Localisation of uranium in roots by chemical extractions and by a short term uptake study. Influence of phosphate.

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Abstract. Pea roots were exposed to nutrient solution containing 25 µmol L^{-1} of uranium in presence or absence of phosphate. The uptake of U was followed during the first 90 minutes of exposure. Fifty percent of the uranium was removed after 45 minutes and after between 60-90 minutes in absence and in presence of phosphate respectively. After 24 hours exposure, root were extracted, to remove uranium from the root apoplasm. Copper extracted similar amounts of uranium than EDTA for root exposed to a solution with phospate while copper extracted only 18% of uranium extracted by EDTA for root exposed to a solution devoid of phosphate. Phosphate decreased the availability of uranium to roots of pea. The presence of phosphate decreased also the amounts of uranium extracted.

Introduction

The transfer of uranium from soil to plants depends on uranium speciation in soil and in soil solution, and on the capacity of plants to take up and detoxify uranium. One mechanism to detoxify a toxic metal in plants consists in the storage of the considered metal in the roots of plants and more precisely in the apoplasm of roots. The apoplasm consists of the plasma membrane, the cell wall (specific structure of the cells of plants), and the inter-cell wall and intercellular free spaces. Carboxylic and peptic compounds present in the cell walls create negative charges onto which cations can adsorb (Dufey et al. 2001). These negative charges confer to the root cation exchange capacity which is between 20 and 50 $cmol_{(+)}\ kg^{-1}$ for the dicotyledons and between 10 and 20 $cmol_{(+)}\ kg^{-1}$ for the monocotyledons.

Two methods are usually applied to determine the importance of the adsorption of a metal onto the cell wall. The first one consists in following the uptake kinetics

of the metal by the plant: the adsorption of the metal onto the cell wall is a rapid process; the entrance of the metal in the cell is slower. The second approach consists in chemical extractions with other cations (Rengel and Robison 1989). Some authors used copper which has a high affinity for root cell walls and which is present as trace elements in plants (Dufey et al. 2001). Adsorption of uranium onto cell wall of algae was also quantified performing extraction with EDTA (Fortin et al. 2004).

Roots of plants can rapidly remove high amounts of uranium from contaminated solution: Ramaswami et al. (2001) showed that different plant species (Sunflower, Vetch, Juniper, Mustard, and Bean) can remove between 60 and 90% of uranium from a solution containing 210 µmol L^{-1} of uranium in demineralized water within 48 hours. Significant uranium retention by root was also observed for smaller exposure time: Eapen et al. (2003) obtained a 50% reduction in uranium solution concentration (initial concentration up to 500µmol L^{-1}) after 2-3 hours of exposure for hairy roots of *Brassica juncea* and after 2 to 8 hours for roots of *Chenopodium armanticolor*. Bhainsa and D'Souza (2001) observed 54% retention after 4 minutes contact time between dried roots of water hyacinth and a solution containing 840 µmol L^{-1} uranium. The rapid uptake of uranium from the solution could indicate an important adsorption of uranium onto the cell wall of plants.

Phosphate could play a role in uranium retention by roots of plants at two levels. First, metals could also precipitate with phosphate in the apoplasm or around the root (Sarret et al. 2002; Straczek 2003) and uranium has been shown to be associated with phosphate in lupine (Gunther et al. 2002). However phosphate could also decrease the bioavailability of uranium for plants (Ebbs et al. 1998).

The main objectif of this work was to compare uranium uptake of pea in presence or absence of phosphate. Two methods were applied: short term uptake studies and chemical extractions on the roots with copper and EDTA. Here preliminary results are shown.

Material and Methods

Pre-culture

Plants were grown in a growth chamber (16 / 8 h light / dark cycle, 65% humidity 24°C). Seed of pea were disinfected with H_2O_2 10% during 30 minutes. After one week of germination, pea was grown on Hoagland nutrient solution during 2 weeks.

Exposure to uranium

Following the pre-culture, plants were grown for a additional day on a nutrient solution containing as much iron and oligoelements as the Hoagland solution and the concentrations of the major elements were at fourth strength (diluted Hoagland nutrient solution). The roots were then briefly rinsed with desionised water. Plants were exposed during 24 h to the diluted Hoagland nutrient solution containing 23 µmol L^{-1} of 238 uranium and 2 µmol L^{-1} of 233 uranium, and 0 or 5 µmol L^{-1} of phosphate.

Uptake of uranium as a function of time

A sample of the nutrient solution was taken at different time intervals (0,3,5, 10, 15,20, 30,45, 60, 90 minutes) to determine the kinetic of the uptake of uranium by roots.

Chemical extractions

After 24 h of exposure to uranium, roots were harvested and divided in two parts. The first part was extracted with EDTA 250µmol L^{-1} during 90 minutes. The second part was extracted with $CuSO_4$ 10 mmol L^{-1} during 30 minutes.

Analyses

The concentration of uranium was assayed by a scintillation counter.

Results and discussion

Short term uptake study

Roots of pea rapidly removed uranium from solution (Fig. 1). In presence of phosphate, 50% of uranium was removed after between 60 and 90 minutes. The removal of uranium was faster in the solution devoid of phosphate; in this case, 50% of uranium was removed after 45 minutes of contact between the solution and the roots of pea. This result indicate that phosphate decreases the bioavailability of uranium for the roots of pea. The removal of uranium by roots of pea was faster than that observed by Eapen et al. (2003) for *B juncea* and *C. amaranticolor* and lower than that observed by Bhainsa and D'Souza (2001) for dried root of hyacinth. However the biomass volume ratio (1g dried biomass by one liter) used

by these authors was higher than our biomass volume ratio (0.3 g dried biomass by one liter)

EDTA and copper extraction

Copper is supposed to desorb uranium adsorbed onto the cell wall while EDTA could remove metal adsorbed and complexed by the roots. Copper and EDTA extracted similar amounts of uranium from roots exposed to solution containing phosphate (Table 1). In opposite, copper was less efficient than EDTA in remaining uranium from the roots in absence of phosphate: only 18% with EDTA was removed with copper. This indicates that without phosphate in solution, uranium was rather precipitated or complexed in the apoplasm than adsorbed onto the cell wall. EDTA and copper extracted more uranium in absence of phosphate than in presence of phosphate (respectively 10 and 35 times). These results indicate that after 24 hours, like following the short exposure time, less uranium was removed by root from the solution containing phosphate than from the solution devoid of phosphate. This result could also indicate that in the case of phosphate in solution, uranium was precipitated in root with phosphate and that this precipitated was not extractable by copper or EDTA.

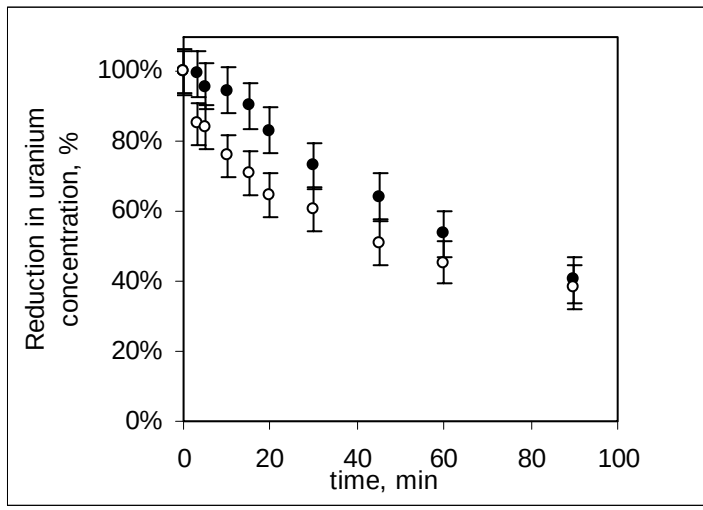

Fig. 1. Reduction in uptake concentration in solution as a function of the exposure time . Roots of pea were exposed to a diluted Hoagland solution containing 25 µmol L^{-1} of uranium and 0 (white symbols) or 5 (black symbols) µmol L^{-1} of phosphate.

Table 1. Uranium extracted by copper or EDTA from pea roots (µmol g^{-1} fresh weight) exposed during 24 hours to a diluted Hoagland solution containing 50µmol L^{-1} of uranium and 0 or 5µmol L^{-1} of phosphate.

Phosphate	Copper extraction	EDTA extraction
0 µmol L^{-1}	18,8 ± 10,7 nmol L^{-1}	105.1 ± 58.7 nmol L^{-1}
5 µmol L^{-1}	1,8 ± 0,8nmol L^{-1}	2.9 ± 0.5 nmol L^{-1}

Conclusion and perspectives

This study showed that roots of pea could remove uranium from contaminated solution in a short time. In absence of phosphate, EDTA extracted more uranium than copper, indicating that other mechanisms than adsorption onto the cell walls occurred. Phosphate decreased the uranium bioavailability for the plants. But on other hand, phosphate could increase the precipitation of uranium in the apoplasm. Roots after extraction will be analysed for the remaining uranium content to determine total uranium uptake after 24 hours exposure and to quantify the proportion of total uranium removed from the nutrient solution which was extracted by copper and EDTA.

These experiments were performed with pea which is a dicotyledon species. A similar experiment will be performed with maize, a mocotyledon species.

References.

Bhainsa KC and D'Souza SF (2001). Uranium(VI) biosorption by dried roots of Eichhornia crassipes (water hyacinth). Journal of environmental science and health A36: 1621-1631.

Dufey JE, Genon JG, Jaillard B, Calba H, Rufyikiri G, Delvaux B (2001). Cation exchange on plant roots involving aluminium: experimental data and modeling. In: Gobran, GR, Wenzel, WW , Lombi, E (Eds.), Trace Elements in the Rhizosphere, CRC Press LCC, Boca Raton, Florida, USA, 2 pp. 228-252.

Eapen S, Suseelan KN, Tivarekar S, Kotwal SA, Mitra R (2003). Potential for rhizofiltration of uranium using hairy root cultures of Brassaica juncea and Chenopodium amaranticolor. Environmental research 91: 127-133.

Ebbs SD, Brady DJ, Kochian LV (1998). Role of uranium speciation in the uptake and translocation of uranium by plants. Journal of experimental botany 49: 1183-1190.

Fortin C, Dutel L, Granier-Laplace (2004). Uranium complexayion and uptake by a green alga in relation to chemical speciation: the importance of the free uranyl ion. Environmental Toxicology and Chemistry 23: 974-981.

Gunther A, Bernhard G, Geipel G, Reich T, Rossberg A, Nitsche H 2002. Uranium speciaiton in plants. Radiochemical Acta 91 319-328.

Ramaswami A, Carr P, Burkhardy M (2001): Plant-uptake of uranium: hydroponics and soil system studies. International journal of phytoremediaition 3: 189-201.

Rengel Z, Robinson DL (1989). Determination of cation exchange capacity of ryegrassroots by summing exchangeable cations. Plant & Soil 116:217-222.

Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazeman JL, Traverse An Marcus MA, Manceau A (2002). Forms of zinc accumulated in the hyperaccumulator Arabidopsis halleri. Plant Physiology 130: 1815-1826.

Straczek A 2003. Mobilisation accumulation et distribution de Zn par et dans des tabacs modifiés pour leur capacité d'excrétion de protons et d'accumulation des métaux. PhD, University of Montpellier France, 130pp