ESRF	**Experiment title:** Uranium speciation in plants	**Experiment number**: 30-02-857
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Aims of the experiment and scientific background

Uranium is a naturally occuring heavy metal. Uranium contamination of surface soils mainly results from the use of phosphate fertilizers but also development of the nuclear industry and military applications. Plants have a remarkable ability to absorb and accumulate metals and organic compounds from soil, water, and air. Over the last 10 years, there has been increasing interest in developing plant-based technologies (phytoremediation methods) to remediate soils contaminated with heavy metals and radionuclides. Current efforts are notably focusing on agronomic plant species which can produce a lot of biomass while accumulating large amounts of heavy metals. Among the Brassicacees family (including oilseed rape), numerous species have been identified as accumulators, and can be potentially used in phytoextraction. On the other hand, plants with large root system, like sunflower, are used to remove metals from water by rhizofiltration.

It is established that U bioavailability for plants depends on the speciation, which is modulated by pH and the presence of ligands such as phosphate, carbonate or organic acids. However, knowledge about U speciation *intra planta* and its modification during plant translocation are scarce. EXAFS has been used to determine uranium speciation after uptake in lupine plants [1]. This study indicates a transformation of U speciation when accumulated in the plant, as compared to U speciation in exposure medium, and that U complexes *intra planta* were identical in the roots, shoot axis, and leaves.

The aim of our experiment was triple. First we wanted to explore a possible difference of U speciation when accumulated in different plants, and we chose oilseed rape (*Brassica napus*) and sunflower (*Helianthus annuus*) as model species, since they are assumed to accumulate large amounts of U. Secondly we wanted to address the possible transformation of U speciation during the translocation of the element from the roots to the shoots in both plant species. Finally we wanted to evaluate the influence of the presence of several lignads and of the pH of the nutrient solution on U accumulation, translocation and speciation transformation in plants. To ensure that U speciation was not modified by sample preparation, EXAFS oscillations were recorded on fresh samples which were not lyophilized.

Experimental method

Four weeks seedlings of oilseed rape (*Brassica napus*) and sunflower (*Helianthus annuus*) were grown in a classical nutrient solution, in controlled conditions of light, temperature and humidity, and then exposed for 72 h to 50 µM of uranyl nitrate. Exposure media were supplemented with U ligands: bicarbonate, phosphate or citric acid, and pH was adjusted to 4 or 7. At the end of the exposure period, plant roots were rinsed to eliminate adsorbed U. Then, shoot and roots were yielded and stored in liquid nitrogen. Fresh plant samples were then ground and pressed as 5-mm diameter pellets, which were stored in liquid nitrogen until the experiment, which was performed in a helium cryostat. 30 µL of each exposure medium was frozen and

sealed in a plastic bag (we used solide state analysis for these samples). XAS spectra were recorded at U L_{III}-edges on BM30B beamline, in fluorescence mode, using a 30 elements solid state Ge detector (Canberra). The monochromator was a Si(220) double crystal. At least 3 spectra (depending on the uranium concentration of the sample) for each sample were recorded 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 k^3 and qualitatively compared to reference curves [2, 3]. In parallel, U theoretical speciation in each conditions was calculated with JCHESS modeling software, using BASSIST thermodynamic constants database [4, 5].

Results

Theoretical U speciation calculation

Plants were exposed to U in 7 different media, containing no ligand, or phosphate, or carbonate, or citrate, at pH 4 or 7 (except for U-phosphate in which pH is set to 5). U theoretical speciations in these media are presented in Table 1.

Exposure medium	**U species**	%
U-phosphate (phosphate 0.55 mM; pH=5)	$(UO_2)_3(PO_4)_2$	100
U-no_ligand4 (no ligand ; pH=4)	UO_2^{2+}	56
	UO_2SO_4	38
	UO_2OH^+	6
U-no_ligand7 (no ligand; pH=7)	$CaUO_4$	94
	UO_2-EDTA	6
U-carbonate4 (carbonate 10 mM; pH=4)	UO_2^{2+}	56
	UO_2SO_4	36
	UO_2CO_3	4
	UO_2OH^+	4
U-carbonate7 (carbonate 10 mM; pH=7)	$UO_2Ca_2(CO_3)_3$	88
	$UO_2(CO_3)_2^{2-}$	6
	$UO_2Ca\,(CO_3)_3^{2-}$	6
U-citrate4 (citrate 10 mM; pH=4)	UO_2-cit_2^{4-}	100
U-citrate7 (citrate 10 mM; pH=7)	UO_2-cit_2^{4-}	100

Table 1. U species distribution in exposure media, theoretical speciations calculated by JCHESS modelisation using BASSIST database.

Experimental determination of U speciation

U speciation in these exposure media was experimentally determined by EXAFS (Figure 1). First observations allow us to confirm that U speciation in U-phosphate, U-no_ligand, U-carbonate and U-citrate media are not similar. U-no_ligand4 and U-carbonate4 oscillations are very close, in accordance with the theoretical speciation (combination of UO_2^{2+} and UO_2SO_4). U-phosphate and U-no_ligand7 oscillations are also quite similar: both contain in theory 100 % of precipitated uranium forms (respectively $(UO_2)_3(PO_4)_2$ and $CaUO_4$). EXAFS curves of U-citrate4 medium could not be obtained. Qualitative comparison with EXAFS oscillations of reference compounds suggests that U-phosphate and U-no ligand7 contain a majority of uranyl phosphate complexes, and that U-no ligand4 and U-carbonate4 contain uranyl carbonate complexes. Medium U-citrate7 could be composed of a mixture of these two compounds. However, the curves of some reference compounds are missing: $CaUO_4$, UO_2SO_4, UO_2OH, UO_2-EDTA.

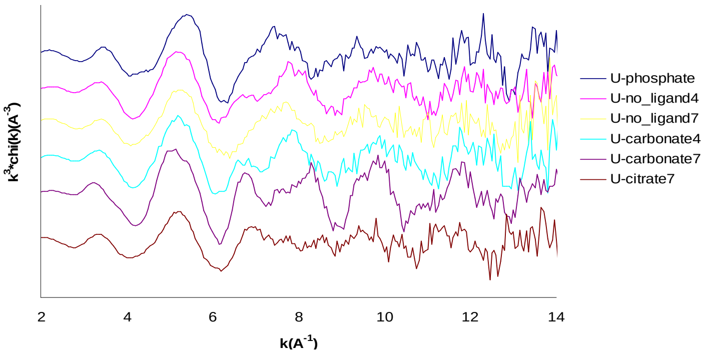

Figure 1. k^3-weighted EXAFS oscillations of U exposure media.

EXAFS oscillations were then analysed in shoot and roots of oilseed rape grown in U-carbonate7 medium (Figure 2). While these curves are quite similar, they both differ from U-carbonate 7 medium oscillations. This point is in accordance with previously published results [1]. However U concentrations in samples are quite low and more spectra have to be recorded in order to obtain sufficient statistics for correct data analysis.

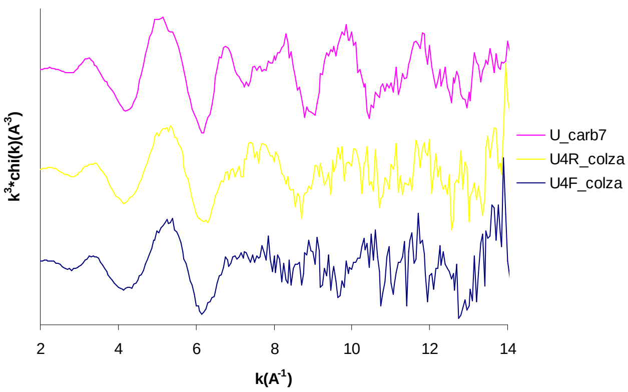

Figure 2. k^3-weighted EXAFS oscillations of roots and shoot of oilseed rape plants grown in U-carbonate7 with U-carbonate7 medium.

EXAFS oscillations of oilseed rape roots exposed to all the exposure media are quite similar (Figure 3), even if differences can be noticed in the 7 Å^{-1} oscillation for U-phosphate and U-carbonate7.

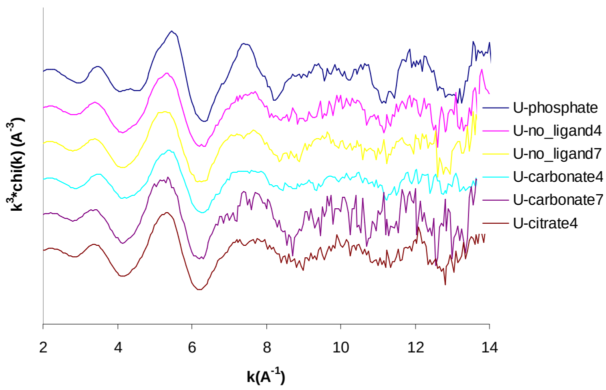

Figure 3: k^3-weighted EXAFS oscillations of oilseed rape roots grown in various contaminated media.

Finally, U EXAFS oscillations in oilseed rape and sunflower roots grown in U-no_ligand7 and U-carbonate4 were compared (figure 4). It is interesting to note that EXAFS oscillations of oilseed rape and sunflower roots exposed to U-carbonate4 are different. Indeed sunflower sample presents a sharper peack at 7Å^{-1}, and an additionnal peak at 12Å^{-1}. This fact is in contradiction with Günther *et al*, 2003 who found identical uranium speciation in dandelion, lamb's lettuce and lupine plants. Furthermore, in a recent study, Vera Tome *et al.*, (2008) showed that seedling of sunflower are able to induce the precipitation of uranium, probably in form of mixed precipitates Ca-U-P, bound to the cell wall of the roots. It would be interesting to analyse if these precipitates are visible in our EXAFS speciation results.

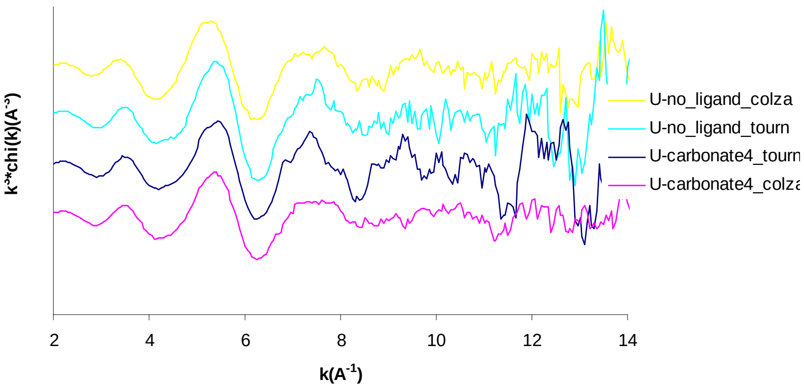

Figure 4: k^3-weighted EXAFS oscillations of rapeseed oil and sunflower roots grown in U-no_ligand7 and U-carbonate4 media.

Conclusions and perspectives

During this experiment, it was possible to confirm differences and similarities in U species distributions in various plant exposure media, previously modeled by theoretical speciation. We also confirmed that speciation do not seem to be modified in plants by root-to-shoot translocation. Conversely, U speciation in plant is different from U speciation in exposure medium, which means that plant roots modify U complexation before or during root accumulation. In oilseed rape roots, uranium speciation is the same whatever the culture medium. The more surprising result is that we noticed marked differences between U speciation in oilseed rape and sunflower roots. In order to enrich this study, we need in the future to analyse the missing reference compounds (for instance U-citrate at pH 4 or 7, U-sulfate and U-EDTA) and to complete the set of sample data. These newly acquired data would permit to perform linear combination fitting of reference spectra and to better understand the influence of plants in U speciation modification.

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