ESRF	**Experiment title:** Interaction between bacteria and uranium in natural soils: population diversity and biotransformation mechanisms	**Experiment number**: 30-02-948
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Introduction

Uranium (U) is a chemical toxicant for Humans, naturally present in soils but also susceptible to be disseminated by industrial activities. In France the Bessine region, in Haut Limousin, has been identified as a contaminated region in which soils contain high concentrations of this toxicant, which then may be transferred to the foodchain. Soil plays a key role in toxic metals bioavailability, by modulating their transfer to surface waters and plants. These transfer properties depend on multiple parameters and among them the presence of bacterial populations.

This experiment is part of an ANR project (EC2CO 2008-2010), CYTRIX, whose purpose was to evaluate the potential role of soil bacteria in complex phenomena of immobilization or transfer of U to the foodchain. In parallel, the influence of U concentration on the structure of bacterial communities was investigated. The project is based on the analysis of Bessine region soils, sampled around the areas of Villard and Vénachat, which contain high concentrations of uranium (15000 ppm and 4700 ppm respectively). A library of bacterial strains, isolated from these soils, has been constructed. Some of them have been identified as very resistant to U, since they are able to grow in a medium containing up to 5 mM of this element. One of the mechanisms explaining this resistance can be their ability to modify U speciation, which would be the demonstration of their ability to modify uranium bioavailability in soils. The aim of this experiment was to analyze U speciation in these soil, and to identify any speciation modification due to bacterial activity.

Experimental method

For the analysis of soil samples, 5-10 mg of each soil was homogenized and pressed as 5-mm pellet. Bacteria isolated from the U-rich soils were grown in classical culture medium and exposed to 0.5-2 mM of U (uranyl acetate), prepared in culture medium. Some of these bacteria were also exposed to U prepared in water. At the end of the exposure period, bacteria were collected by centrifugation and freeze-dried. These samples will be pressed as 5-mm pellets, which were packed and sealed. U exposure solutions were also sampled, and placed in quartz capillaries for analysis.

XANES and EXAFS spectra of these samples were collected at U L_{III} edge on BM30B beamline, in fluorescence mode, using the 30 elements solid state Ge detector (Canberra). 3-8 spectra (depending on the uranium concentration of the sample) were recorded for each sample and averaged to improve the statistics.

EXAFS oscillations were isolated from the raw, averaged data by removal of the pre-edge background, approximated by a first-order polynomial, followed by μ_0-removal *via* spline fitting techniques using Athena software. The resulting EXAFS curves in the wavevector (k) space were weighted by k^3 and qualitatively compared to reference curves.

Results

As shown in Figure 1A, U speciation can be identified by qualitative observation of k3 weighted EXAFS spectra, particularly in the region 6.5-9 $Å^{-1}$. U-carbonate and U-malate can be identified be a characteristic beat at 6.6-6.8 $Å^{-1}$. In U-phosphate reference compound, this region shows the typical feature, sharper than in other compounds, with a maximum around 7.4 $Å^{-1}$. Finally, UO_2^{2+} and U-citrate (pH1) also show characteristic feature with a maximum at 7.9-8.1 $Å^{-1}$. The EXAFS spectrum of U-citrate is modified when pH is increased, U-citrate pH1 spectrum is very similar to UO_2^{2+} spectrum while U-citrate pH7 shows a smoother feature in the region 6.5-9 $Å^{-1}$. Altough these conclusions are only qualitative, they permit to draw first conclusions on U speciation in soils and bacteria. Moreover these reference spectra have high similarities with thodse described in the literature. Data adjustement is under process.

Comparison of soil spectra (Figure 1B) with the spectra of these reference compounds led to the conclusion that, in Villard, U speciation is related to U-phosphate. Moreover, small yellow precipitates were extracted from this soil, and directly analyzed. The EXAFS spectrum of these precipitates is closely related to Villard soil spectrum, and even more closely related to U-phosphate spectrum. In a first glance, we can thus conclude that in this soil, U is in the U-phosphate speciation. In the soil from Vénachat area, the speciation is more complex, and cannot be directly compared to one reference compound. A deeper data processing is thus required in order to draw first conclusions.

Finally, the first conclusion which can be drawn from qualitative inspection of bacterial EXAFS spectra (Figure 1C) is that all the strains seem to have accumulated U in the same speciation as U speciation in exposure medium (Figure 1C, black curve), and this chemical form is related to U-phosphate.

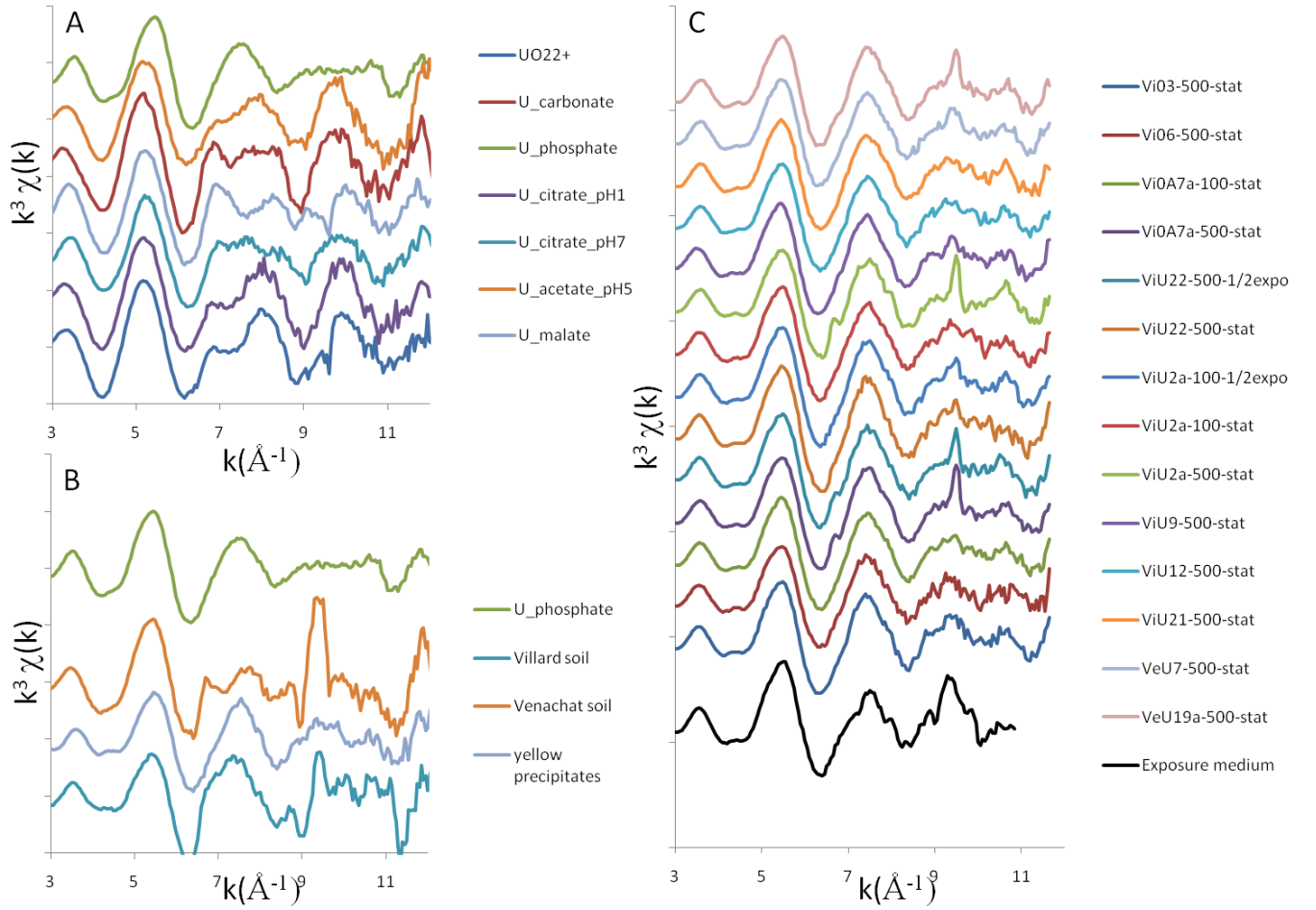

Figure 1. U LIII-edge k3 weighted EXAFS spectra of reference compounds (A), soil samples (B) and bacteria exposed for 24h to U prepared in culture medium (C).

One hypothesis permitting to explain that all bacterial strais have accumulated U-phosphate is that U may have precipitated as U-phosphate in exposure medium (the latter containing a high concentration of phosphate), this precipitates may thus be adsorbed on baterial walls. Consequently these adsorbed precipitates may mask minor U chemical forms accumulated in bacteria during U metablization process. To confirm this hypothesis, two of these bacterial strains were also exposed to U prepared in watre rather than

in exposure medium. EXAFS spectra of the resulting bacterial pellets is shown in Figure 2B. Two exposure pH were tested, pH1 and pH7. A first interesting point is that the two strains do not exhibit the same U speciation transformation. Secondly U speciation in ViOA7a differs when bacteria are exposed at pH1 as compared to exposure at pH7. EXAFS spectrum of Vi0A7a at pH1 is related to UO22+ or U-citrate pH1; at pH7 it is related to U-phosphate. EXAFS spectrum of ViU22, at pH1 and pH7, is more related to U-citrate pH7. Fitting of the EXAFS structural parameters, which is presently under process, will permit a better interpretation of these results.

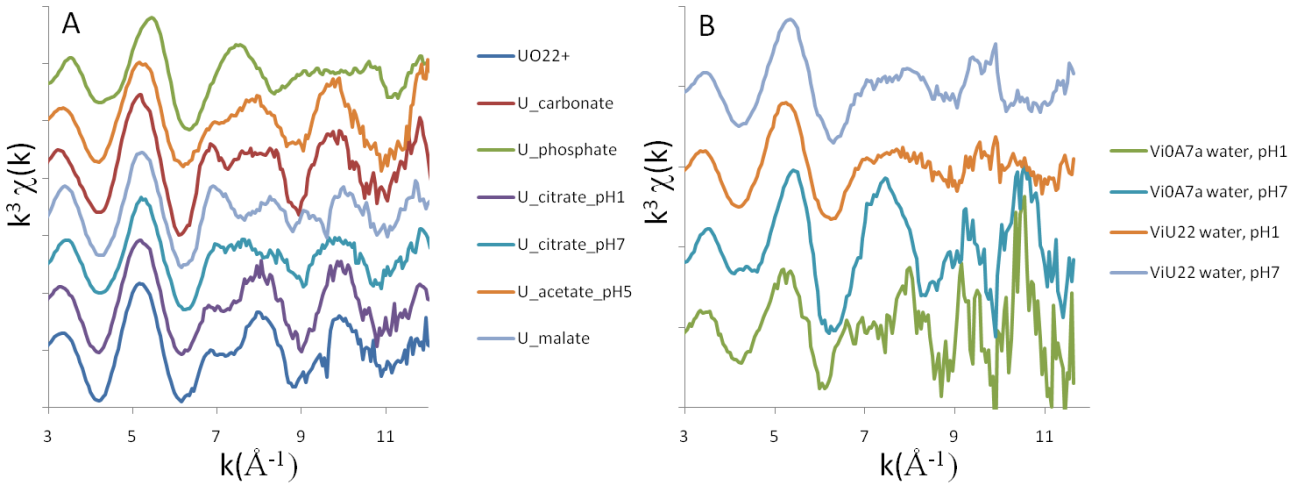

Figure 2. U LIII-edge k3 weighted EXAFS spectra of reference compounds (A) and bacteria exposed for 24h to U prepared in water, either acidified (pH1) or neutral (pH7) (C).

Conclusions and perspectives

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