

## Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:  
<https://www.esrf.fr/misapps/SMISWebClient/protected/welcome.do>

### Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

#### Experiment Report supporting a new proposal (“relevant report”)

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a “*preliminary report*”),
- even for experiments whose scientific area is different from the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as “relevant report(s)” in the new application form for beam time.

### Deadlines for submitting a report supporting a new proposal

- 1<sup>st</sup> March Proposal Round - **5<sup>th</sup> March**
- 10<sup>th</sup> September Proposal Round - **13<sup>th</sup> September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

#### Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

#### Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

### Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.



**Experiment title:** Characterization of sulfide precipitates related to acidophilic microorganisms: mineral neoformation and metal mobility with environmental implications.

**Experiment number:** ES-896

<b>Beamline:</b> BM20 ROBL	<b>Date of experiment:</b> from: 29 October 2020 at 08:00 to: 03 November 2020 at 08:00	<b>Date of report:</b> 09/02/2021
<b>Shifts:</b> 15	<b>Local contact(s):</b> Prieur Damien and Scheinost Andreas	<i>Received at ESRF:</i>

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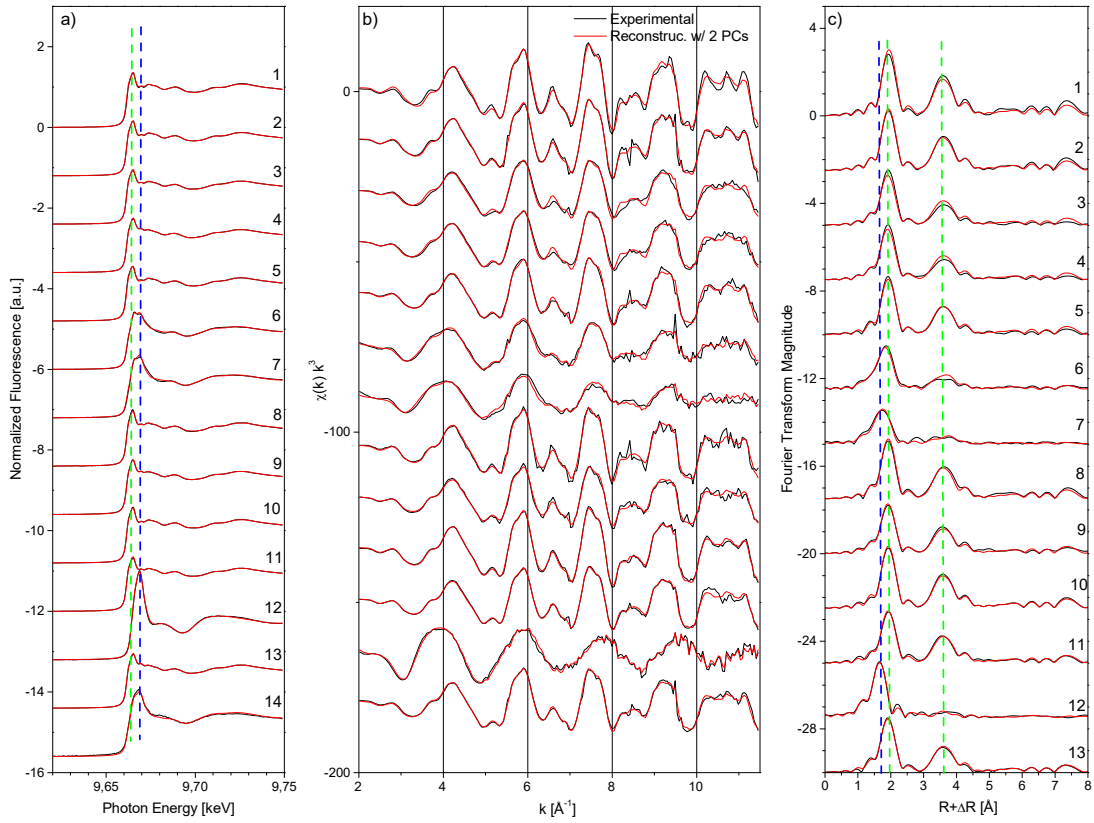
**Report:**

In this report we display the results of the experiment number ES-896 conducted in ESRF facilities between 29 of October and 3 of November of 2020. The experiment was focused on the identification of solid neoformed phase as a result of metal-microbe-mineral interaction in diverse acidic anaerobic environments. Samples were successfully prepared under anoxic conditions (glovebox) and shipped to ESRF in LN2.

As it was proposed, we managed to perform X-ray Absorption Spectroscopy (XAS) measurements (both XANES and EXAFS regions) of 16 samples at liquid He temperature. In particular we measured k-edges of Fe (30 spectra), Ni (21 spectra), Cu (28 spectra), Zn (21 spectra) and As (5 spectra). Unfortunately, the flux loss at higher energies coupled with the low concentration of Cd in our samples, made it impossible to obtain a good k-edge spectra with high enough relation of peak to background.

Moreover, for the mineral phase identification basing on spectral fitting, a total of 21 references of natural minerals were measured at corresponding elemental k-edges (10 Fe, 6 Cu, 2 Zn, 2 As and 1 Ni).

The results of Zn k-edge XAS spectra are summarized in the Figure 1. Data analysis revealed that all spectra are well reproduced by two principal components, which allowed us to assume that there are only two major Zn species present in all samples. Zn sulfide (wurtzite/sphalerite) has formed in larger amounts in all samples except for 7 and 12. Apparently, the range of crystallinity (i.e. the size of crystalline domains) in the sample 6 is smaller, compared to the rest of samples, which are representative of more evolved systems. In addition, as it was expected, the sulfide precipitation is progressively higher among samples 7, 4 and 5, being totally coherent with the treatment of each incubation column. Similar effect is observed in sample number 2 versus 11 and 12. Finally, we could conclude that these neoformed precipitates are rather stable to the oxidation by comparing the sample 11 and 13, which represent the same sample being treated in the glovebox and under oxic conditions respectively.



**Figure 1** Zinc K-edge XAS spectra of the incubation column samples (from #2 top to #14 bottom), along with a wurtzite reference (#1). Left: XANES spectra, center:  $k^3$ -weighted  $\chi(k)$  EXAFS spectra; right: corresponding Fourier transform magnitude. Black lines are the experimental spectra, red lines their reconstruction by two principal components. Blue hatched lines represent wurtzite/sphalerite characteristics, green hatched lines characteristics of Zn-O coordination.

On the other hand, in samples 7, 12 and 15 Zn is prevalently coordinated by oxygen, without a significant amount of long-range order, suggesting water coordination (e.g. outer sphere sorption complex) or less likely extremely disordered solid. The sample 6 shows an intermediate composition between sulfidic and oxide coordinated Zn. This shows that the ZnS neof ormation is more extended in the sediment and minor in the water column. The proportion of these components are shown in the Table 1.

#	SAMPLE	SULFIDE	OXIDE
1	Wurtzite	1.00	0.00
2	TincC	0.91	0.09
3	Bru21cC	0.69	0.31
4	BruShcR	0.69	0.31
5	BruShcC	0.80	0.20
6	BruSh2cCs	0.44	0.56
7	BruShcl	0.24	0.76
8	FC18cC	0.87	0.13
9	FCShcC	0.70	0.30
10	GuShcC	0.90	0.10
11	TincR	0.75	0.25
12	Tin2cR	0.00	1.00
13	TincRox	0.74	0.26
14	BruSh2cCw	nd	nd

**Table 1.** Zn K-edge XAFS samples and EXAFS-derived component speciation (ITT).

Regarding to Cu coordination, the results might be divided in three groups: Cu-O, Cu-S and Cu-Cu as shown in the Figure 2. The former is clearly detected in the sample 43 and corresponds to the same system as the sample 12 from the Figure 1. The absence of H<sub>2</sub>S, as a result of scarce sulfate reduction due to biological activity in the column, makes impossible the sulfide neoformation. This also corresponds to our previous observations under electron microscopy. Furthermore, the samples 61, 62, 63, 71 and 72 also show some Cu in coordination with O, likely showing that not all of the Cu was immobilized from the original waters.

The second group of samples, where Cu is coordinated to S could be subdivided in two groups. The first three sample show a very characteristic pre-edge feature in XANES, only observed in chalcopyrite reference pattern. Though the coexistence with other Cu sulfides should not be discarded. By contrary, samples 41 and 42 showed no pre-edge features and are more likely to be covellite or chalcocite Cu sulfides (CuS and Cu<sub>2</sub>S respectively). Samples 41 and 41-ox were prepared from the same original sample, being the former prepared in the glovebox and the latter in the normal oxidic environment. The aim of this duplication was to establish the stability of our sulfides to the oxidation and show us the high stability of neoformed sulfides to the oxidation. It is also worth mentioning that the range of order of sulfides in samples 41 and 42 is shorter, than that of samples 1, 2 and 3.

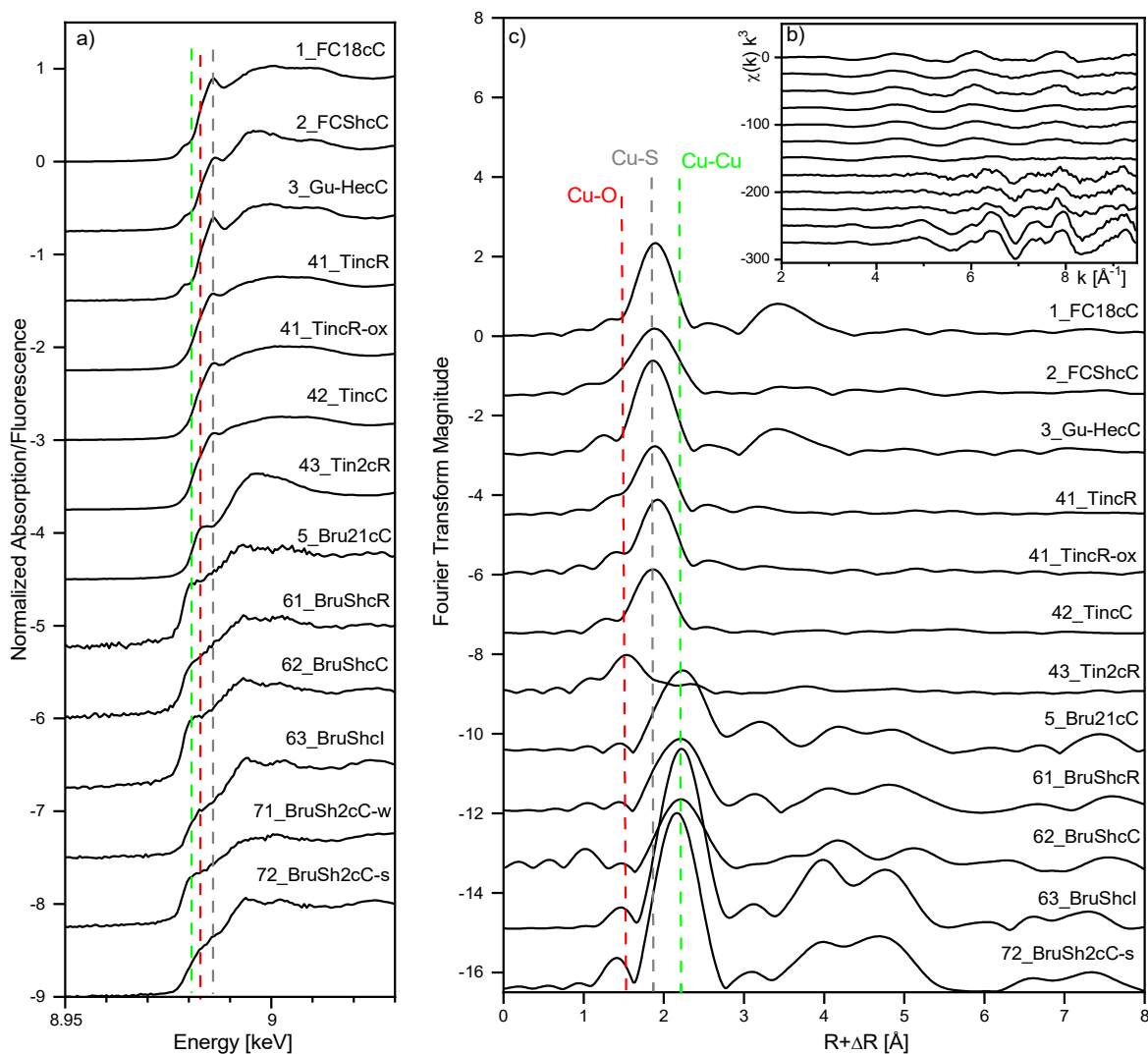


Figure 2.

Finally, the third group include samples 5, 61, 62, 63 and 72. It shows the prevalence of Cu-Cu coordination, most likely evidencing the precipitation of Cu as metallic Cu<sup>0</sup>. Interestingly, in the column with low bacterial activity we observe a higher Cu-Cu peak, compared with the same peak shown by the rest of the samples with higher bacterial activity. This behavior possibly evidences that the reduction might be mainly abiotic, perhaps as an environment response to the creation of very negative redox potential (ORP).

## **Conclusion**

We still have to work on the Ni, As and Fe data, but Zn and Cu k edge measurements already allow us to draw important conclusions on the sulfide neoformation in highly acidic anaerobic environments. Some spectra confirm our previous observations (e.g. under electron microscopy), but others suggest mineral phases, the precipitation of which was not spotted by other techniques. This evidences the great importance of XAS, being representative of the whole sample and not dependent on particle size, which is the weak spot of the microscopy.

The SXRD, included in the original proposal, was not completed due to reduction of granted shifts and therefore limited allocation time. Given the weak point of the identification of some mineral phases using XAS, along with constrains in the discrimination between two zinc sulfide polymorphs (i.e. between sphalerite and wurtzite), it will be interesting to run the diffraction experiment to clarify these uncertainties in future proposals.