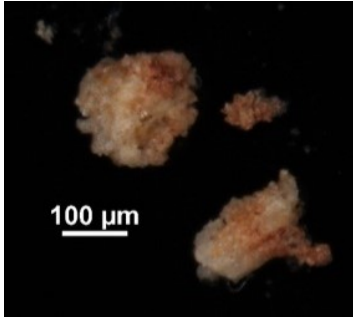
e)

Fig. XII For sample Testigo 5: **a)** optical micrograph (x10 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

Sample Testigo 6

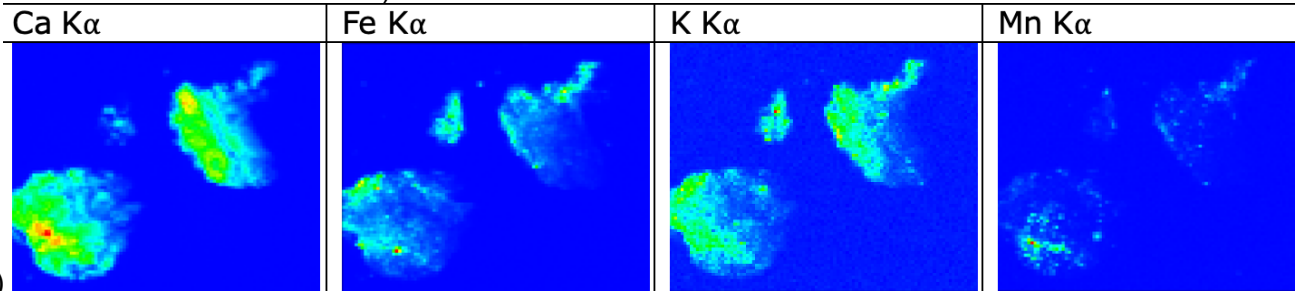
Representation: Claviform sign

Drop location name: (no name)

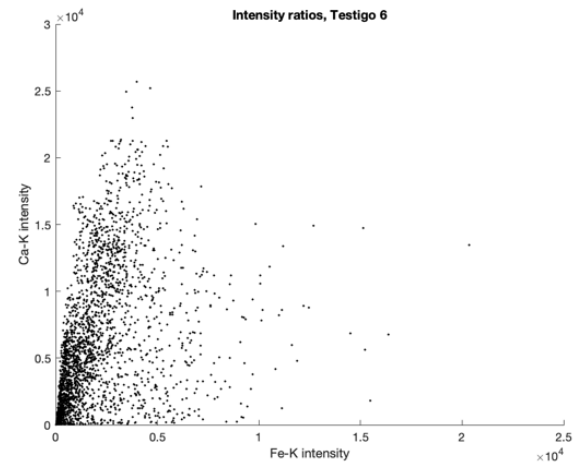


a)

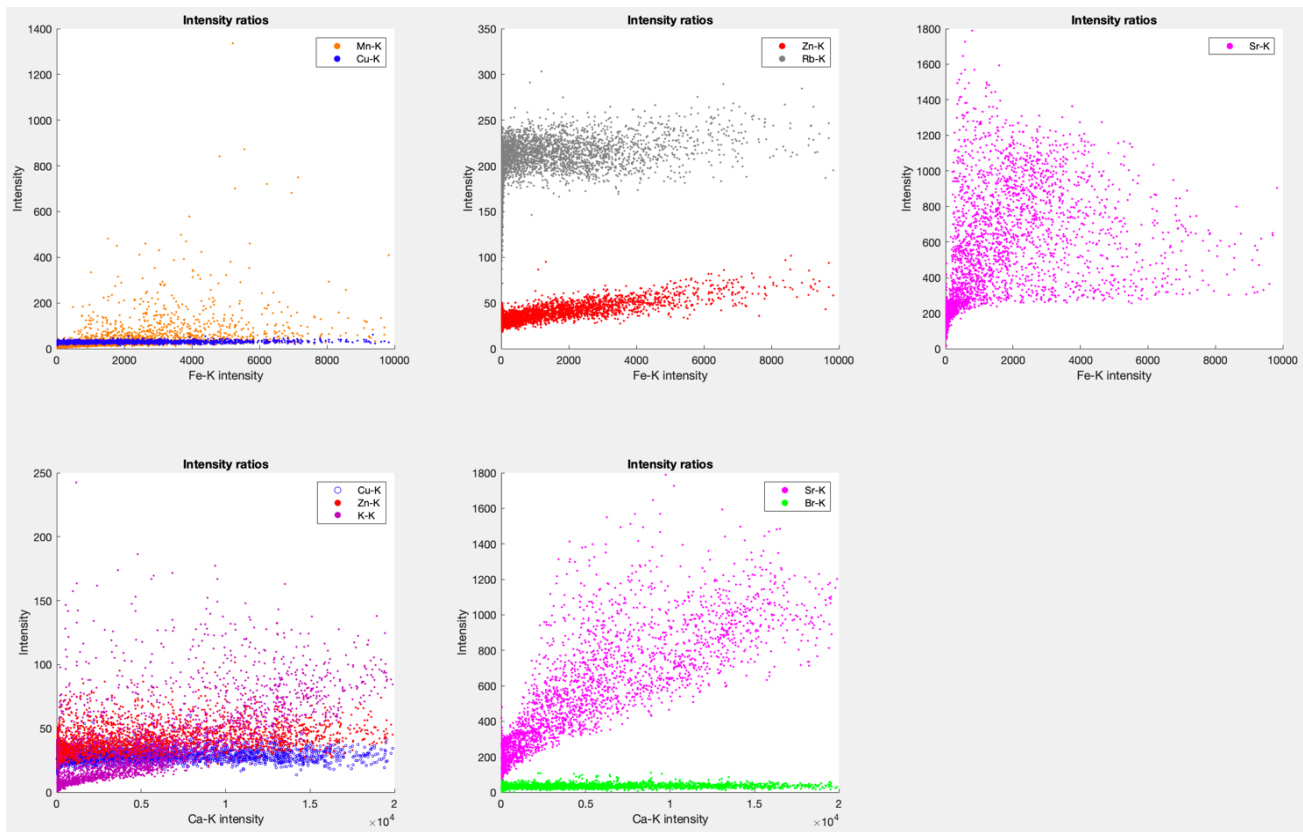
b)



c)



d)



e)

Fig. XIII For sample Testigo 6: **a)** optical micrograph (x10 magnification) of the sample, selected from its recipient and deposited over carbon tape © C2RMF Myriam Eveno, **b)** sum spectra of the SR- μ XRF spectra (one spectrum per pixel) of the analysis of the sample. In red, the PyMCA fit. The green and pink lines are the background of the spectrum taken into account by PyMCA when performing the fit, **c)** the corresponding chemical maps of several elements **d)** intensity of the Ca-K plotted against the intensity of Fe-K, for each pixel of the SR- μ XRF 2D scan, **e)** intensity of different elements plotted against the intensity of Fe-K or Ca-K, to find possible correlations.

Figure XIV presents the X-ray diffraction (XRD) spectra obtained using the ID22 beamline of the European Synchrotron Radiation Facility (ESRF) of a sample of red coloring matter from drop location ALT1_1. The black line represents the experimental XRD data collected from the sample. The superimposed colored lines correspond to the reference diffraction patterns for calcite (CaCO_3 , red), hematite (Fe_2O_3 , blue), and quartz (SiO_2 , green), obtained from the Crystallography Open Database (COD) with reference codes 1010928, 1011240, and 1011097, respectively. The spectra therefore suggests that significant amounts of calcite and hematite are present in the sample. Goethite, nevertheless, was not identified in this sample. The use of the ID22 beamline at ESRF, known for its high resolution and sensitivity, ensures precise and reliable data, enhancing the robustness of the analysis.

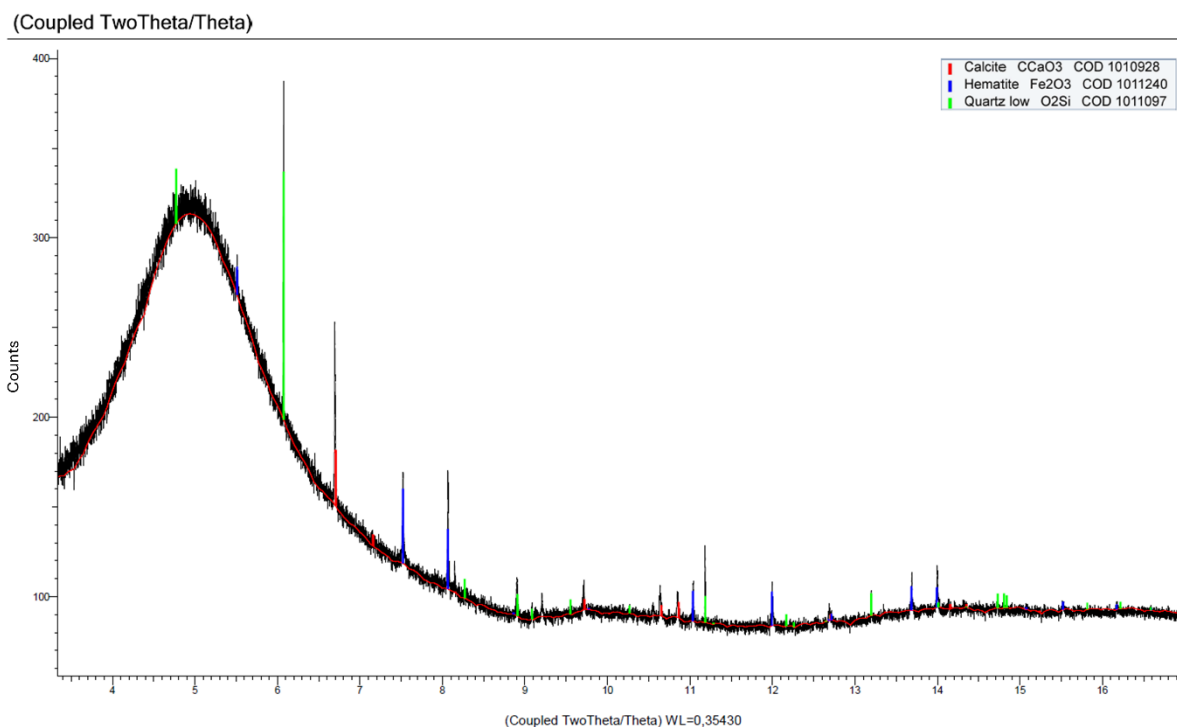


Fig. XIV XRD spectra obtained at the ID22 beamline of the ESRF synchrotron on a red sample from the ALT1_1 drop location. The black curve represents the experimental data, while the colored lines indicate the reference patterns for calcite (red), hematite (blue), and quartz (green) according to the Crystallography Open Database (COD). The experimental data shows peaks corresponding to these mineral phases, confirming their presence in the sample.

Table I displays the step size, acquisition time, and area of the SR- μ XRF maps.

Table I Step size, acquisition time and area parameters of the SR- μ XRF maps of the thirteen samples

Sample name	Step size (μm)	Acquisition time (s/point)	Area (mm^2)
ALT1_1A	10	0.6	0.5 x 0.5
ALT1_1B	10	1	0.24 x 0.24
ALT1_1C	10	1	0.4 x 0.4
ALT1_1	5	0.5	1 x 0.7
ALT1_2	10	1	0.2 x 0.58
ALT1_4	7	0.5	0.35 x 0.3
ALT12	10	0.7	0.7 x 0.6
ALT12_3	3	0.6	0.4 x 0.45
ALT12_4	5	0.5	0.1 x 0.07
ALT15	10	0.5	0.7 x 0.7
ALT19	5	0.5	0.22 x 0.21
Testigo 5	7	0.5	0.35 x 0.35
Testigo 6	5	0.5	0.25 x 0.2

Table II presents the presence of correlation between the intensity of different elements and that of Fe or Ca.

Table II Presence of correlation between the intensity of different elements and that of Fe or Ca. If the correlation is clear, it appears marked with "xx". If a slight correlation exists, "x". In cases of negative correlation (element decreases with increasing Fe or Ca), "↓". The main elements that seem to correlate with Fe and Ca appear in bold.

Sample (ALT_)	1_1	1_1A	1_1B	1_1C	1_2	1_4	12	12_3	12_4A	15	Testigo 6	Testigo 5	19
Correlation with Fe:													
Ti		x	x	x	x	x		x	x	x			
Mn	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	x	xx	xx
Co		xx	xx	xx	xx	xx	xx	xx		xx			
Cu	xx	x	xx	xx	x	x		xx				x	xx
Zn	x	xx	↓(xx)	x	xx	x		xx		x	xx		xx
Pb		x	xx	xx		xx	xx	xx		xx			
Rb	xx		xx	xx	x	xx	xx	xx		x		xx	xx
K	x	x	x	x	x	x		x		x	x		x
Sr	↓(x)	x	↓(xx)	↓(xx)	x		↓(x)	↓(x)				xx	x
Cr	x					x		x			x	x	x
Br		x	x	xx	x		x	x		x		xx	
Correlation with Ca:													
Mn		x			x			x				x	
Cu			x		x		x						
Rb								x				x	
K	x	x	xx		x			x	xx	x	xx		
Sr	x	xx	x		xx	x	x	xx	xx		xx		
Br							x					x	

Figure XV displays the score plots of different PCs. It complements Figure 5 in the article.

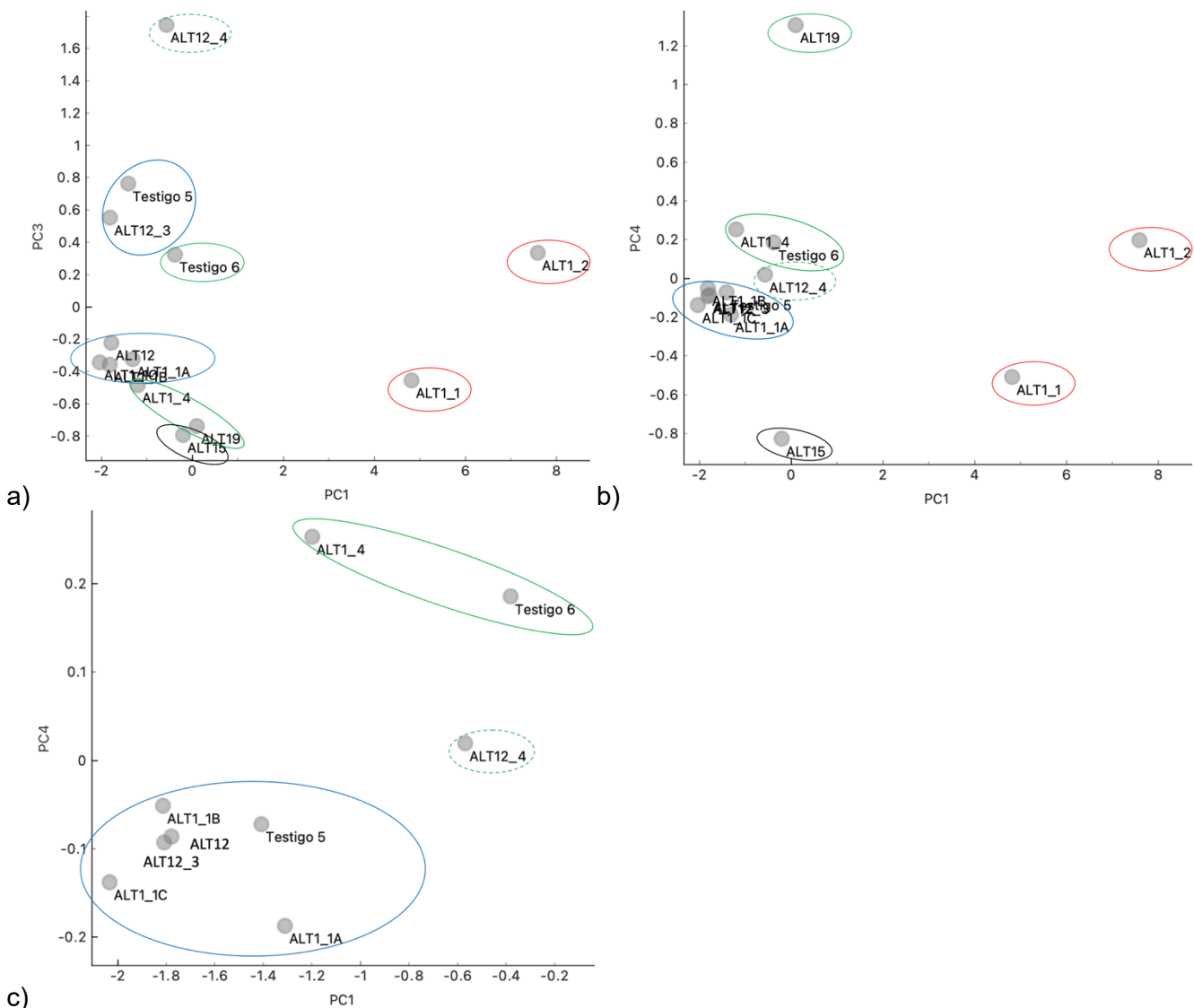


Fig. XV Score plots of different PCs with observed clusters marked (blue, green, red, black): **a)** PC1 vs PC3, **b)** PC1 vs PC4, **c)** zoom of PC1 vs PC4

Figure XVI illustrates a reduced version of the sum spectra for the thirteen samples from the eight locations studied, using the findings from Fig. 5 and the PCA analysis. Nearly identical or spectra presenting the same characterizing features are simplified, showing just one per group. This allows a simplified comparison: ALT1_1A, ALT1_1C and Testigo 5 have been simplified with ALT1_1B (two different figures); ALT19 and Testigo 6 with ALT1_4 (three different figures, although ALT19 could also be on its own); ALT12_3 and ALT12_4 with ALT12 (same drop location, ALT12_4 judged not representative); ALT1_1 with ALT1_2. ALT15 stands alone. The main differentiating features between the sum spectra are portrayed in red.

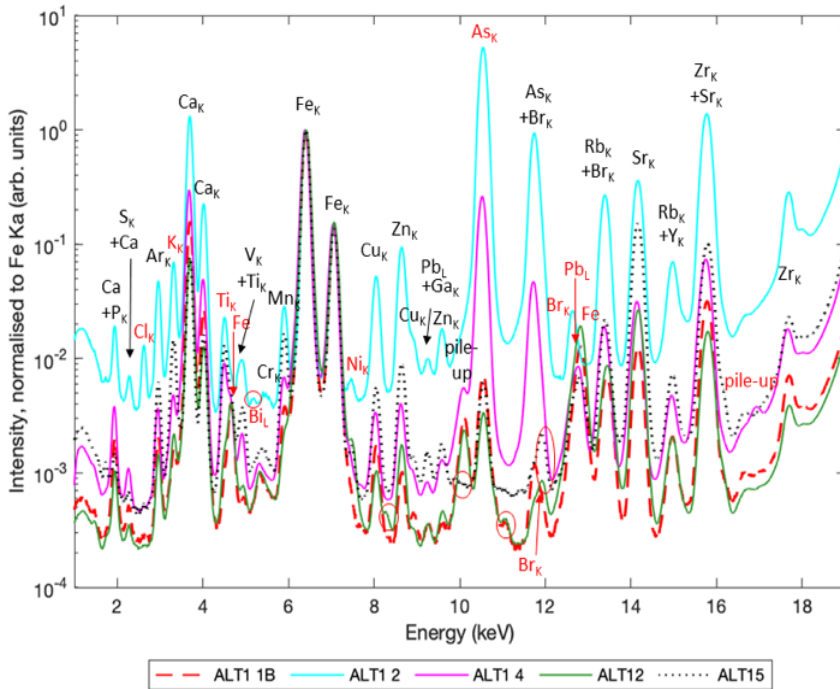


Fig. XVI Simplified representation of the thirteen sum spectra with samples ALT1_1B, ALT1_2, ALT1_4, ALT12 and ALT15. The characterizing differences arising are marked in red