Accumulation of actinides in the mushroom *Podospora anserina*

Pôle Énergie & Environnement – Équipe RAPHYNEE

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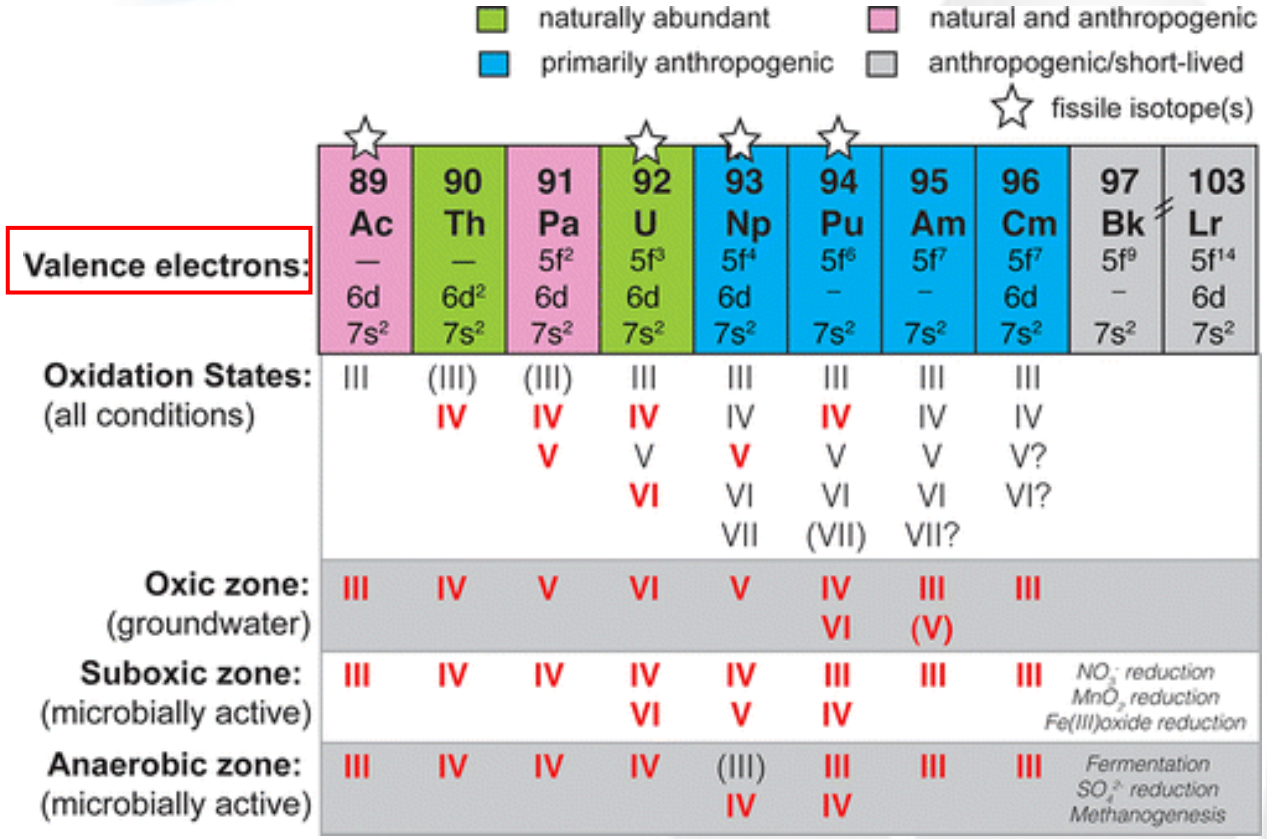
Actinide Behavior in the Environment

Sources of An in the environment are various (natural and anthropogenic)



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

Oxidation states : one of the most important actinide mobility-governing factors

$$Pu^{4+} > Pu^{3+} > PuO_2^{2+} > PuO_2^{+}$$

strong complexes
weak mobility

weak complexes
strong mobility

different actinide behavior in the hydrosphere, geosphere and biosphere

Mushroom

1. ubiquitous and good metal accumulator
2. important role (decomposer) in the food chain
3. very limited study

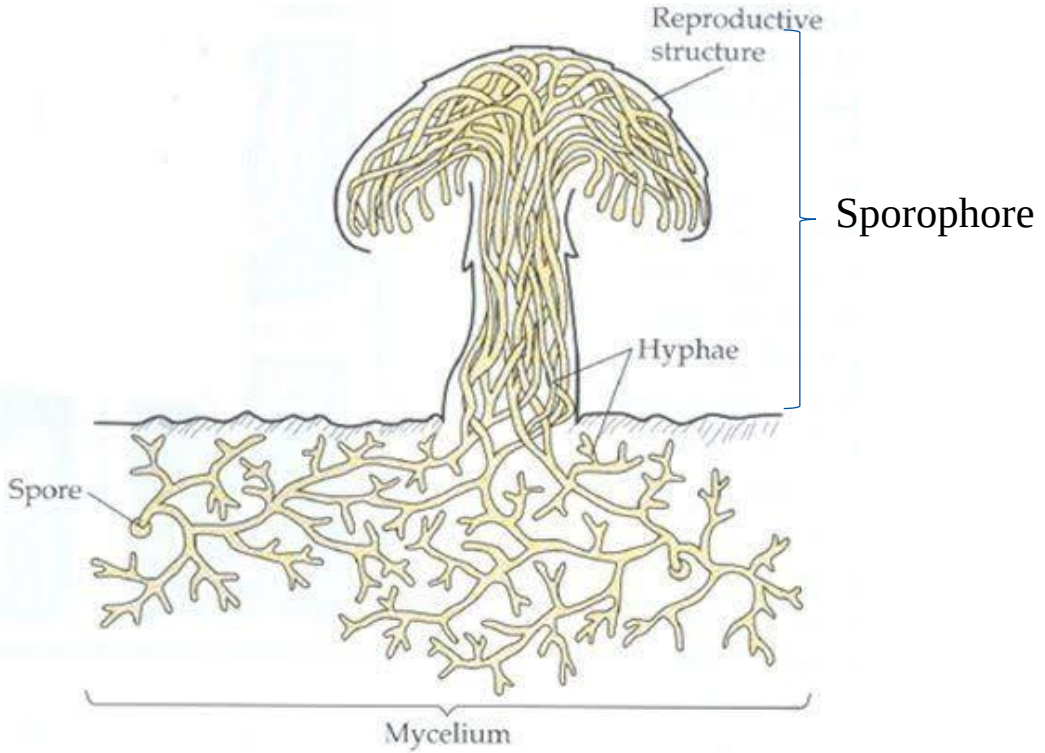
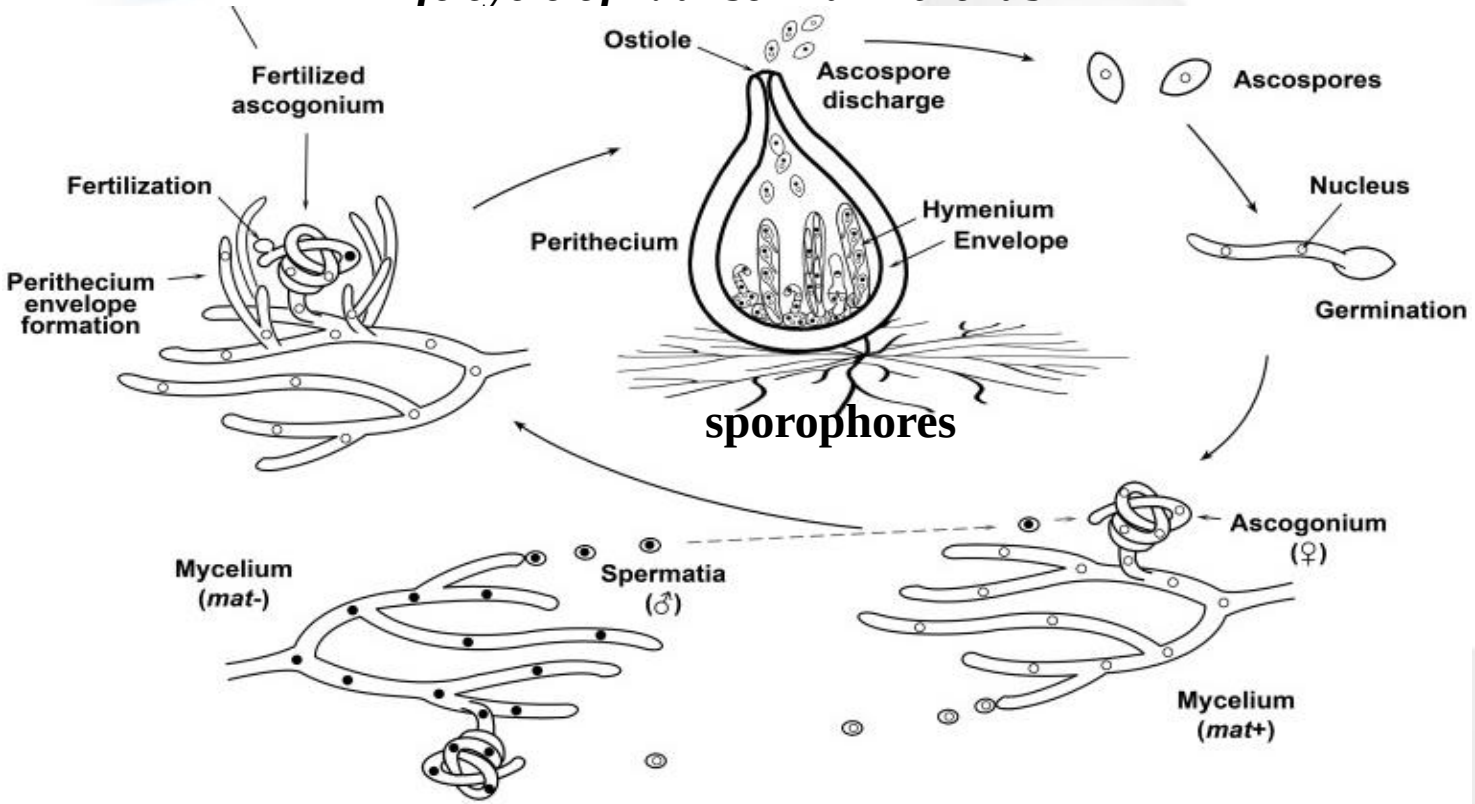


Illustration of basic morphology of mushroom

Species selected : *Podospora anserina*

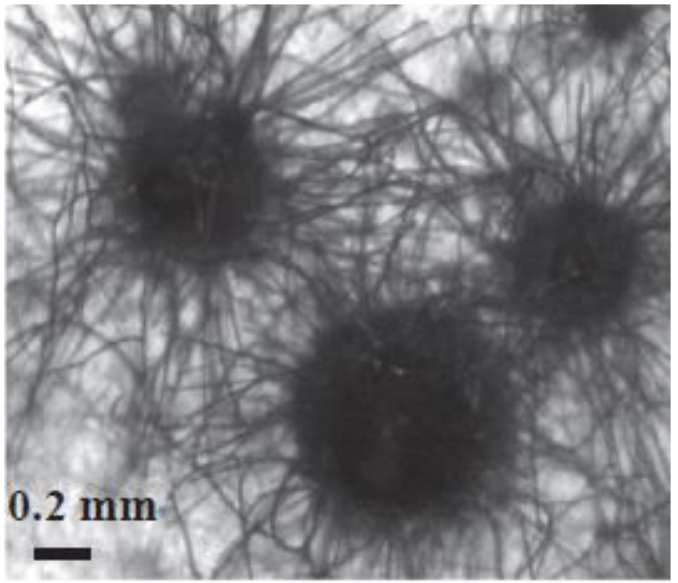
P. anserina: filamentous ascomycete mushroom, usually be observed on the excretion of herbivorous animals.

Life cycle of P. anserina in the lab



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

a complete cycle (mycelial growth and sexual reproduction) in the lab: 7 to 10 days at 27 ° C



Xie *et al.*, Fungal Genetics and Biology, 2018, 116

advantage

- Simplicity of culture, short life cycle and similar physiology with macro mushrooms (extrapolation)
- Expertise in the culture and the biochemistry of *P. anserina*

Methodology

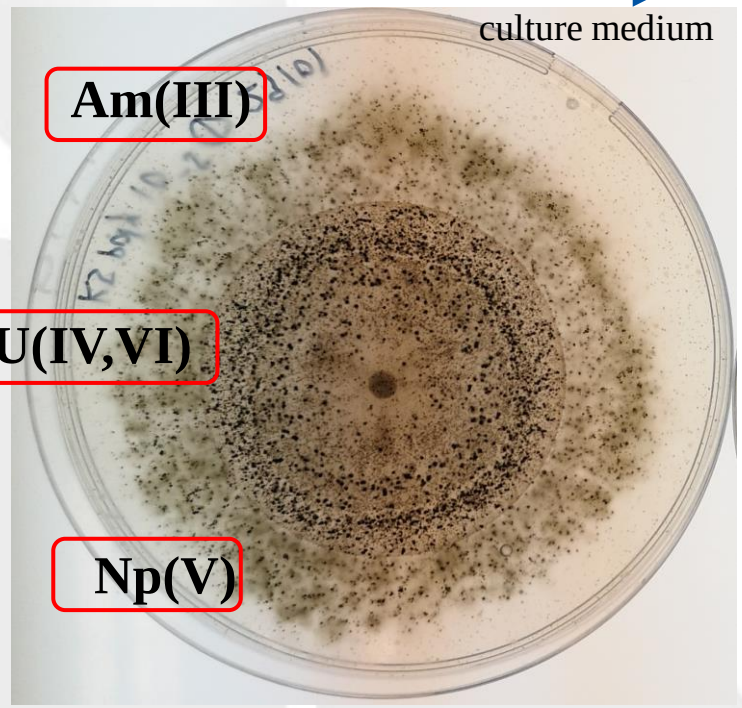
Speciation in the culture medium

Composition	Concentration (mg/L)	Concentration (M)
$MgSO_4$	250	2.08×10^{-3}
Urea	500	8.33×10^{-3}
Biotine	0.05	2.05×10^{-7}
Thiamine	0.05	1.89×10^{-7}
Citric acid	5	2.60×10^{-5}
$ZnSO_4$	5	3.11×10^{-5}
$CuSO_4$	0.25	1.56×10^{-6}
$MnSO_4$	0.05	3.31×10^{-7}
H_3BO_3	0.05	8.06×10^{-7}
$Na_2MoO_4 \cdot 2H_2O$	0.05	2.07×10^{-7}
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	1	2.55×10^{-6}
Dextrin	5500	-

culture medium of P. anserina (pH7)

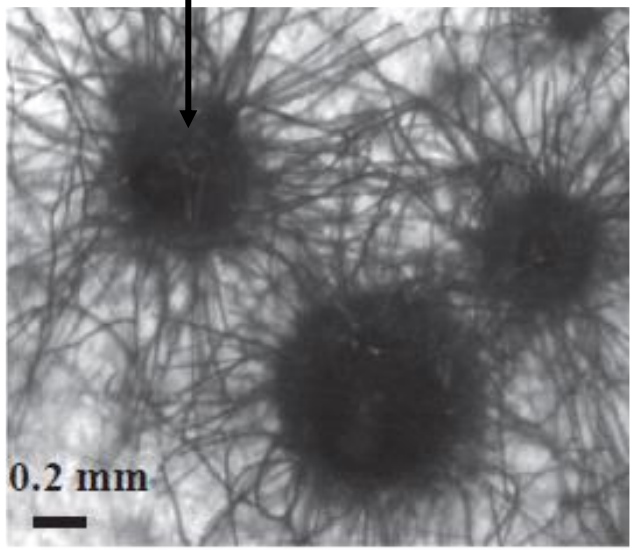
Theoretical speciation,
spectroscopic techniques: XAS, UV-Vis, FTIR

Accumulation



*Eu(III) as analog of Am(III) for the moment

Speciation/Localization in the mushroom



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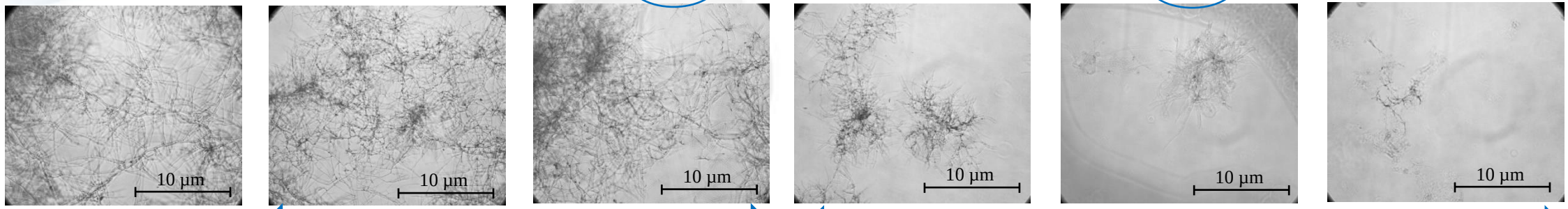
Microscopy / spectroscopic techniques

Influence of Europium concentration on the mycelium growth

2 days after inoculation

C_{Eu} (M) 0 | 10^{-6} | 10^{-5} | 10^{-4} | 5.10^{-4} | 10^{-3}

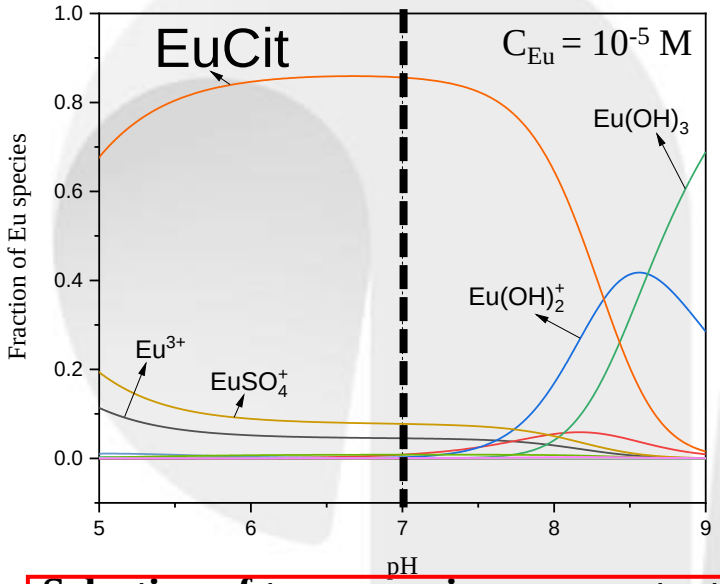


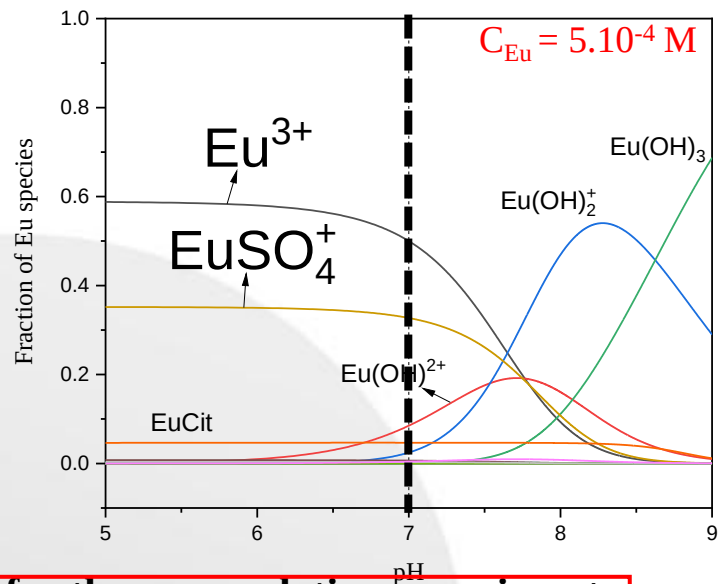
Low impact

Strong impact

Increase of C_{Eu} in the medium → Decrease of mycelium growth

Theoretical speciation of the europium in M2 medium at $pCO_2 = 0$, I = 0, T = 25° C





c(Citrate)=2.6×10^{-5}M

Selection of two europium concentrations for the accumulation experiments 10^{-5} M (low impact) and 5.10^{-4} M (strong impact)

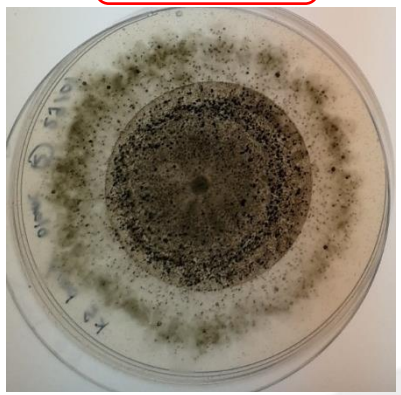
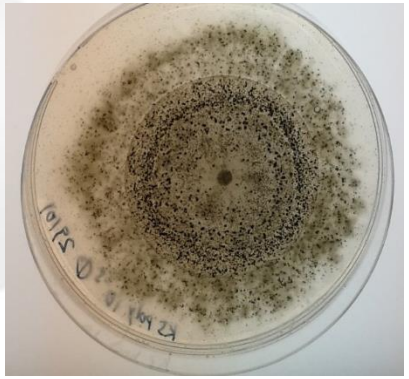
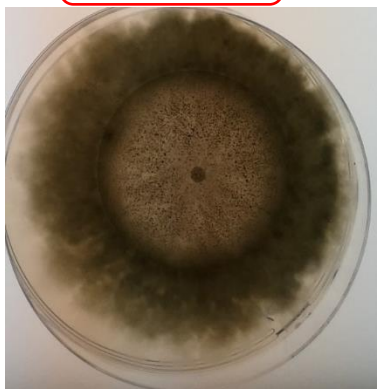
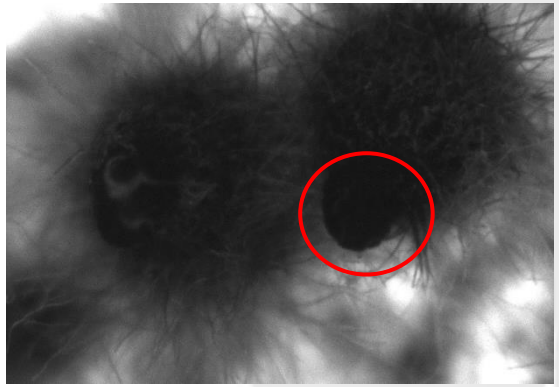
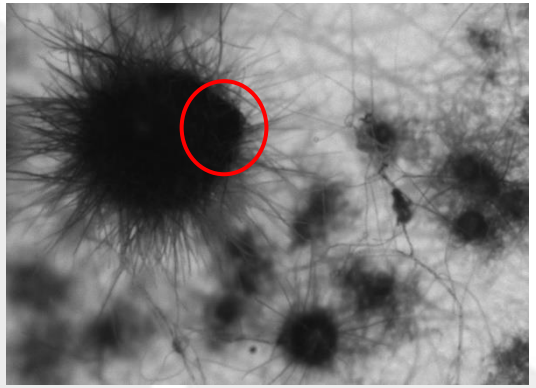
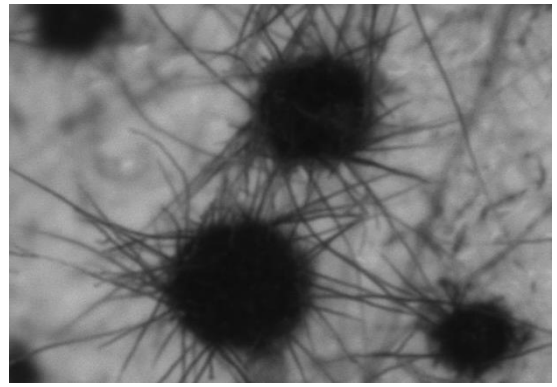
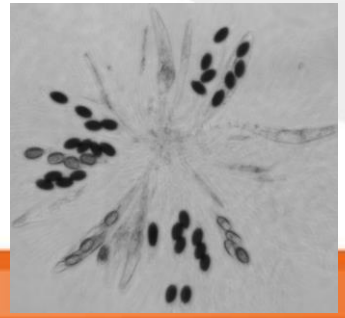
Influence of Europium concentration on the sexual reproduction

10 days after inoculation

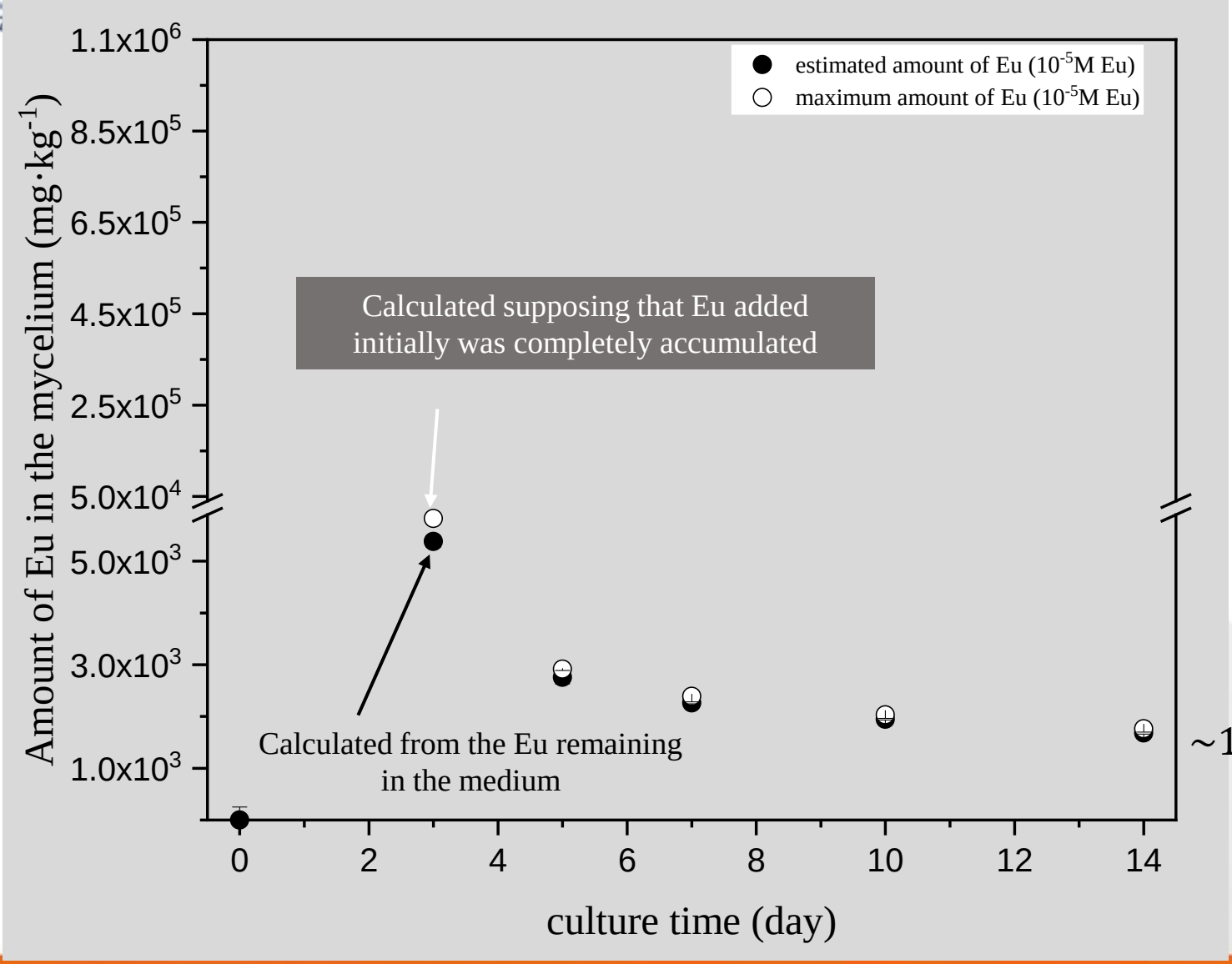
perithecia

Open the perithecia and observe the spores

spores

Blank	10^{-5}M Eu	5.10^{-4}M Eu
		
		
	fewer perithecia, less mature	few perithecia, not mature
		**No spore was observed**

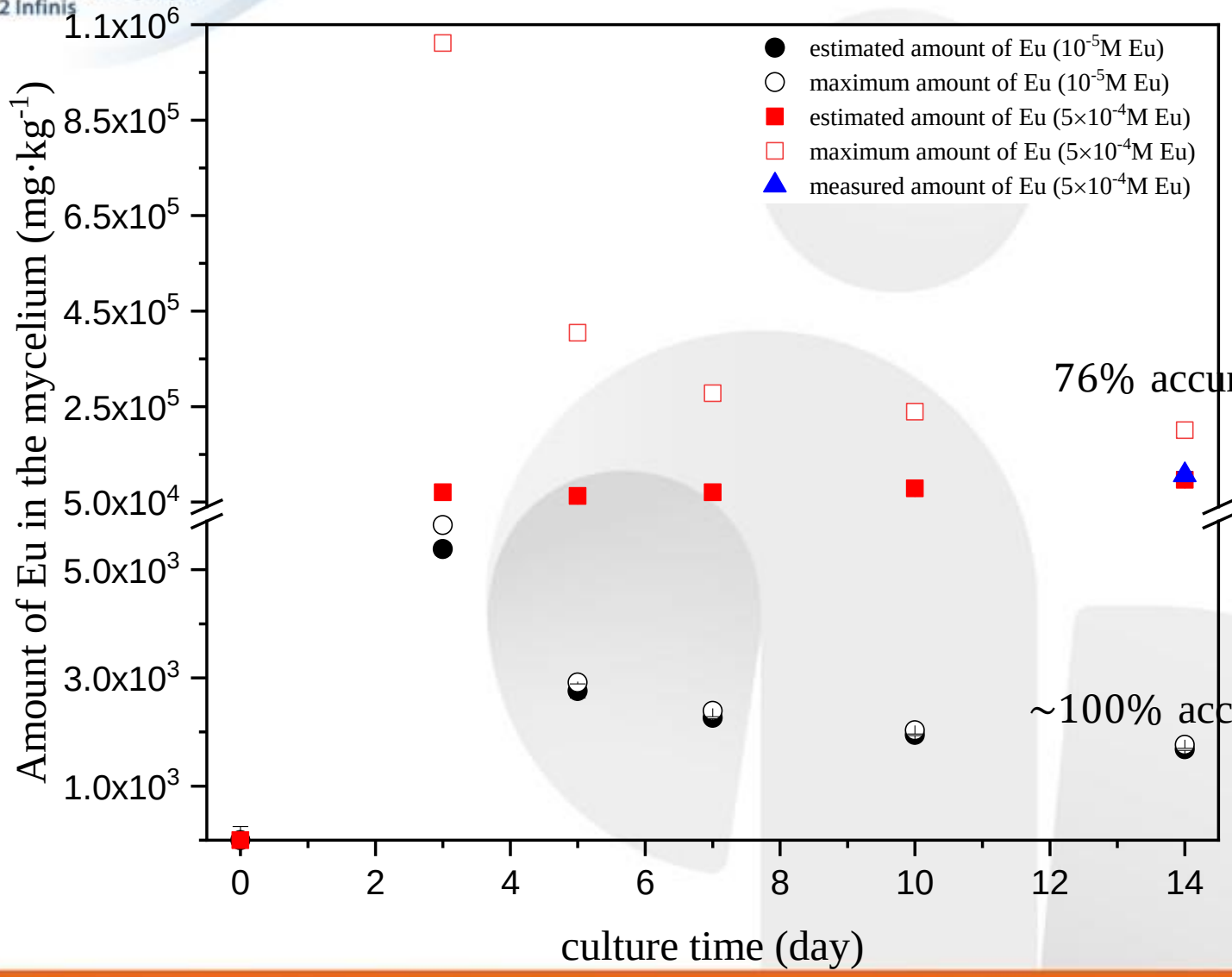
Eu accumulation monitoring in the mycelium



- Bioaccumulation of Eu estimated by $[Eu]_{D0}$ - $[Eu]_{measured}$ in the medium related to the mycelial biomass
- The maximum amount corresponds to the calculated amount when the mycelium accumulate all of the Eu present in the medium

~100% accumulation

Eu accumulation monitoring in the mycelium

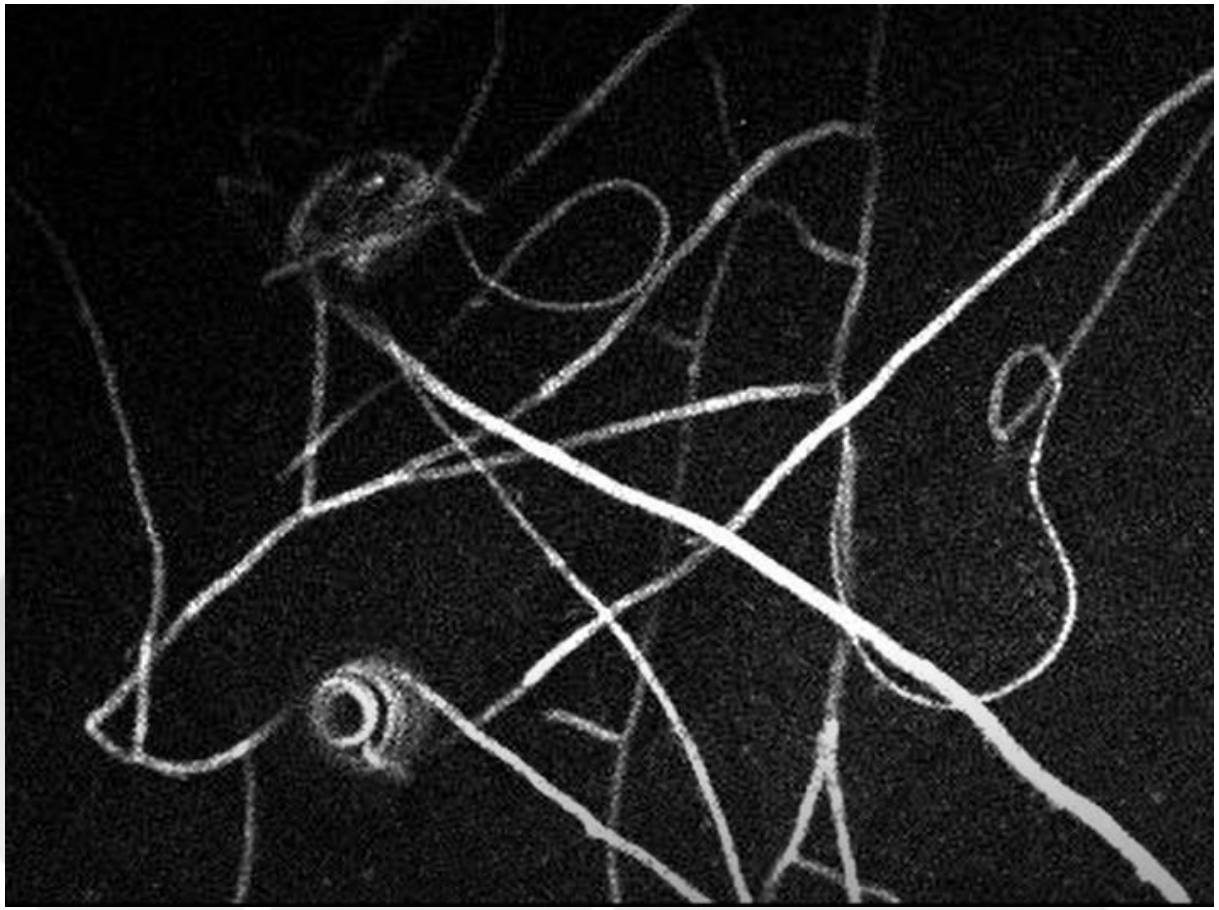


76% accumulation

~100% accumulation

- Bioaccumulation of Eu estimated by $[Eu]_{D0}$ - $[Eu]_{mesured}$ in the medium related to the mycelial biomass
- The maximum amount corresponds to the calculated amount when the mycelium accumulate all of the Eu present in the medium
- The amount of Eu estimated from the medium was verified by the amount measured directly in the mycelium: the mycelium effectively accumulated Eu.

Understanding the transfer mechanisms by imaging technology



Imaging of Eu-loaded (5.10^{-4}M Eu) mycelia by Fluorescence microscopy

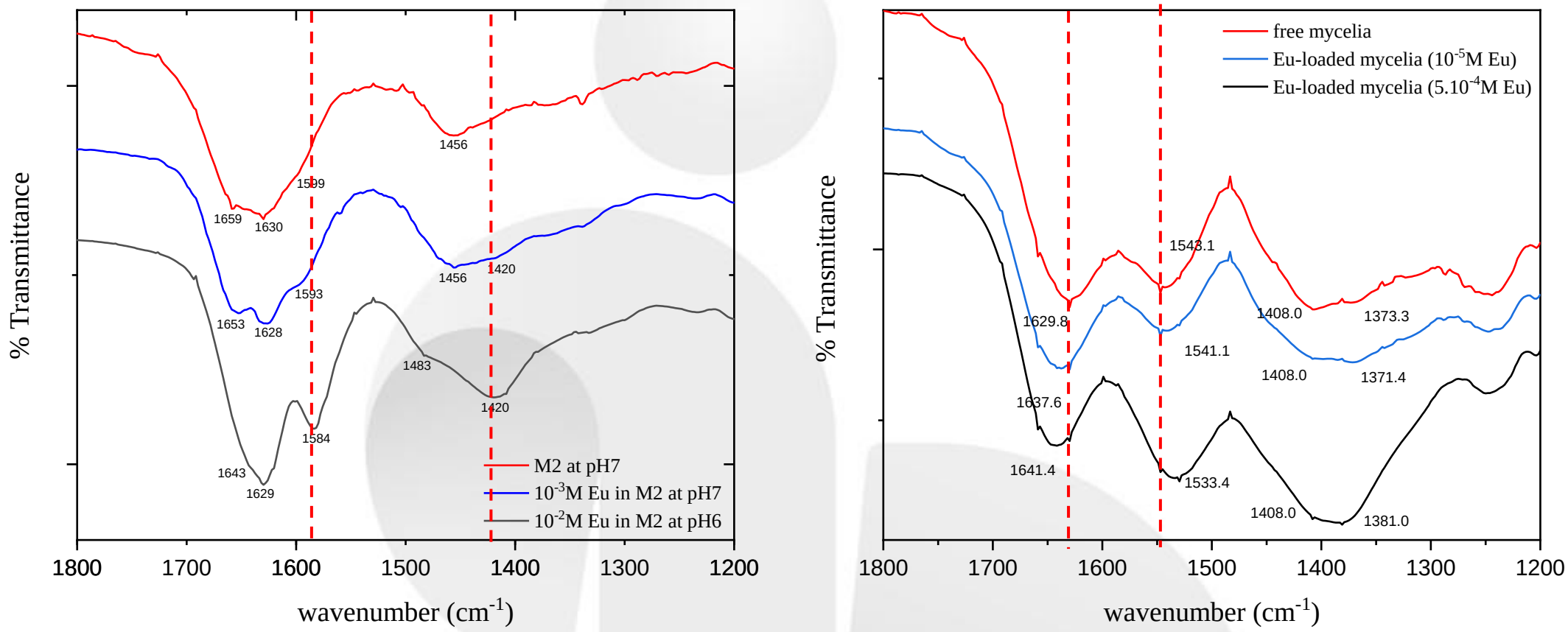
➡ Eu: homogeneously distributed in the mycelia

Understanding the transfer mechanisms by spectroscopic study

culture medium

mycelia

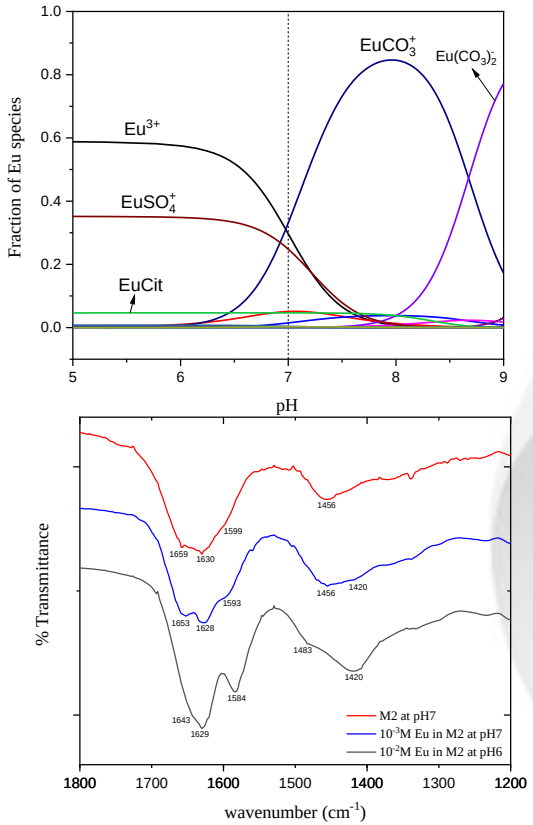
(100 mg Eu·g^{-1} DW)



ATR-FTIR spectra of Eu in the culture medium (left) and Eu-loaded mycelia (right)

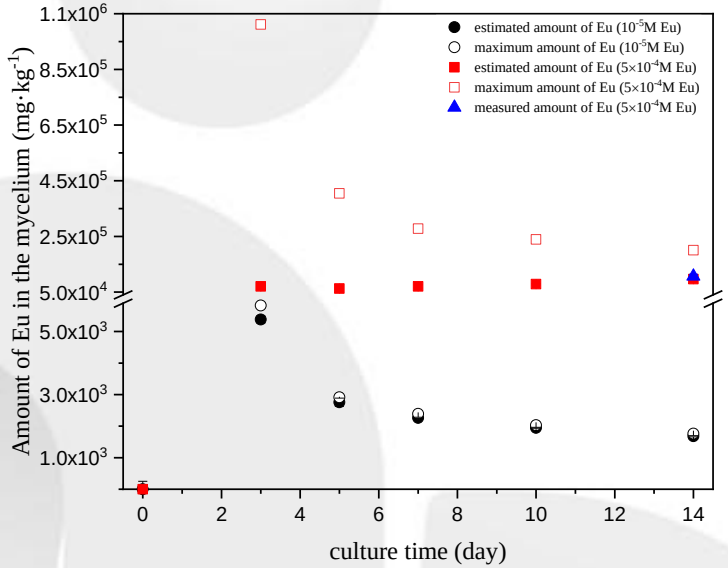
Conclusion and perspectives

Speciation in the culture medium



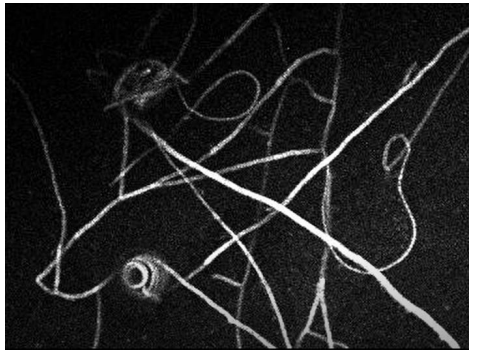
Theoretical speciation in comparison with spectroscopic techniques

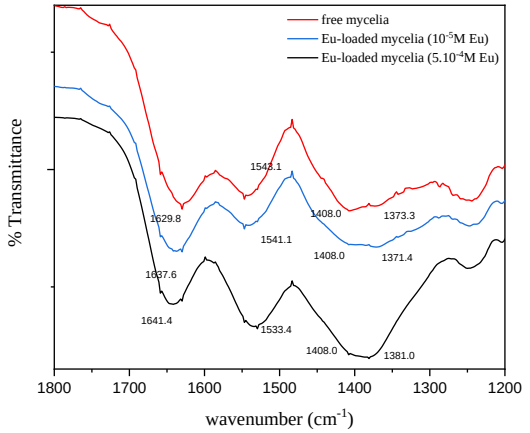
Accumulation



Eu accumulation monitoring in the culture medium/mycelium

Speciation/Localization in the mushroom





Imaging technology in comparison with spectroscopic techniques

Conclusion and perspectives

Mechanisms of accumulation ?

- Speciation study: characterization by spectroscopic techniques (UV-vis spectrophotometry, XAS, and TRLIFS)
- Investigation of Eu concentration in the perithecia/spore (next month)
- Adaptation of the protocol in our laboratory for further study with uranium and neptunium.

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