EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

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://wwws.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 form 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

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th 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: Uranium interaction with plant cells	Experiment number: 20-01-814
Beamline: BM20	**Date of experiment**: from: 12.02.21 to: 16.02.21	**Date of report**: 09.11.21
Shifts: 12	**Local contact(s)**: André Rossberg, Stephen Bauters, Damien Prieur	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Jenny Jessat

Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, Bautzner Landstr. 400, 01328 Dresden, Germany

Report:

For the safety assessment of a potential nuclear waste repository, accident scenarios have to be considered in which radionuclides can be released, transported via groundwater and interact with the biosphere, including plants. To understand these interactions in more detail, suspension plant cell cultures with model organisms such as *Brassica napus* (*B. napus*, canola), which is a representative of typical crops in Europe, are suitable. The reduction and binding of uranium(VI) by *B. napus* plant cells was investigated with high energy-resolution fluorescence detection X-ray absorption near-edge structure (HERFD-XANES) spectroscopy and extended X-ray absorption fine structure (EXAFS) spectroscopy. These methods allow insight into the oxidation states and the chemical environment of the cell-associated uranium, respectively.

B. napus cells were incubated with 20, 100, and 200 µM U(VI). After 1, 24, 48, and 72 h they were separated from the supernatants and washed. Like cultivation and incubation, sample preparation was performed aerobically. For HERFD-XANES measurements, the biomass was transferred into polyethylene sample holders sealed with Kapton tape. The biomass for EXAFS measurements was transferred into a 3 mm thick polyethylene double confined sample holder. All data were collected on samples under cryogenic conditions.

Previous experiments showed that uranium is immobilized by interaction with plant cells. [1] At the same time, there was evidence of formation and/or release of metabolites that could complex U(VI). In the literature, a transient reduction of U(VI) by *B. napus* plant cells was observed and linked to the glutathione metabolism. [2] The HERFD-XANES measurements performed here showed that there are indications of a reduction of U(VI) to U(V) and U(IV) to a small extent, supporting the results of the mentioned study. The obtained results also suggest that the reduction of U(VI) to U(IV) occurs indirectly via U(V) by a single electron transfer and a subsequent disproportionation of U(V) to U(VI) and U(IV). Furthermore, with the help of EXAFS measurements, the insights into the binding of U(VI) to plant cells via organic-phosphate binding motifs were deepened.

For evaluation of the EXAFS spectra a shell fit **(**Figure 1) was performed and the EXAFS shell fit parameters for the plant cells exposed to 20 and 200 µM U can be found in **Table 1**. For a structure assignment reference spectra for the U(VI) interaction with various biological systems were used: adenosine monophosphate (AMP) and adenosine triphosphate (ATP) [3], fructose-1,6-bisphosphate [4], meta autunite [4–6], as well as structural data for bacteria and plants (root).

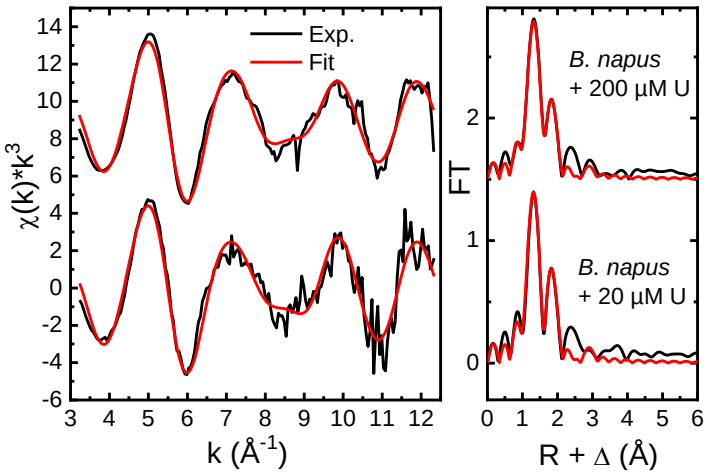

Figure 1: U L_{III}-edge k^3-weighted EXAFS spectra (left) and corresponding Fourier transforms (right) for both *B. napus* cell samples.

Table 1: EXAFS shell fit parameters for *B. napus* cells exposed to 20 and 200 µM U.

Path	N	R (Å)	σ^2 (Å^2)	ΔE_0 (eV)
		Cells + 20 µM U		
U-O$_{ax}$	2*	1.773(3)	0.0014(2)	1.1(9)
MS-O$_{ax}$	2/	3.546/	0.0028/	1.1/
U-O$_{eq}$	5*	2.321(7)	0.0081(7)	1.1/
		Cells + 200 µM U		
U-O$_{ax}$	2*	1.776(3)	0.0020(2)	0.5(7)
MS-O$_{ax}$	2/	3.552/	0.0040/	0.5/
U-O$_{eq}$	5*	2.306(7)	0.0091(5)	0.5/

*: fixed parameter, /: linked parameter, N: coordination number, R: radial distance, σ^2: Debye-Waller factor, ΔE_0: shift in energy threshold, MS: multiple scattering. Estimated standard deviations of the variable parameter as given from EXAFSPAK in parenthesis.

A clear structural assignment was not possible. For both cell samples the best match was found with reference spectra for the binding of U(VI) to lupine roots [7] and AMP [3]. From this information, it can be concluded that the binding of U(VI) to *B. napus* cells occurs via organic-phosphate binding motifs. This is in agreement with the expectation, since U has a high affinity for P. Moreover, these data fit previous spectroscopic results with *B. napus* callus cells. [8]

A manuscript summarizing the results of this study is in preparation, but will be supplemented beforehand with further analytics on metabolite release (HPLC, MS, NMR) and proteomics studies. The submission of this manuscript is planned for 2022.

References:

[1] J. Jessat, S. Sachs, H. Moll, W. John, R. Steudtner, R. Hübner, F. Bok, T. Stumpf, *Environ. Sci. Technol.* **2021**, DOI 10.1021/acs.est.0c05881.

[2] K. Viehweger, G. Geipel, G. Bernhard, *Biometals* **2011**, *24*, 1197–1204.

[3] C. Hennig, G. Geipel, G. Bernhard, *EXAFS Investigation of Uranium(VI) Adenosine Phosphate Complexes*, Forschungszentrum Rossendorf, Dresden, **2003**.

[4] A. Rossberg, T. Abe, K. Okuwaki, A. Barkleit, K. Fukuzawa, T. Nakano, Y. Mochizuki, S. Tsushima, *Chem. Commun.* **2019**, *55*, 2015–2018.

[5] C. Hennig, P. J. Panak, T. Reich, A. Roßberg, J. Raff, S. Selenska-Pobell, W. Matz, J. J. Bucher, G. Bernhard, H. Nitsche, *Radiochim. Acta* **2001**, *89*, 625–632.

[6] C. Hennig, G. Reck, T. Reich, A. Roßberg, W. Kraus, J. Sieler, *Zeitschrift für Krist. - Cryst. Mater.* **2003**, *218*, 37–45.

[7] A. Günther, G. Bernhard, G. Geipel, T. Reich, A. Roßberg, H. Nitsche, *Radiochim. Acta* **2003**, *91*, 319–328.

[8] H. Moll, S. Sachs, G. Geipel, *Environ. Sci. Pollut. Res.* **2020**, *27*, 32048–32061.