ESRF	**Experiment title:** Uranium speciation in plants	**Experiment number**: 30-02-926
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Names and affiliations of applicants (* indicates experimentalists):

Marie CARRIERE* CEA/DSM/IRAMIS/LPS
Julien LAURETTE* CEA Saclay
Bât 637
F-91191 Gif sur Yvette, France

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. Especially, citric acid was demonstrated to be the most efficient chelatant agent for inducing a large uranium accumulation in plant shoot and for improving the uranium root to shoot transfert [1].
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 [2]. 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* was identical in the roots, shoot axis, and leaves. However, there is no available data about uranium speciation and its evolution in the presence of organic acids like citric acid in exposure medium.

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 root to the shoot. Finally we wanted to evaluate the influence of the presence of ligands like carbonate or citric acid 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 100 µM of uranyl nitrate. Exposure media (in which phosphate was removed) were supplemented or not with 10 mM of citric acid or 10 mM of carbonated species, and pH was adjusted to 4 (nothing and citric acid) or 7 (carbonate). 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 both exposure media (with or without citric acid), and solutions containing reference compounds (uranyl sulfate or uranyl citrate for instance) 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 [3, 4]. In parallel, U theoretical speciation was calculated in each condition with JCHESS modeling software, using BASSIST thermodynamic constants database [5, 6].

Results

Theoretical U speciation calculation

Plants were exposed to 100 µM of U in the 3 media (containing 10 mM of citric acid, 10 mM of carbonated species or neither). U theoretical speciations in each medium are presented in Table 1.

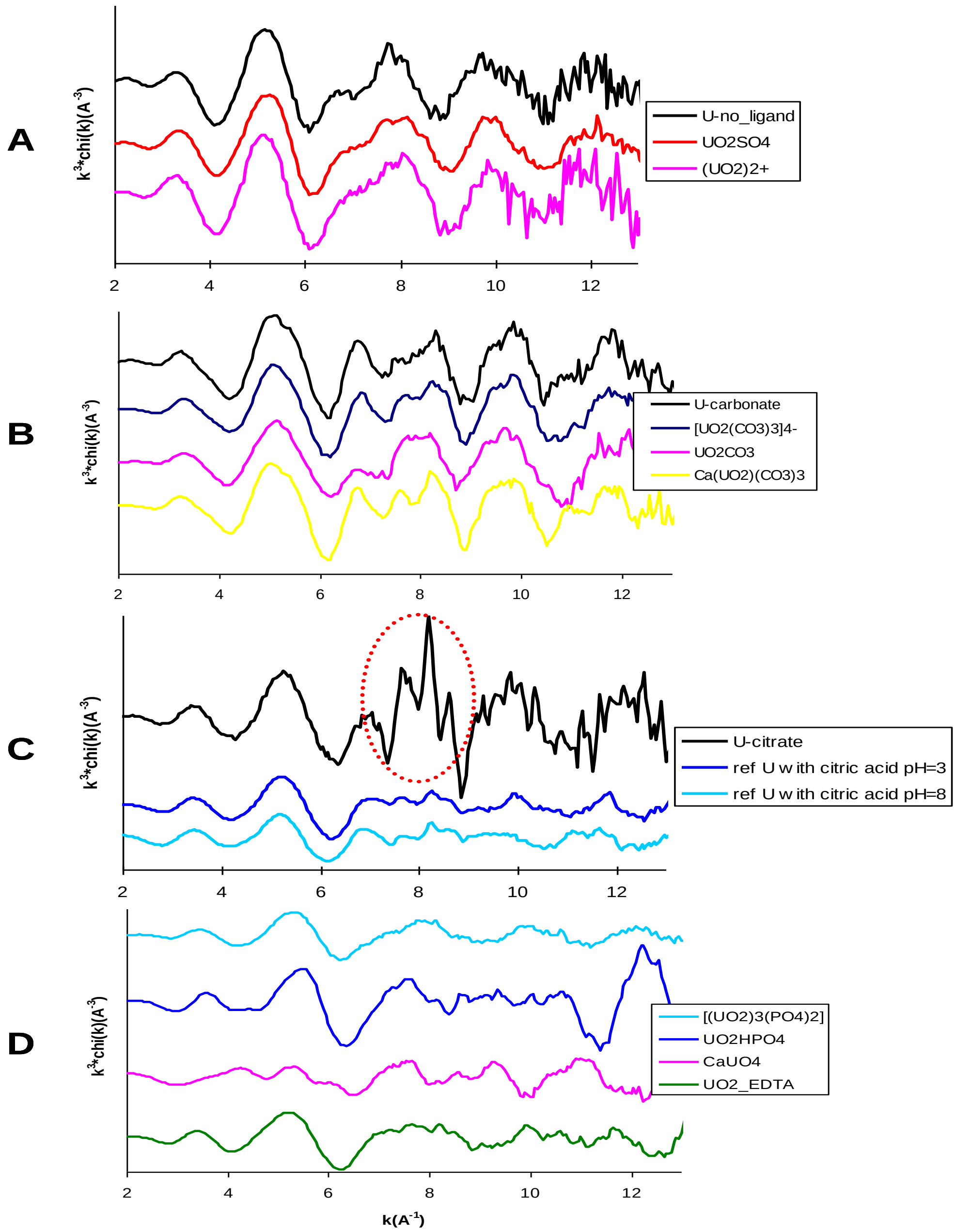
Exposure medium	**U species**	%
U-no_ligand (pH=4)	UO_2^{2+} UO_2SO_4 UO_2OH^+	56 38 6
U-carbonate (10 mM carbonated species ; pH=7)	$UO_2Ca_2(CO_3)_3$ $UO_2(CO_3)_2^{2-}$ $UO_2Ca(CO_3)_3^{2-}$	88 6 6
U-citrate (10 mM citric acid; pH=4)	$UO_2\text{-cit}_2^{4-}$	100

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

Experimental determination of U speciation

<u>Exposure media</u>

In each exposure medium, U speciation was experimentally determined by EXAFS (figures 1A, 1B et 1C), and compared with corresponding reference compounds oscillations. Other references uranyl phosphates, uranyl-EDTA and $CaUO_4$ are also displayed (figure 1D).



Figures 1. k^3-weighted EXAFS oscillations of exposure media, compared with corresponding : U-no_ligand (1A), U-carbonate (1B), U-citrate (1C), and others reference compounds (1D).

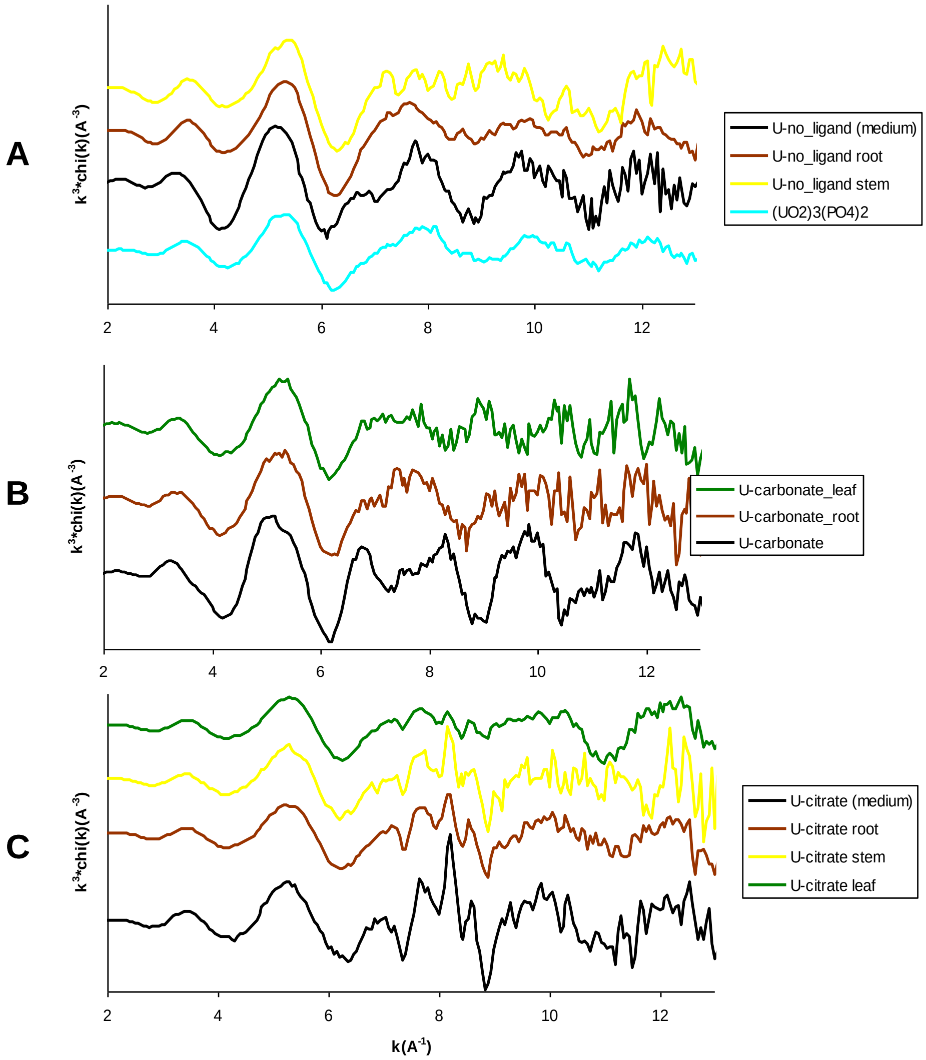
First observations allow us to confirm that U speciations in U-no_ligand, U-carbonate and U-citrate media are not similar. On the one hand, U-no_ligand oscillations are very close to UO_2^{2+} and UO_2SO_4 oscillations, (uranyle et U-sulfate se ressemble entre eux… ??), and U-carbonate oscillations look like to the three reference U-carbonate complexes, in accordance with the theoretical speciation.

On the other hand, U-citrate medium oscillations don't match with citric acid reference compounds curves, but we have encountered unexpected artefact in 7-9 $Å^{-1}$ area (figure 1C: 3 sharp peaks surrounded by red dotted line).

Moreover, wa can assert that reference U-citrate at pH=3 is not the same that U-citrate at pH=8 (différences at 7, 10 and 12 $Å^{-1}$). Pasilis and Pamberton [7] notably conclued that stoechiometry of uranyl-citrate complexes depends on pH. The 2 uranium phosphate compounds also differ each other (sharper peak at 7 $Å^{-1}$ for UO_2-HPO_4 and additional peak at 12 $Å^{-1}$).

Medium / *intra planta* speciation:

EXAFS oscillations were then analysed in shoot, stem and roots of oilseed rape grown in U-no_ligand, U-carbonate and U-citrate media (figures 2A, 2B and 2C). Stem grown in U-carbonate and leaf grown in U-no_ligand couldn't be recorded because lack of time and too low U concentration, repectively.



Figures 2. k^3-weighted EXAFS oscillations of medium (in black), root (brown), stem (yellow) and shoot (green) of oilseed rape plants grown in U-no_ligand (2A), U-carbonate (2B) and U-citrate (2C) media.

EXAFS oscillations corresponding to organs of oilseed rape (root, stem and leaf) grown in U-citrate medium are not exploitable because of artefacts in 7-9 $Å^{-1}$ area. In the case of U-carbonate, the curve corresponding to the root sample is clearly different from the medium curve (particularly at 6.5 and 9.5 $Å^{-1}$), while oscillations of roots grown in U-no_ligand slightly differ at 7-8 $Å^{-1}$ peak from U-no_ligand medium oscillations. We noticed that spectra of root and stem exposed in U-no_ligand are close among themselves, and spectra of root and leaf exposed in U-carbonate are very similar. Results suggest that a transformation of U speciation occurs when accumulated in the plant roots, as compared to U speciation in exposure medium (i) and that uranium speciation remains the same *intra planta* (ii). These 2 facts are in accordance with Günther *et al*, 2003 [2].
Interestingly we notice that oscillations of U-no_ligand roots are close to U-phosphate reference compounds.

<u>Oilseed rape / sunflower:</u>

Finally, U EXAFS oscillations of roots grown in U-no_ligand between oilseed rape and sunflower (figure 3).

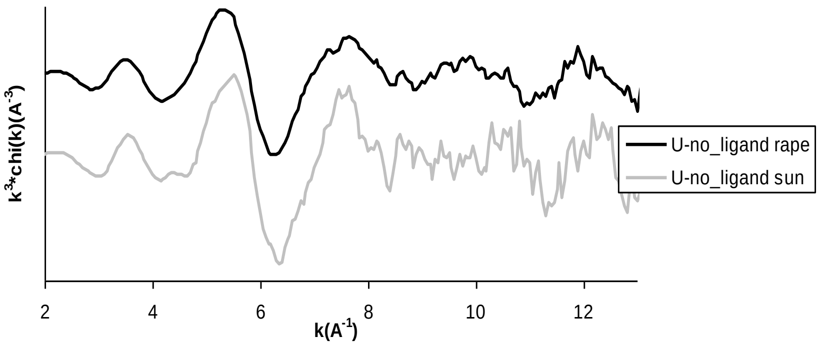


Figure 3: k^3-weighted EXAFS oscillations of oilseed rape (black curve) and sunflower (grey curve) roots grown in U-no_ligand.

Little differences could be found in EXAFS oscillations between oilseed rape and sunflower roots samples. Roots of sunflower grown in U-no_ligand display a sharper peak then oilseed rape at 7.5 $Å^{-1}$. This result confirm previous records which showed marked difference between U speciation in oilseed rape and sunflower roots grown in U-no_ligand medium.

Conclusions and perspectives

During this experiment, it was possible to confirm differences in U species distributions in various plant exposure media, previously modeled by theoretical speciation. We also assert that U speciation in plant is different from U speciation in exposure medium when no strong ligand or when carbonate are present in the medium, which means that plant roots modify U complexation before or during root accumulation. Furthermore, speciation seems not to be modified in plants by root-to-shoot translocation. Finally, we noticed little differences between U speciation in oilseed rape and sunflower roots, at least in case of U-no_ligand exposure medium. Further investigations from all these data would permit to perform linear combination fitting of reference spectra in order to better understand U speciation and it modification by plants and presence of ligand.

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