Accumulation of Europium(III) in the filamentous fungus *Podospora anserina*

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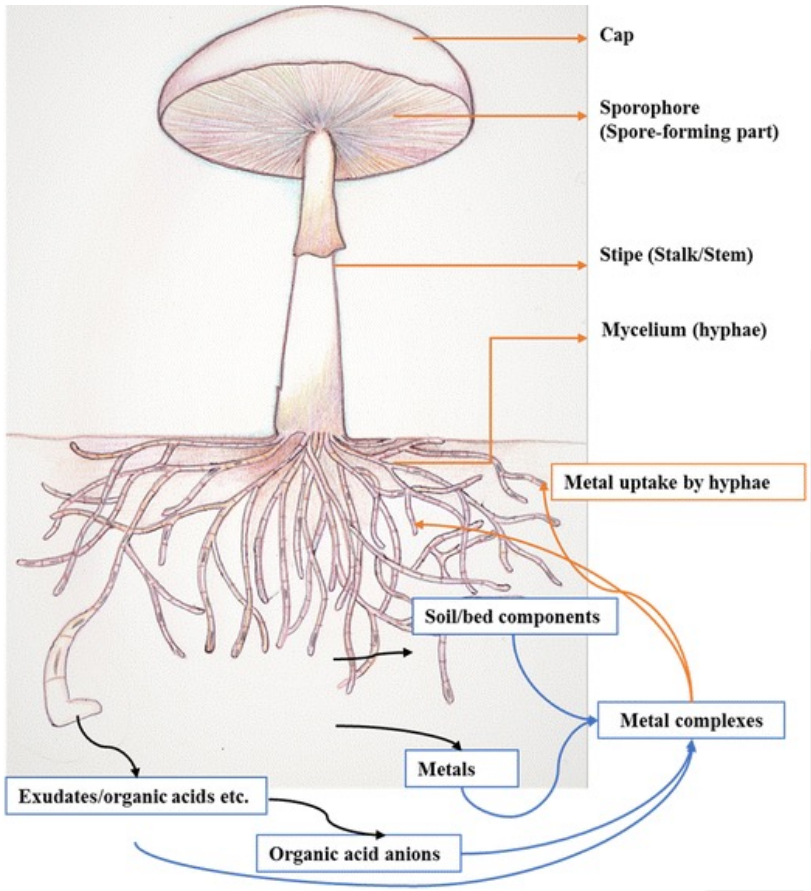
Journées plateforme MOSAIC

Context

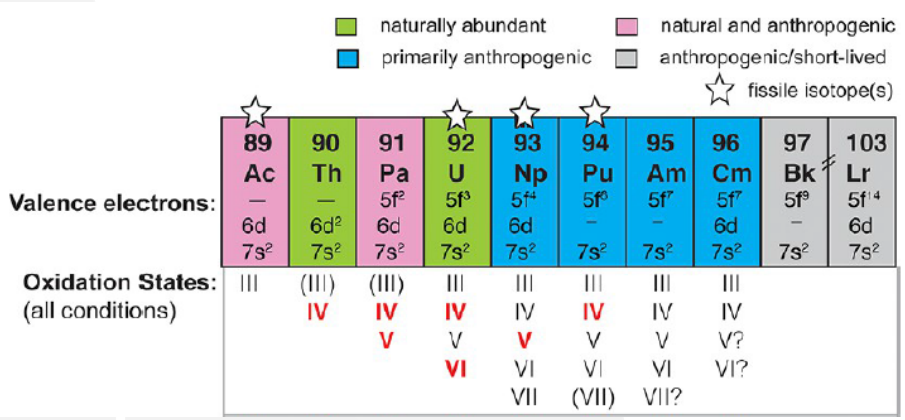


- Within the biosphere, fungi play a major role in the ecosystem : wide geographic dispersion, abundance, heavy metal accumulation ability

  ⇒ good bio-indicators of environmental pollution?

- Natural and anthropogenic radionuclides are present in the different environmental compartments

  → **chemical form governs mobility and bioavailability**

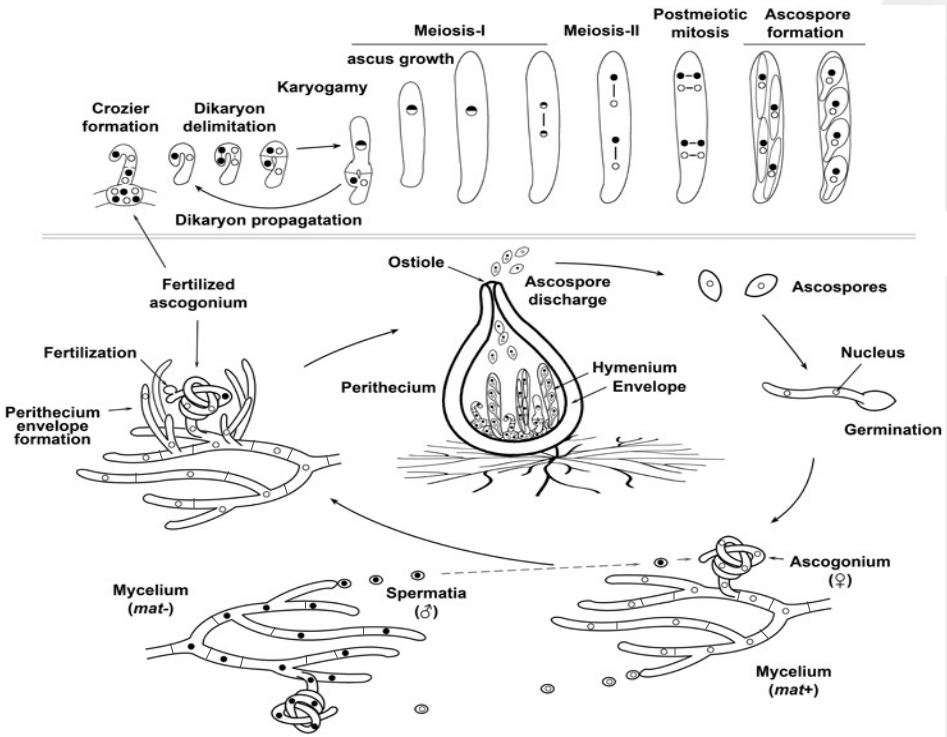


Chatterjee, Soumya, et al. *Environmental Science and Pollution Research* 24.24 (2017): 19480-19493.

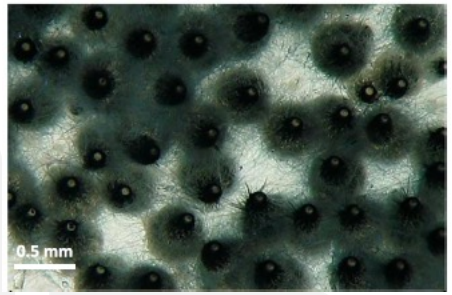
Maher, K. et al., Inorganic Chemistry **2013,** *52(7)*, 3510-3532.

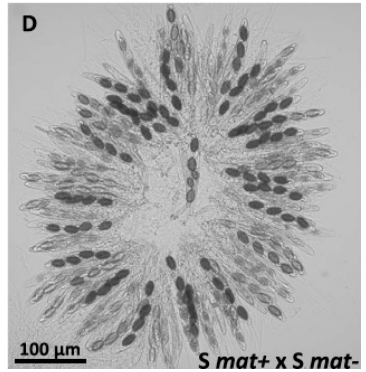
Podospora anserina

Filamentous ascomycete fungus, usually observed on the excretion of herbivorous animals.



- Easily cultivated, short life cycle (7days), non-pathogenic
- In liquid medium(**mycelium growth**) or in solid medium (**sexual reproduction**)





How *P. anserina* adapts to a highly contaminated environment?

Peraza Reyes L *et al.* Frontiers in physiology, 2013, 4: 244.

Methodology : semi-natural system

Composition of the culture medium

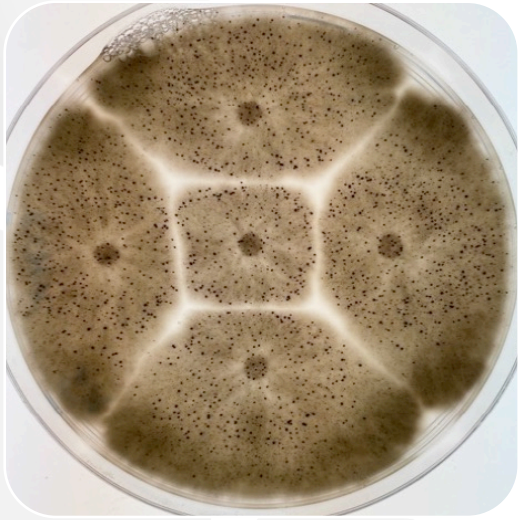
$MgSO_4$	250 mg/L
$ZnSO_4$	5 mg/L
$CuSO_4$	0.25 mg/L
$MnSO_4$	0.05 mg/L
$Fe(NH_4)_2(SO_4)_2.6H_2O$	1 mg/L
Urea	500 mg/L
Citric acid	5 mg/L
Biotine	0.05 mg/L
Thiamine	0.05 mg/L
H_3BO_3	0.05 mg/L
$Na_2MoO_4.2H_2O$	0.05 mg/L
Dextrin	5500 mg/L

Chemical form of Eu(III) in culture medium?

Modelling and experimental speciation

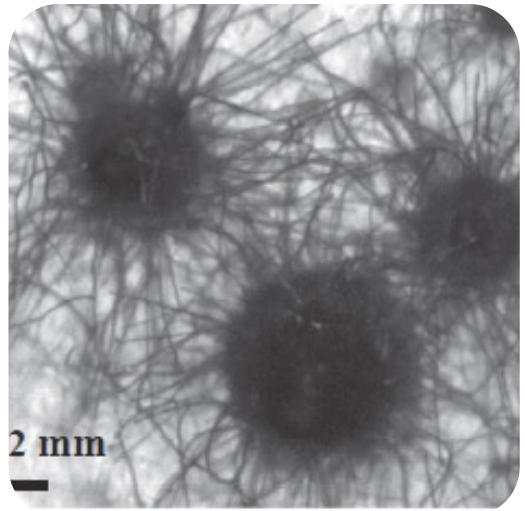
Eu(III)

Analog of Am, Cm and Pu(III)



Effect and accumulation of Eu(III) on *P. anserina*?

Visual observation and ICP-MS



Xie et al., Fungal Genetics and Biology, 116, (2018)

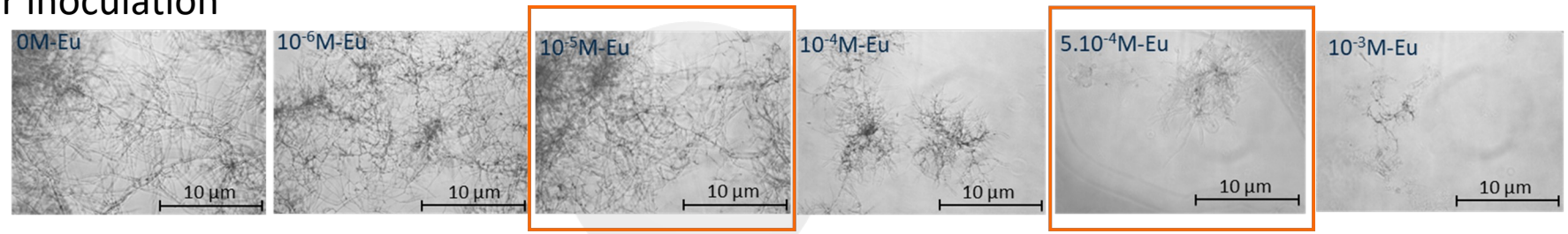
Speciation and localization of Eu(III) in fungus?

Microscopy and spectroscopy

Toward a better understanding of the transfer and accumulation mechanisms…

Influence of Eu(III) concentration on the mycelium growth (liquid medium)

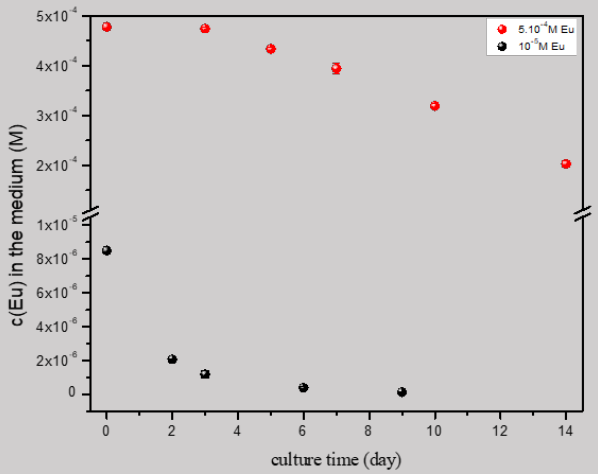
2 days after inoculation



$m_{mycelia}$ (mg)	25	21	22	6	8.5	1.5

Low impact on the culture growth

strong impact on the culture growth

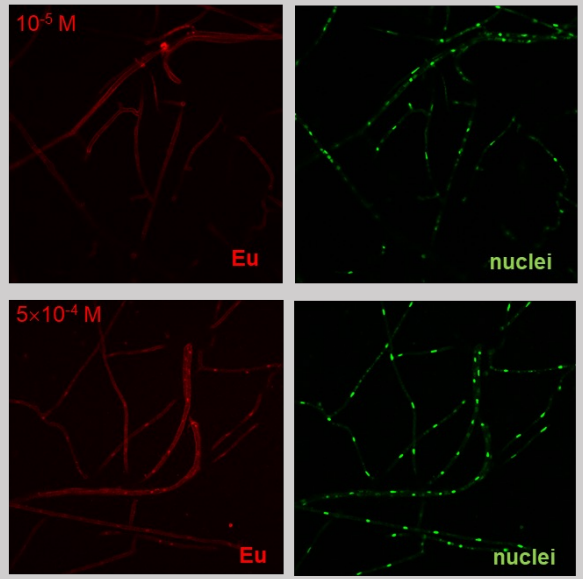


10^{-5}M

c_{Eu} = 1120 ± 88 µg Eu/ g of dry mycelium

5×10^{-4}M

c_{Eu} = 84 140 ± 8600 µg Eu/ g of dry mycelium



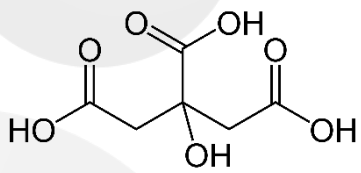
Influence of Eu(III) concentration on the mycelium growth (liquid medium)

Speciation modelling

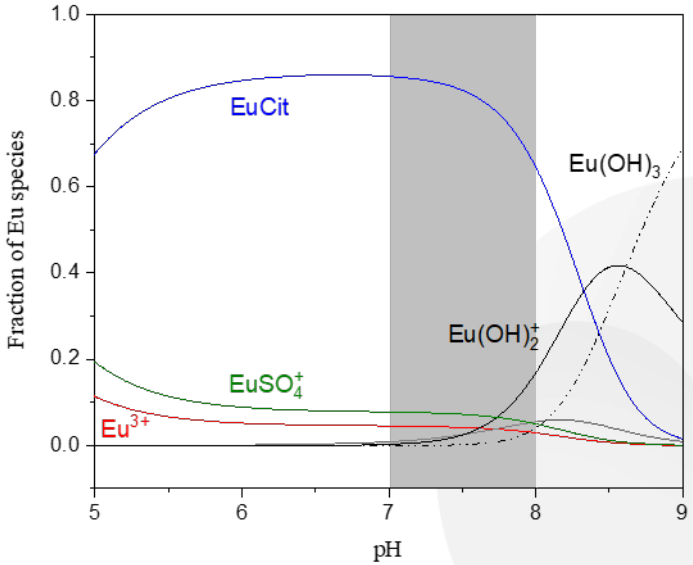
I = 0, T = 25 ° C

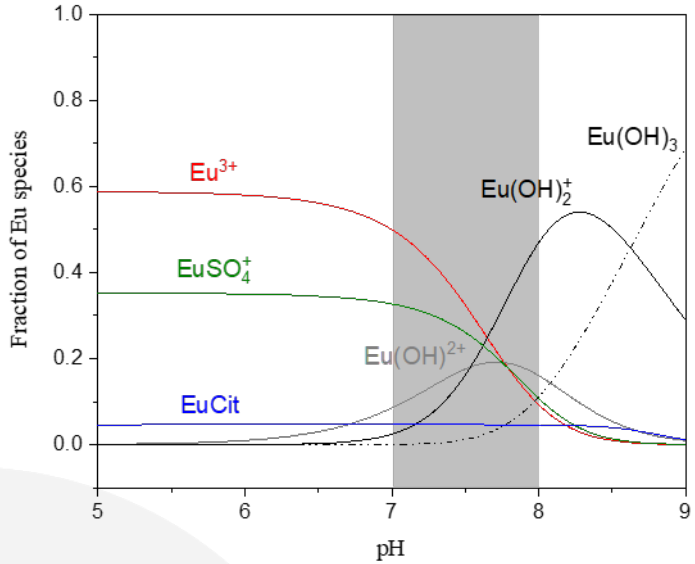
$c_{Eu} = 10^{-5}$ M

$C_{citrate} \sim 2\times10^{-5}$ M



$c_{Eu} = 5\times10^{-4}$ M

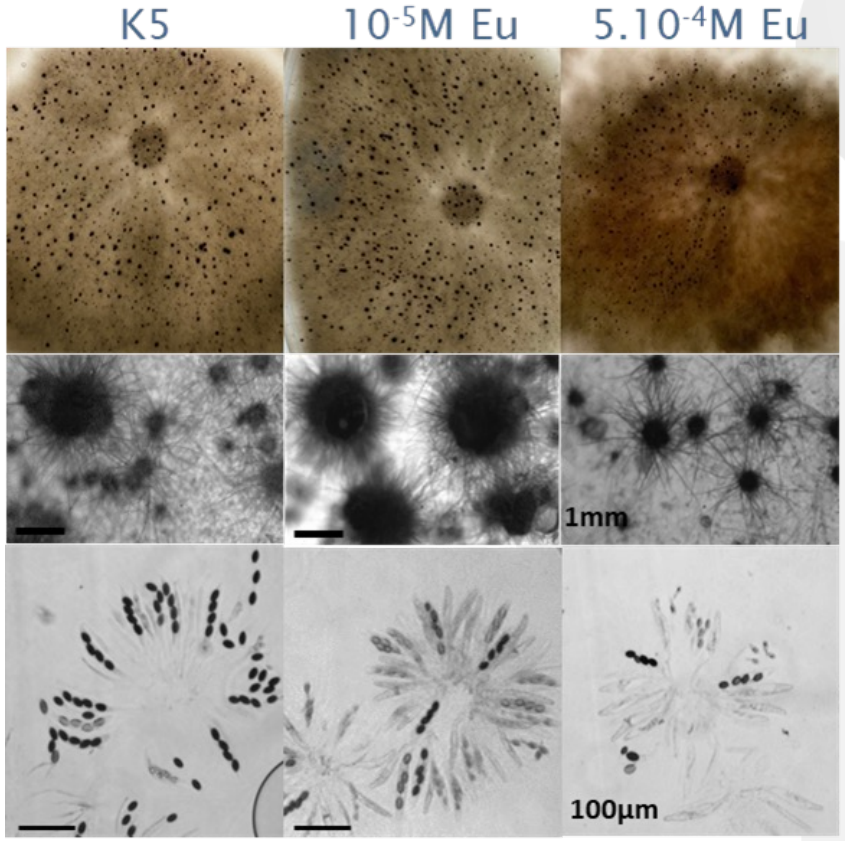




Hypothesis : Different mechanisms due to different Eu speciation in the culture medium, maximum of Eu tolerance or sorption capacity reached

Influence of Eu(III) concentration on the sexual reproduction (solid medium)

The sexual reproduction requires the use of a solid medium (agar) *and the presence of potassium*, which prevents the separation of the mycelium from the medium.



- Eu(III) **delays** the appearance of **perithecia** and **ascospore production** :

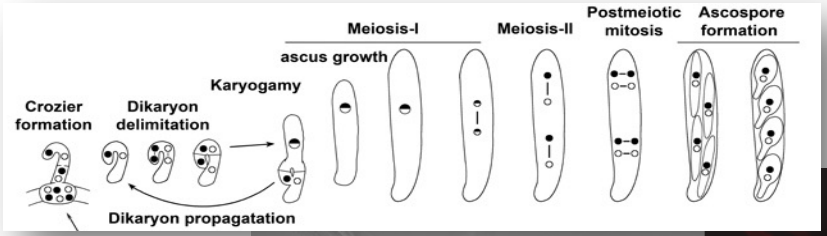
  + 1day at $c_{Eu} = 10^{-5}$ M

  +5 days at $c_{Eu} = 5 \times 10^{-4}$ M

- Eu concentration in perithecia :

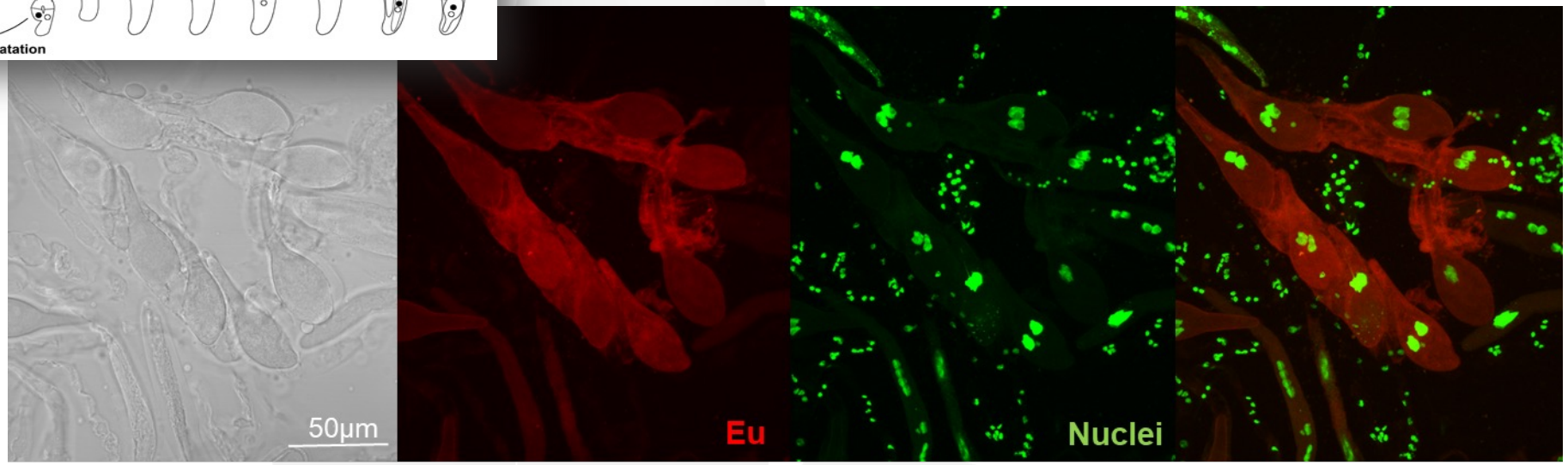
  89 (10^{-5}M) and 1021 (5×10^{-4}M) µg Eu/ g of dry perithecia

Influence of Eu(III) concentration on the sexual reproduction (solid medium)



Fluorescence confocal microsocopy

Nuclei are labelled with MCM1-GFP protein



Visualization of the localization of Eu in perithecia after contamination with c_{Eu}= 10^{-5} M allows to locate Eu in asci and ascospores

→ **Eu accumulation occurs during asci differentiation and is mainly localized in mature ascospore**

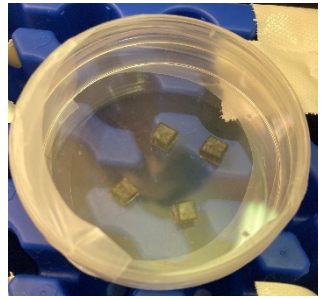
Outlook

The **knowledge of the speciation** in the fungus compared to the initial is **still necessary** for a better understanding of the transfer mechanisms

→ limitations of spectroscopic techniques (detection limits or only first shell of coordination)

Use of TOF-SIMS (ANDROMEDE)

- analysis of europium in different samples of increasing complexity : standards - different parts of the fungus (mycelium and perithecia) at different stages of development
- optimize sample preparation protocols
- Combine with other techniques



Thank you for your attention

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G. Creff
A. Jeanson
C. Den Auwer

MITI adaptation du vivant

NEEDS