Microorganisms of radionuclides-contaminated soils of Chernobyl: in depth analysis of diversity and study of uranium-bacteria interactions.

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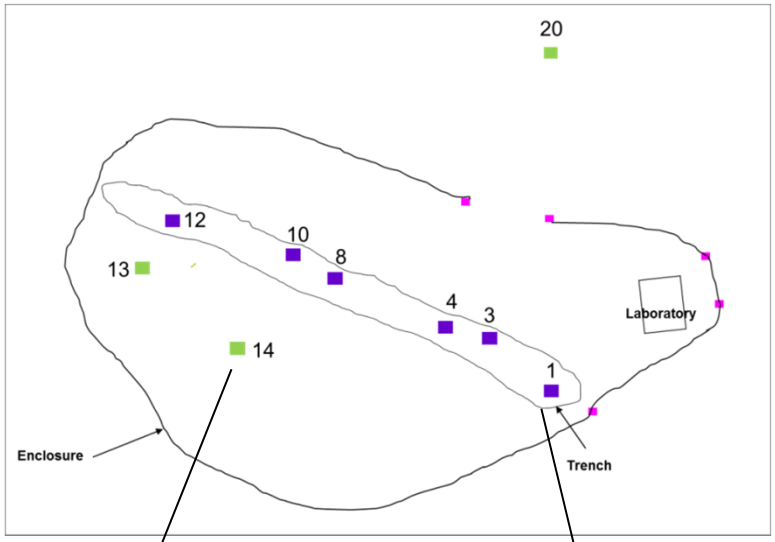
The Trench T22 in the Chernobyl exclusion zone

A nuclear waste storage trench where contaminated debris were buried in 1987.

Experimental platform for the study of RNs transfer.

Impact of RNs on bacterial diversity ?
Impact of bacteria on RNs transfer ?

Impact of RNs on soil microbial diversity



Outside
Low ^{137}Cs (0.3-1.5 Bq/g)
Low Corg content

Inside
High ^{137}Cs (60-750 Bq/g)
High Corg content

50-60 cm depth, aerobic

Metagenomic DNA extraction

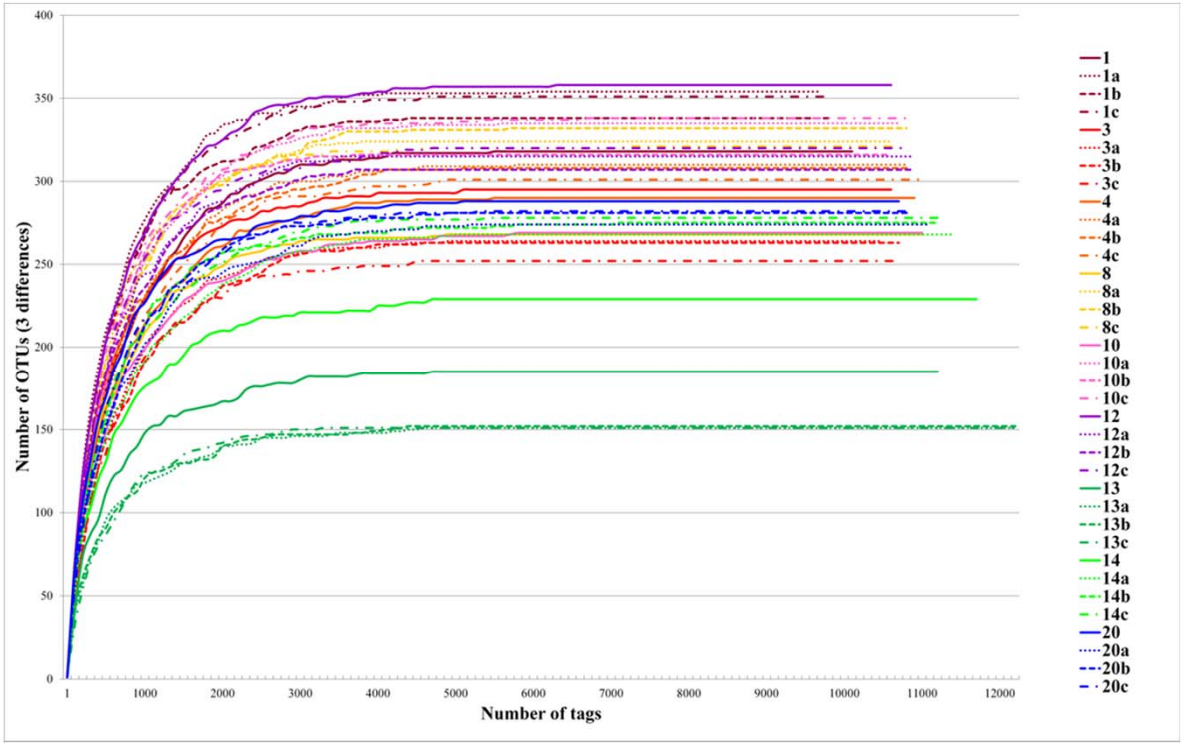
Sampling campaigns in April (single) and October (triplicates) 2009
→ 12 control samples
→ 24 contaminated samples

Chapon *et al.* (2012) Appl Geochem

In-depth analysis of soil bacterial communities

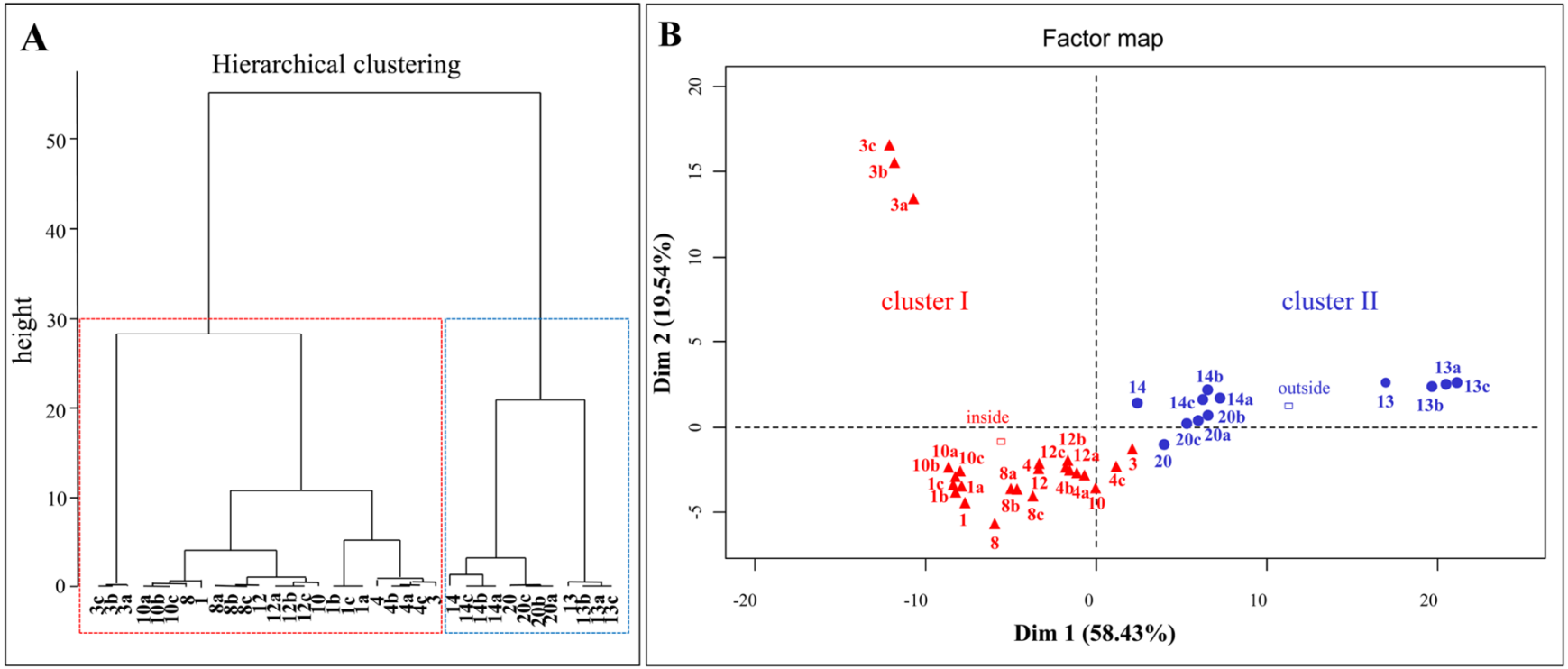
16SrRNA genes (V4 region)
454-pyrosequencing

695,349 high quality reads
13,347 reads per samples



- the diversity is saturated
- high apparent diversity in all samples
- slight positive effect of the trench conditions on bacterial diversity

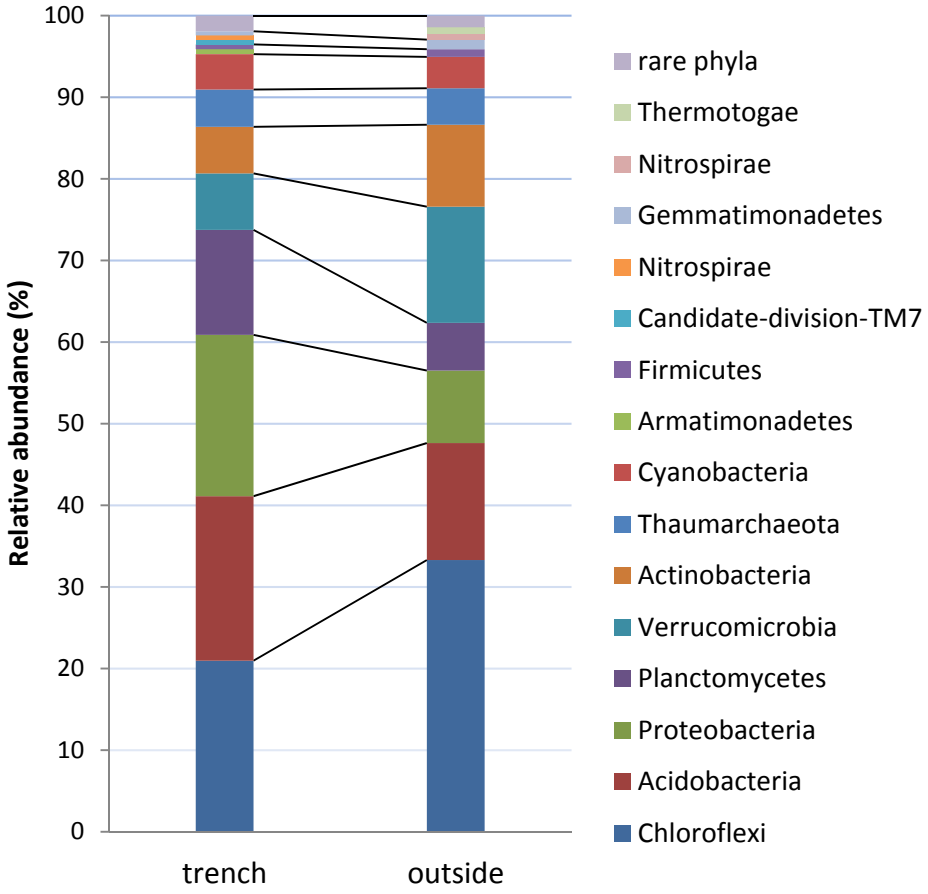
Comparative analysis of bacterial communities



Hierarchical clustering and PCA (OTU at 97%)

- Different species composition and relative abundance
- Impact of the trench conditions on bacterial communities

Comparative analysis of bacterial communities



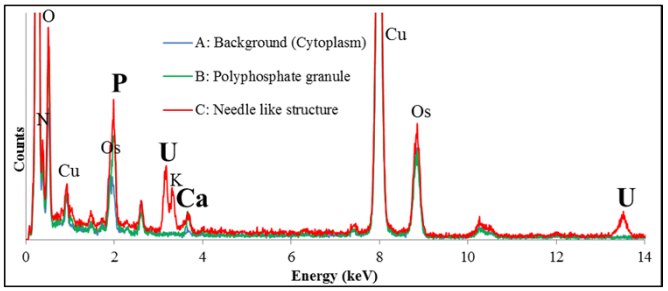
- High abundance of *Chloroflexi*
- Enrichment in *Acidobacteria*, *Proteobacteria* and *Planctomycetes* in the trench.
- Decrease of *Chloroflexi* and *Verrucomicrobia*

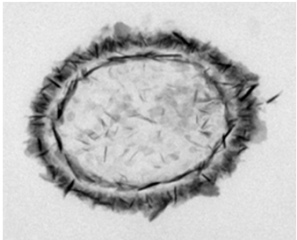
Isolation of a uranium-tolerant species

Collection of cultured isolates

Selection of uranium tolerant isolates

Microbacterium sp. A9





Aerobic culture in
TSB/10 pH7
uranium 500 µM
24h 30°C

Chapon et al. (2012) Appl Geochem

Influence of uranium speciation on its cellular localization

Culture, exponential growth phase

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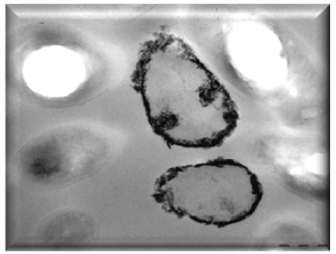
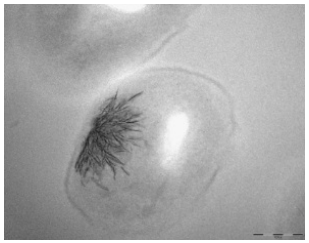
Washing in NaCl 0.1M

Exposure in
NaCl 0.1M **pH7**
uranium 100 µM
24h 30°C

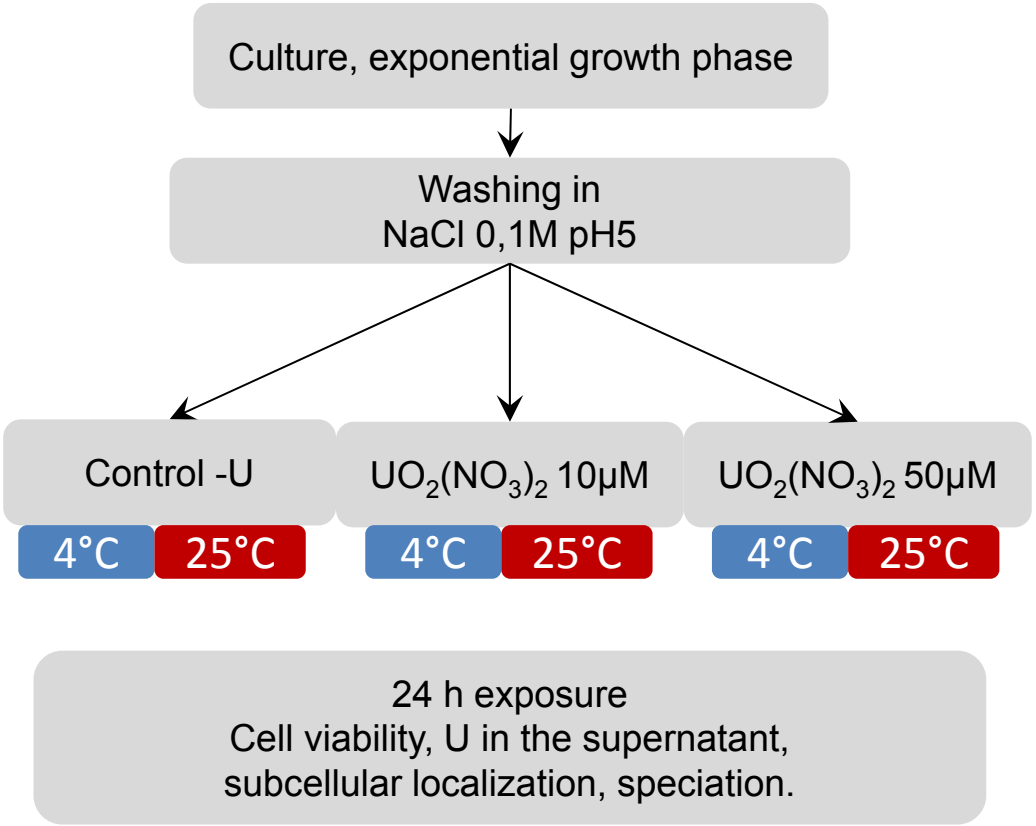


Exposure in
NaCl 0.1M **pH5**
uranium 100 µM
24h 30°C



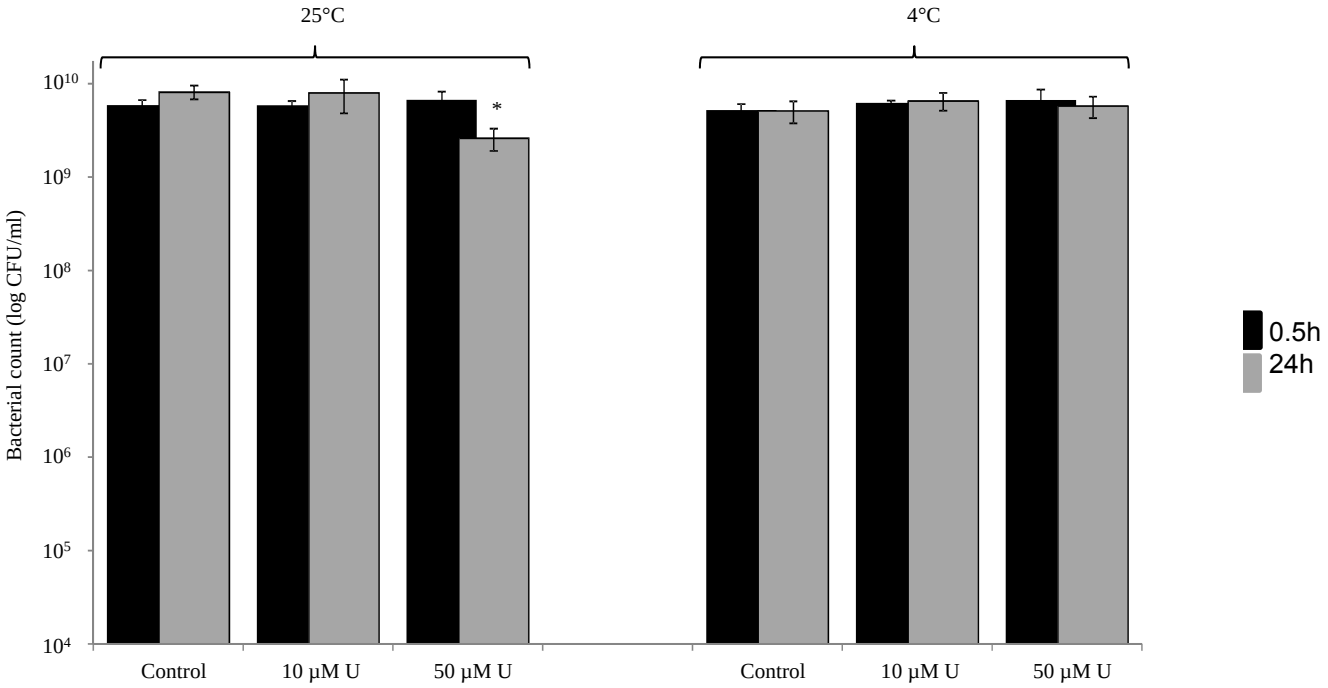
Cell survival?
Active mecanism?

Microbacterium-uranium interactions at pH5



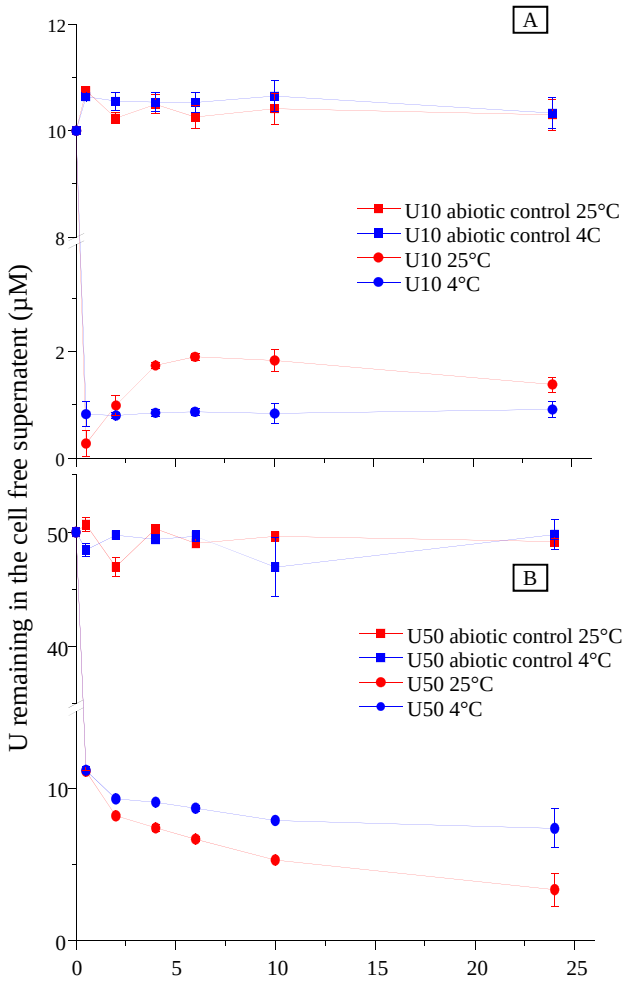
Theodorakopouls *et al.* (2014) J Hazard Metal, minor revisions

Cell survival



- A 4°C: 100% survival
- A 25°C: 100% with 0 and10µM U, 61% with 50µM

Uranium sequestration

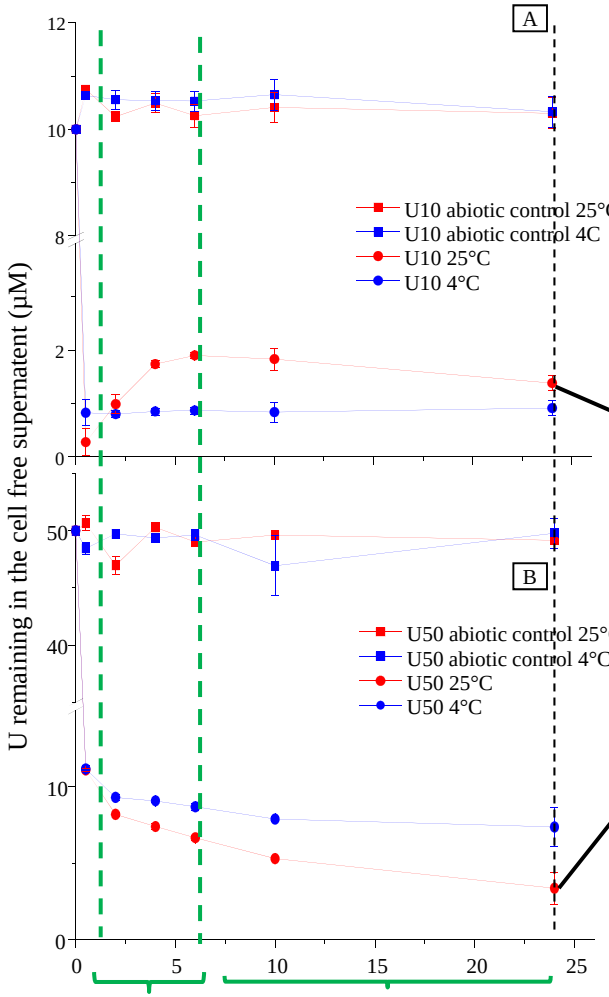


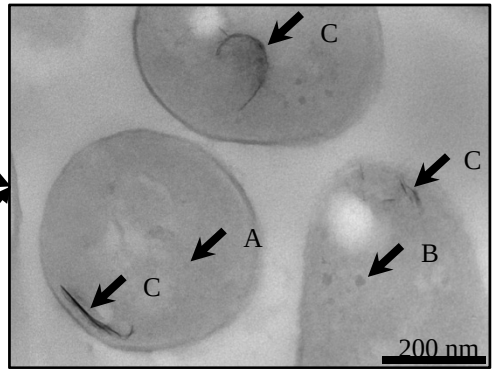
U: 10µM

U: 50µM

- ✓ Rapid sorbtion of U (passive)
- ✓ Transient efflux with 10µM U only (active)
- ✓ Slow U accumulation (active)

Subcellular localization of U (TEM-EDX)



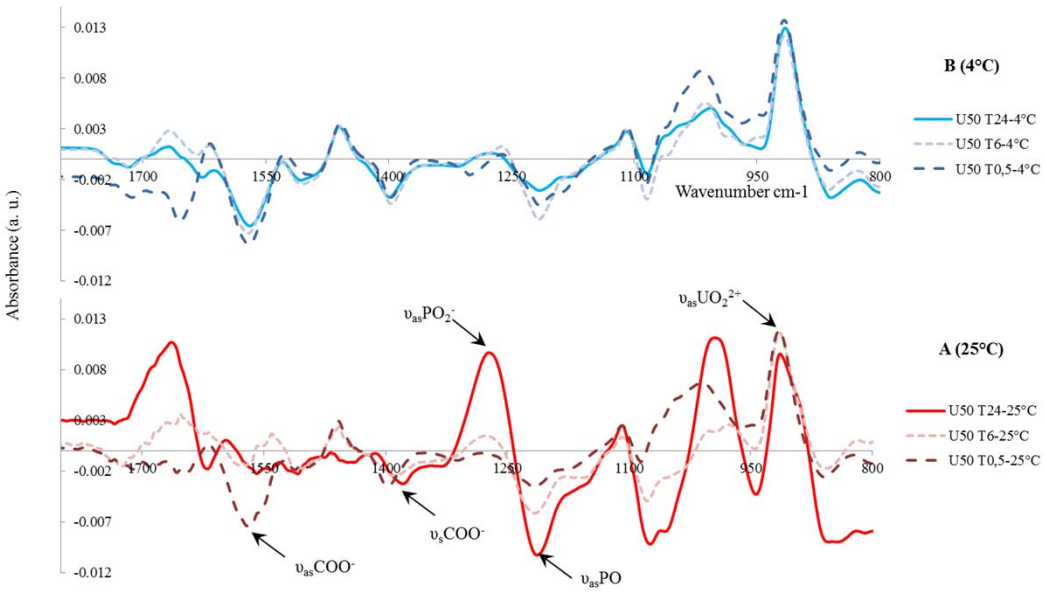


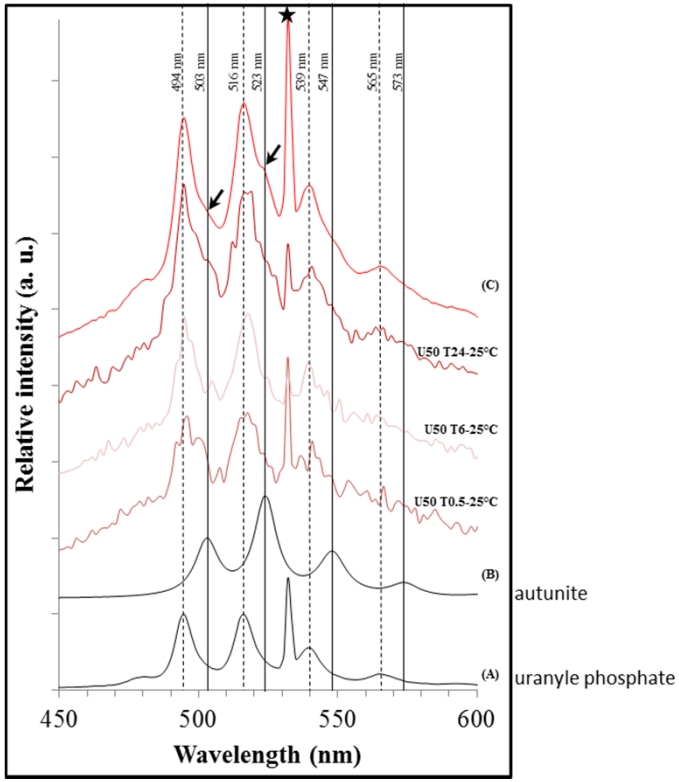
U detectable at 25°C from 6h exposure

U not detectable

U not detectable at 4°C

Uranium speciation (FTIR and TRLF)

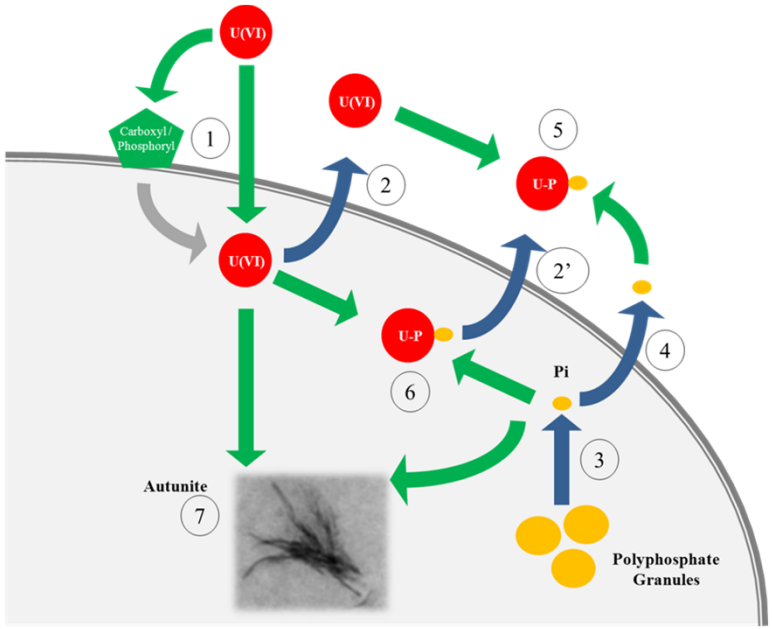




At 6h: 34% U-P + 6% autunite + background
At 24h: 45% U-P + 15% autunite + background

- 4°C: U interacts with carboxyl and phosphoryl groups. Stable
- 25°C: carboxyl groups > phosphoryl/phosphate groups during the first step
  phosphoryl/phosphate groups>carboxyl groups after 6h exposure
- Presence of autunite and U-Phosphate

Ongoing studies…



Working model

Cellular response to uranium stress:
-global approaches (transcriptomic, proteomic)
-identification of the transporter involved in U efflux

Impact on uranium transfert:
-soil columns

Thank you for your attention !

	50 µM 25°C	50 µM 4°C	10 µM 25°C	10 µM 4°C
Species name	% of total concentration			
UO_2^{2+}	46.96	63.17	67.53	73.83
UO_2OH^+	12.66	17.00	18.19	19.84
$(UO_2)_2(OH)_2^{2+}$	19.98	8.39	8.25	2.29
$(UO_2)_3(OH)_5^+$	14.98	0.93	1.77	0.06
UO_2Cl^+	2.51	2.72	3.63	3.19
$(UO_2)_3(OH)_4^{2+}$	1.40	3.63	0.17	0.23
$(UO_2)_4(OH)_7^+$	0.79	2.94	0.03	0.04
$(UO_2)_2OH^{3+}$	0.57	1.02	0.23	0.28
$UO_2(OH)_2$	0.12	0.17	0.18	0.20
$UO_2NO_3^+$	0.01	0.03	0.02	0.03

Uranium speciation in NaCl 0.1M pH5 (MINTEQ):
60 to 93% soluble