Experiment Report Form

ESRF	**Experiment title:** Chemical and structural characterization of metal-organic materials obtained through heavy metal biosorption by exopolysaccharide-producing cyanobacteria	**Experiment number**: EV-514
Beamline: BM-08	**Date of experiment**: from: 04/07/2023 to: 10/04/2023	**Date of report**:
Shifts:18	**Local contact(s)**: Francesco D'acapito	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

XAS investigations at Cu, Ni, Zn K-edges were performed at the beamline BM08-LISA at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). Experiments were carried out in the first experimental hutch (EH1) adopting a non-focused beam (1mmx2mm ca) and Si(311) monochromator crystals. Estimated flux on the sample was ~ $8*10^9$ ph/s. In order to reduce the possibility of beam damage, measurements were performed in vacuum at 80 K using a cold finger LN2 cryostat (T 80 K). XAS spectra were acquired in fluorescence (cyanobacteria samples, summarised in table 1) mode with a photodiode or transmission (reference compounds, summarised in table 2) mode, under vacuum conditions.

In EH2 data on negative control (characterized by the presence of trace metals due to the presence of these elements in cyanobacteria cultivation medium) were collected by adopting a focused beam (150*150 μm^2) with a higher flux (~ $5*10^{10}$ ph/s) and changing the position of the beam during spectra acquisition. In EH2, for the collection of fluorescence spectra, a four-channel silicon drift detector (SDD) was used, in a vacuum chamber with cold finger cryostats at 80 K. A further reference chamber in EH2 was used to align and calibrate energy with a Cu, Ni and Zn foil.

The samples (cells or polysaccharides) were glued on carbon tips and covered with kapton foil to avoid sample's loss before being mounted on an aluminium sample holder. Cellulose pellets were prepared for reference compounds.

Repeated quick XANES spectra were performed on test samples at the beginning of the experiment to accurately check for beam damage. In Figure 1 the spectra acquired on one sample at Cu K-edge is shown as an example. Spectra of the samples were acquired with an energy increment of 1, 0.5, 0.15 eV in the pre-edge regions, with a constant increment of 0.5 eV in the edge region, and 0.05 eV in the post-edge region up to a maximum k value of 16 $Å^{-1}$.

Two-to-four spectra were collected and merged in order to optimize the signal-to-noise ratio. To calibrate energy and to average multiple spectra, ATHENA software was utilized. To extract structural EXAFS signals ($k \cdot \chi(k)$), standard procedures were followed: background removal, edge step normalization, and energy calibration.

XANES spectra and extracted EXAFS of the samples are shown below. This is a preliminary report: since the analysis of the spectra is still ongoing, the data will be updated in the following weeks.

Table 1. Sample summary

	Cyanobacteria	Fraction	Metal – K-edge
1	*D. salina* 16Som2 (16S)	Cells + bound EPS (C)	Ni
2	*D. salina* 16Som2 (16S)	RPS (P)	Ni
3	*D. salina* 16Som2 (16S)	Cells + bound EPS (C)	Cu
4	*D. salina* 16Som2 (16S)	RPS (P)	Cu
5	*D. salina* 16Som2 (16S)	Cells + bound EPS (C)	Zn
6	*D. salina* 16Som2 (16S)	RPS (P)	Zn
7	*D. salina* 16Som2 (16S)	Cells + bound EPS (C)	Control (Cu, Zn)
8	*D. salina* 16Som2 (16S)	RPS (P)	Control (Cu, Zn)
9	*D. salina* 16Som2 (16S)	Cells + bound EPS (C)	Multimetal (M) Ni,Cu,Zn
10	*D. salina* 16Som2 (16S)	RPS (P)	Multimetal (M) Ni,Cu,Zn
11	*Cyanothece* sp. CE4 (CE4)	Cells + bound EPS (C)	Ni
12	*Cyanothece* sp. CE4 (CE4)	RPS (P)	Ni
13	*Cyanothece* sp. CE4 (CE4)	Cells + bound EPS (C)	Cu
14	*Cyanothece* sp. CE4 (CE4)	RPS (P)	Cu
15	*Cyanothece* sp. CE4 (CE4)	Cells + bound EPS (C)	Zn
16	*Cyanothece* sp. CE4 (CE4)	RPS (P)	Zn
17	*Cyanothece* sp. CE4 (CE4)	Cells + bound EPS (C)	Control (Cu, Zn)
18	*Cyanothece* sp. CE4 (CE4)	RPS (P)	Control (Cu, Zn)
19	*Cyanothece* sp. CE4 (CE4)	Cells + bound EPS (C)	Multimetal (M) Ni,Cu,Zn
20	*Cyanothece* sp. CE4 (CE4)	RPS (P)	Multimetal (M) Ni,Cu,Zn

Table 2. Reference compounds summary

Cu k-edge	Ni k-edge	Zn k-edge
CuCl2	NiCl2	ZnCl2
Cu2O	NiO	ZnO
CuO	Ni3S2	ZnS
Cu(CH3COO)2	Ni(CH3COO)2	ZnSO4
Cu(CO3)2_Cu(OH)2		Zn(CO3)2_Zn(OH2)3
Cu2S		Zn(NO3)2
CuNO3		$ZnC_4H_6O_4$
CuSO4		

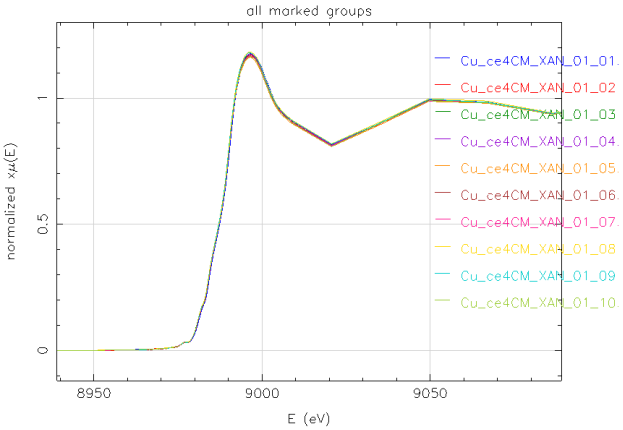
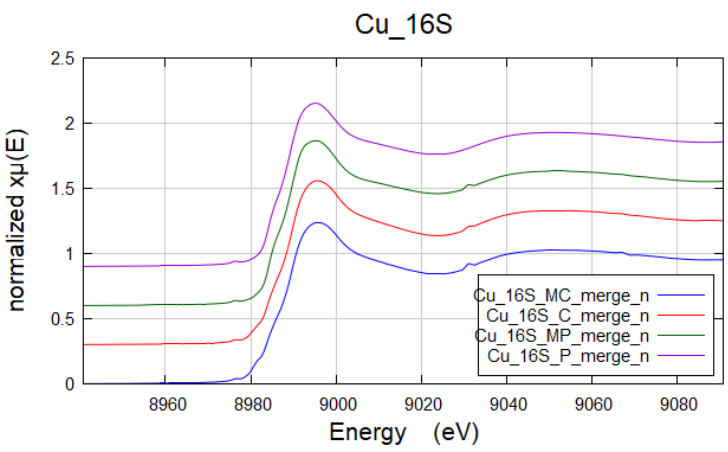
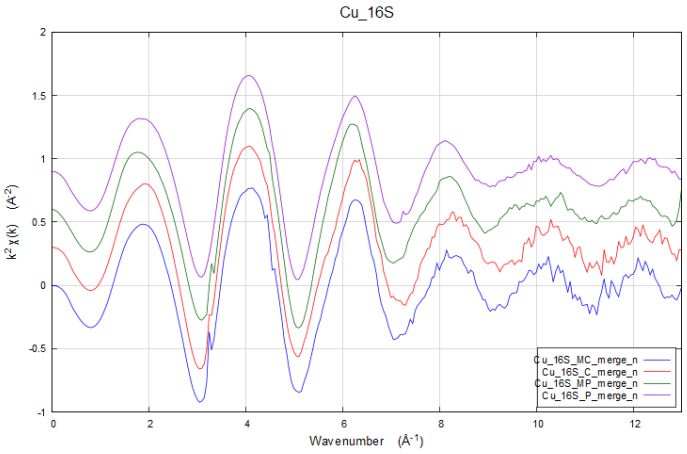


Figure 1 **Repeated short XANES spectra of Cu_CE4CM sample as example indicating the absence of beam damage**

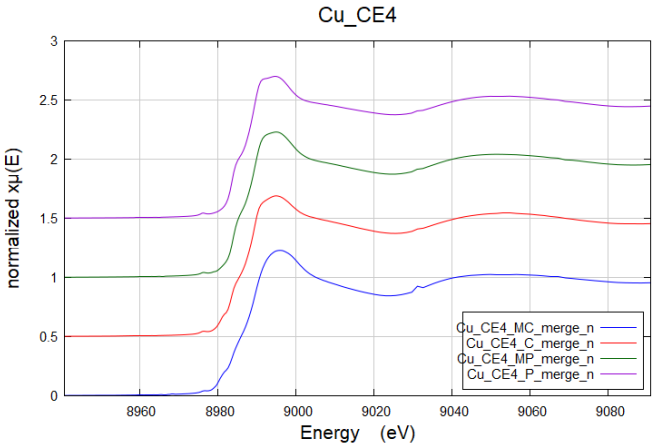
Cu K-edge

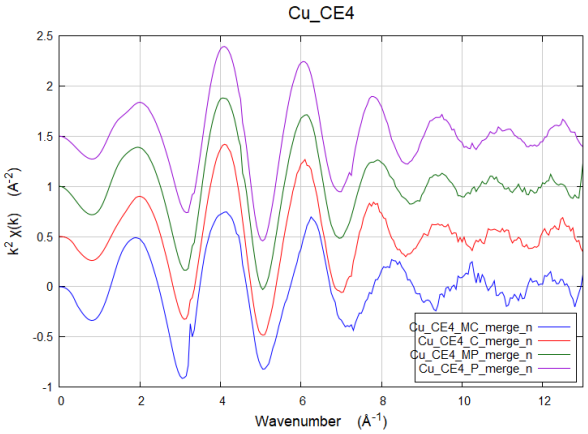
Strain 16Som2: XANES spectra on the left, EXAFS signal on the right ("M" means multi-metal sample, "C" means cellular sample, "P" means polysaccharidic sample)





Strain CE4: XANES spectra on the left, EXAFS signal on the right ("M" means multi-metal sample, "C" means cellular sample, "P" means polysaccharidic sample)

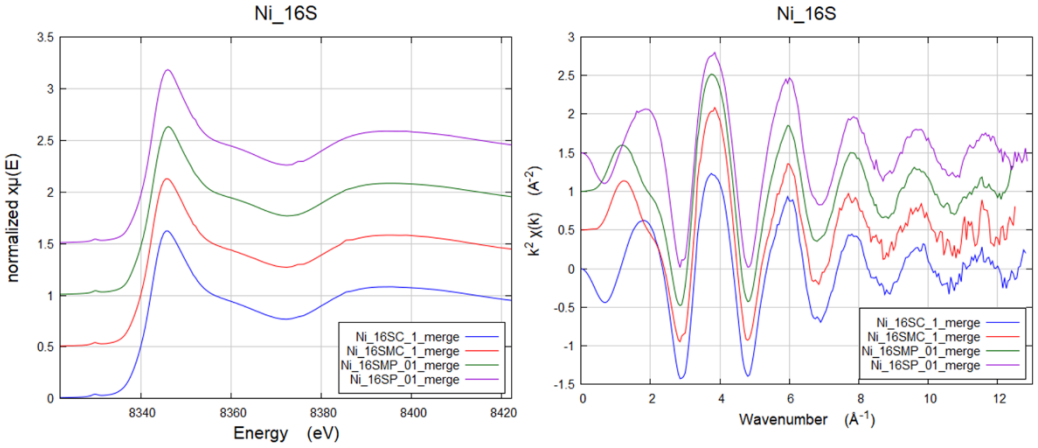




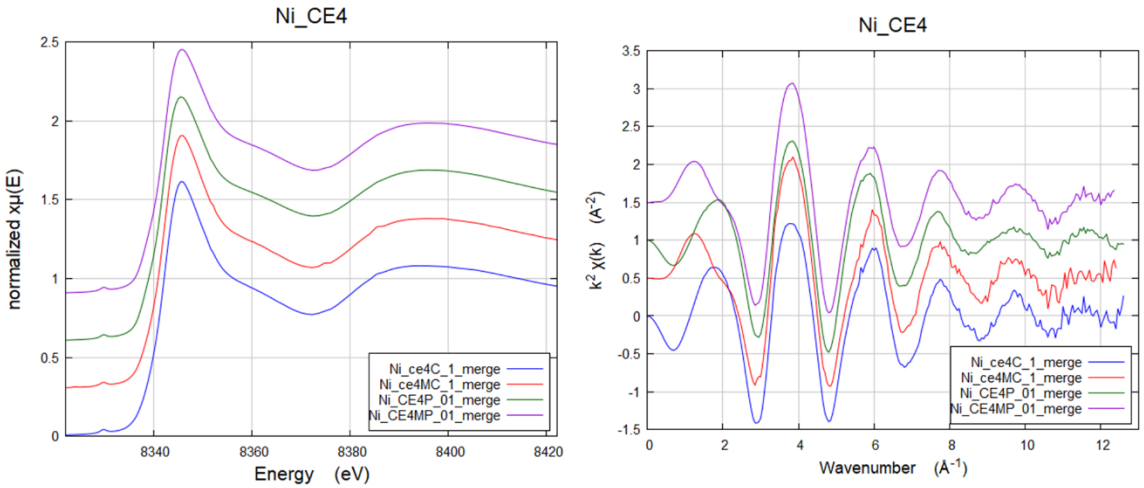
The presence of Cu, Ni, Zn simultaneously seems to affect the chemical environment of the samples. The major differences are visible in CE4 strain. Preliminary fitting on 16Som2 samples suggested the presence of oxygen in the first shell.

Ni K-edge

Strain 16Som2: XANES spectra on the left, EAFS signal on the right ("M" means multi-metal sample, "C" means cellular sample, "P" means polysaccharidic sample)



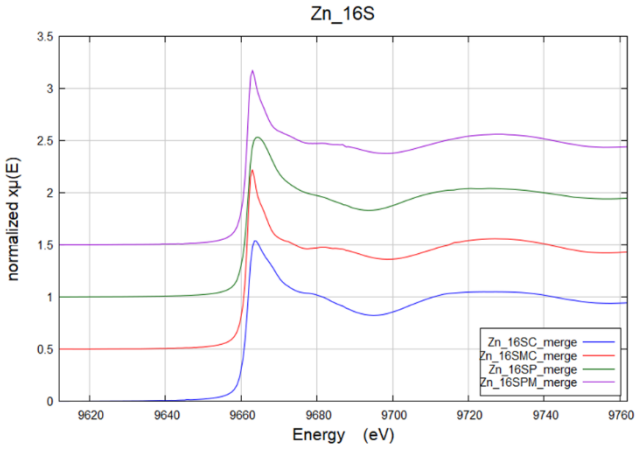
Strain CE4: XANES spectra on the left, EXAFS signal on the right ("M" means multi-metal sample, "C" means cellular sample, "P" means polysaccharidic sample)

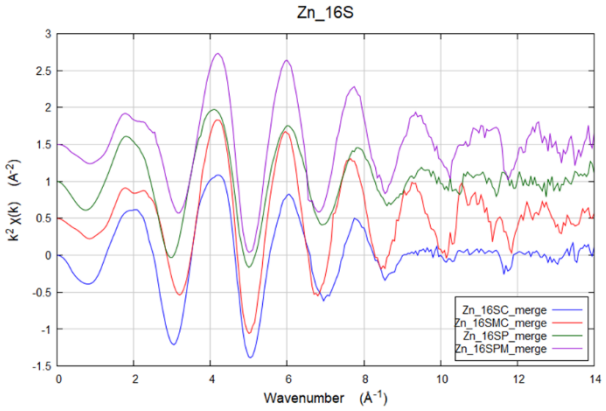


CE4 strain showed the major differences also in Ni samples compared to 16Som2, the main differences are visible comparing cellular and polysaccharidic fractions as well as single and multi-metal solutions.

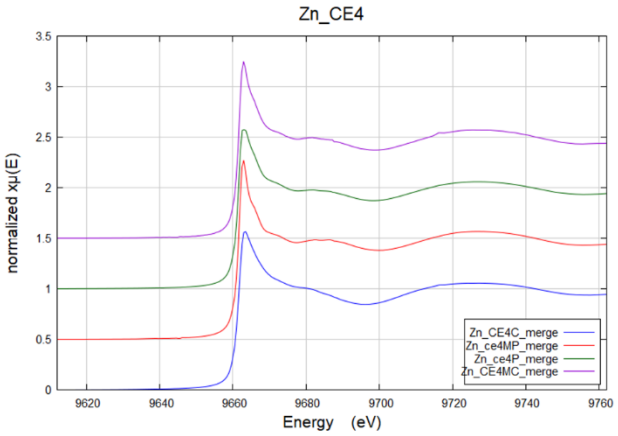
Zn K-edge

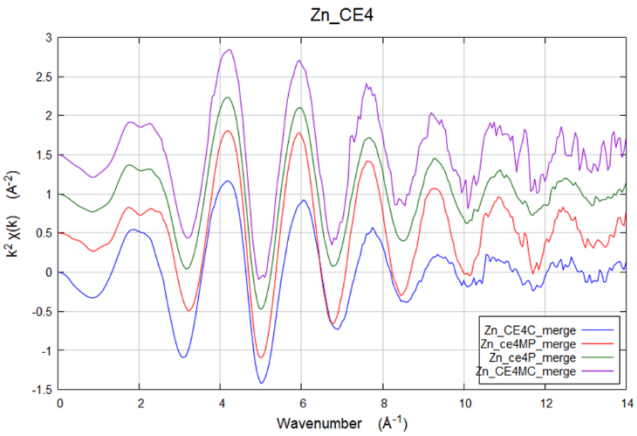
Strain 16Som2: XANES spectra on the left, EAFS signal on the right ("M" means multi-metal sample, "C" means cellular sample, "P" means polysaccharidic sample)





Strain CE4: XANES spectra on the left, EXAFS signal on the right ("M" means multi-metal sample, "C" means cellular sample, "P" means polysaccharidic sample)





Zn spectra are influenced by the strain, the fraction and the presence of the three metals simultaneously. Preliminary fitting on these samples suggested the presence of sulfur in the first shell.

Updated information will be added in the next weeks.