ESRF	Experiment title: **Arsenic oxidation and accumulation by *Euglena mutabilis* in acid mine drainage : XAS imaging of arsenic speciation.**	**Experiment number**: ME 978
Beamline:	**Date of experiment**: 28/01/2005 at 8:00 to 04/02/2005 at 8:00	**Date of report**: 18/03/2004
Shifts:	**Local contact(s)**: Olivier Proux, Jean-Louis Hazemann	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Guillaume MORIN*, Prof. François GUYOT* , Jennyfer MIOT * / IMPMC UMR7590 - Paris6&7 – IPGP, 140 rue de Lourmel 75015 Paris

Corinne CASIOT, Jean-Christian PERSONNÉ, Françoise ELBAZ -POULICHET / HYDROSCIENCES - UMR 5569, Université de Montpellier II

Report:

This report concerns the 15 shifts allocated on the BM30B beamline to run bulk Euglena samples at the As K-edge. Shifts requested in the project to perform X-ray imaging of the samples on ID22 were not allocated. EXAFS and XANES data were recorded at 10K at the As-K edge (11 869 eV) using a Si(220) monochromator at the BM30B/FAME beamline (Figs 1-3). Data were recorded in fluorescence mode using a 30 elements Ge - array detector completed by a 3 to 9 $\Delta\mu$ Ge filter to attenuate elastic scattering and Fe fluorescence from Fe-rich samples. The high flux at the sample allowed us to record good EXAFS data on very dilute samples (< 500 ppm wt. As; Fig. 2). Thanks to horizontal focusing, energy resolution was also about 0.5 eV. EXAFS and XANES data were recorded in step-scan mode after recording few quick-XANES spectra in order to check for unwanted photo-oxidation or reduction of the samples under the beam.

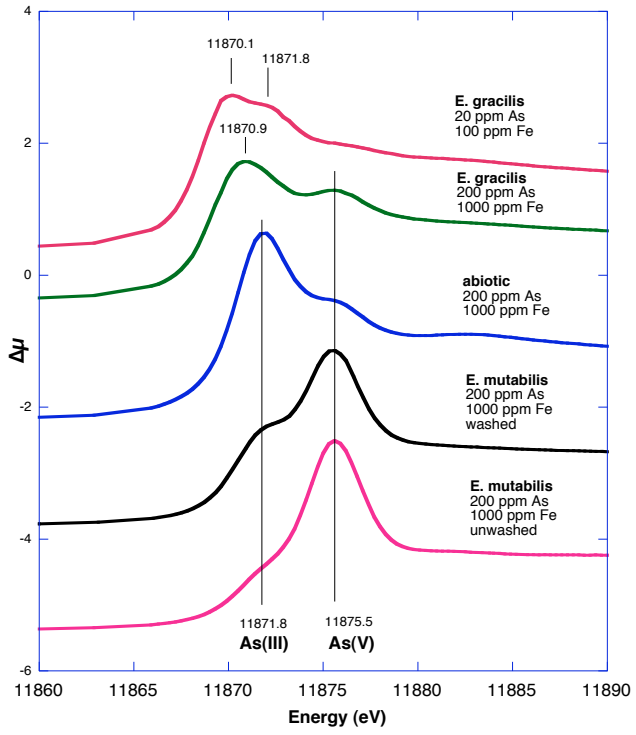

Figure 1. As-K XANES spectra of cell suspensions of *E. gracilis* and *E. mutabilis* grown in Fe^{II} and As^{III} bearing media.
In *E. gracilis,* absorption maximum at 11870.1 eV can be related to MeAsDMPS (monomethylarsenic 2,3-dimercapto-1-propane sulfonic acid) or to $As(Glu)_3$ (arsenic glutathione). Absorption maximum at 11871.8 eV can be related to As(III). Absorption maximum at 11870.9 eV can be related to MMA(III) (monomethyl arsonous acid) or to Me2AsDMPS (dimethylarsenic 2,3-dimercapto-1-propane sulfonic acid). (Smith et al. 2005)
In *E. mutabilis,* absorption maxima at 11871.8 and 11875.5 eV correspond to As(III) and As(V) respectively.

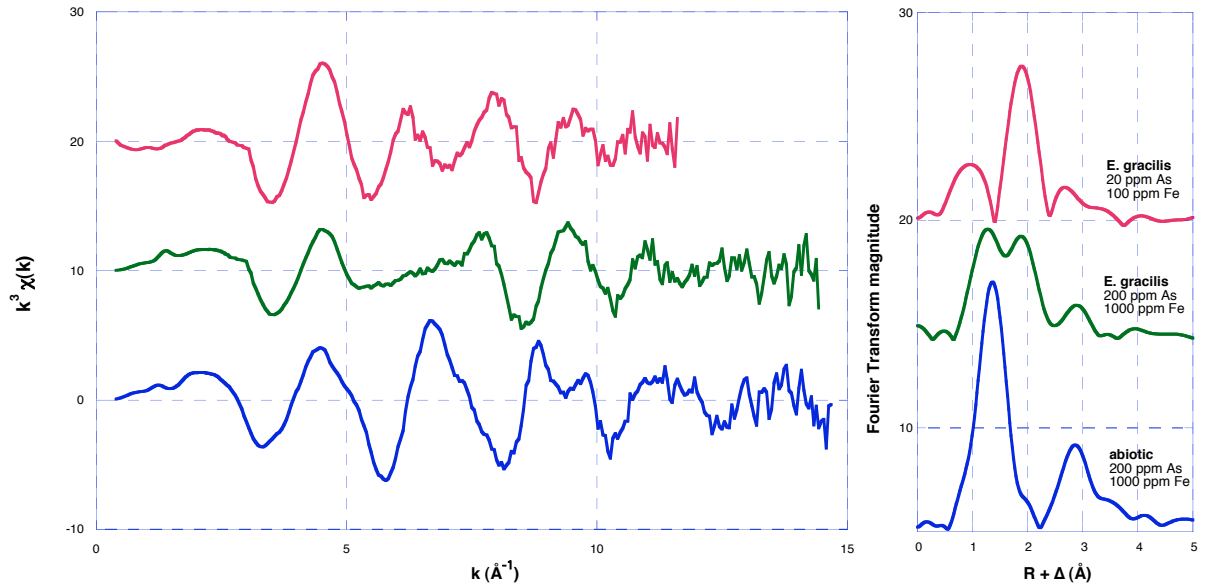

Figure 2. Comparative plot of EXAFS data on cell suspensions of E. gracilis grown in 100 ppm Fe and 20 ppm As^{III} E. gracilis grown in 1000 ppm Fe and 200 ppm As^{III}, and abiotic precipitate from the 1000 ppm Fe and 200 ppm As^{III} solution.

Preliminary shell-by-shell analysis of the EXAFS signal indicates dominant contribution of As-S bonds in *E. gracilis* grown in 20 ppm As(III) and 100ppm Fe medium, which is consistent with metabolization of arsenic as $As(Glu)_3$ and DMPS compounds. The additional presence of inorganic As(III) in *E. gracilis* grown in 200 ppm As(III) and 1000ppm Fe medium is related to the mixture with amorphous abiotic precipitate.

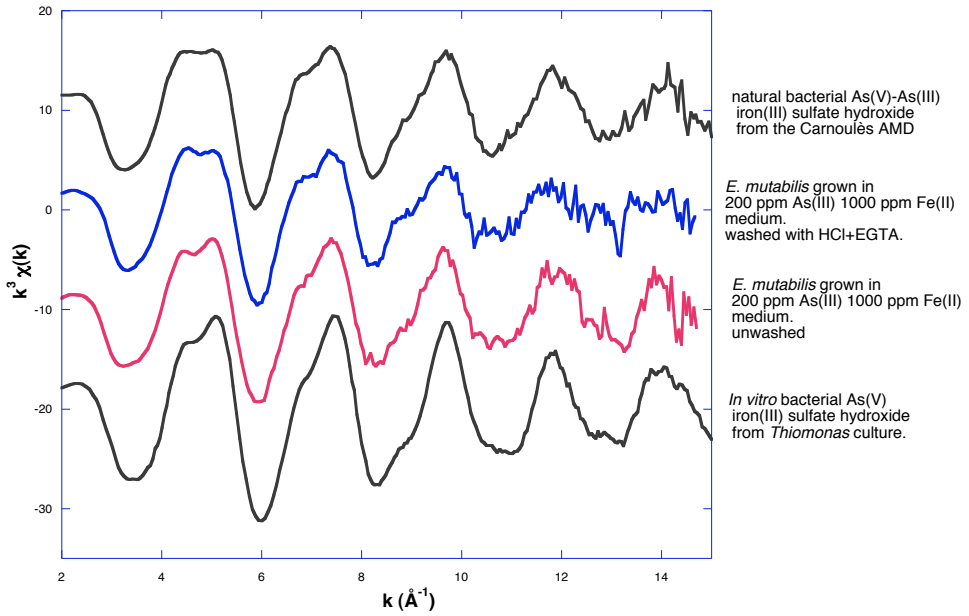

Figure 3. EXAFS data on cell suspensions of *E. mutabilis* grown in Fe^{II} and As^{III} rich media compared with EXAFS data of bacterial precipitates. The latter correspond to poorly ordered mixed As(III)-Fe(III) and As(V)-Fe(III) iron(III) sulfate oxy-hydroxides.

Similarity of the As-EXAFS spectra of *E. mutabilis* with those of bacterial Fe(III)-As(III) and Fe(III)-As(V) sulfate oxyhydroxides suggests that the major part of As(III) is co-precipitated in such mineral phases. These compounds have been already identified as products of the activity of *Acidithiobacillus ferrooxidans* and *Thiomonas* sp. (Morin et al. 2003; Duquesnes et al. 2003). These strains are abundant in the Carnoulès acid mine drainage from which the *E. mutabilis* were collected. The difficulty to separate bacteria from *E. mutabilis* cells upon the isolation procedure might explain the difference in arsenic speciation in *E. gracilis* and *E. mutabilis* samples. This result enlightens the major role of bacteria in detoxifying the Carnoulès water. Imaging arsenic speciation at the micron scale in both species would yield crucial information about the mechanisms of arsenic metabolization and sequestration.

Smith et al. (2005) *Environ. Sci. Technol.* 39, 248-254.
Morin et al. (2003) *Environ. Sci. Technol.* 37, 1705-1712.
Duquesne et al. (2003) *Appl. Environ. Microbiology* 69/10, 6165-6173.