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Ecotoxicological impacts of 17- β -estradiol and related chemicals on soil micro-organisms

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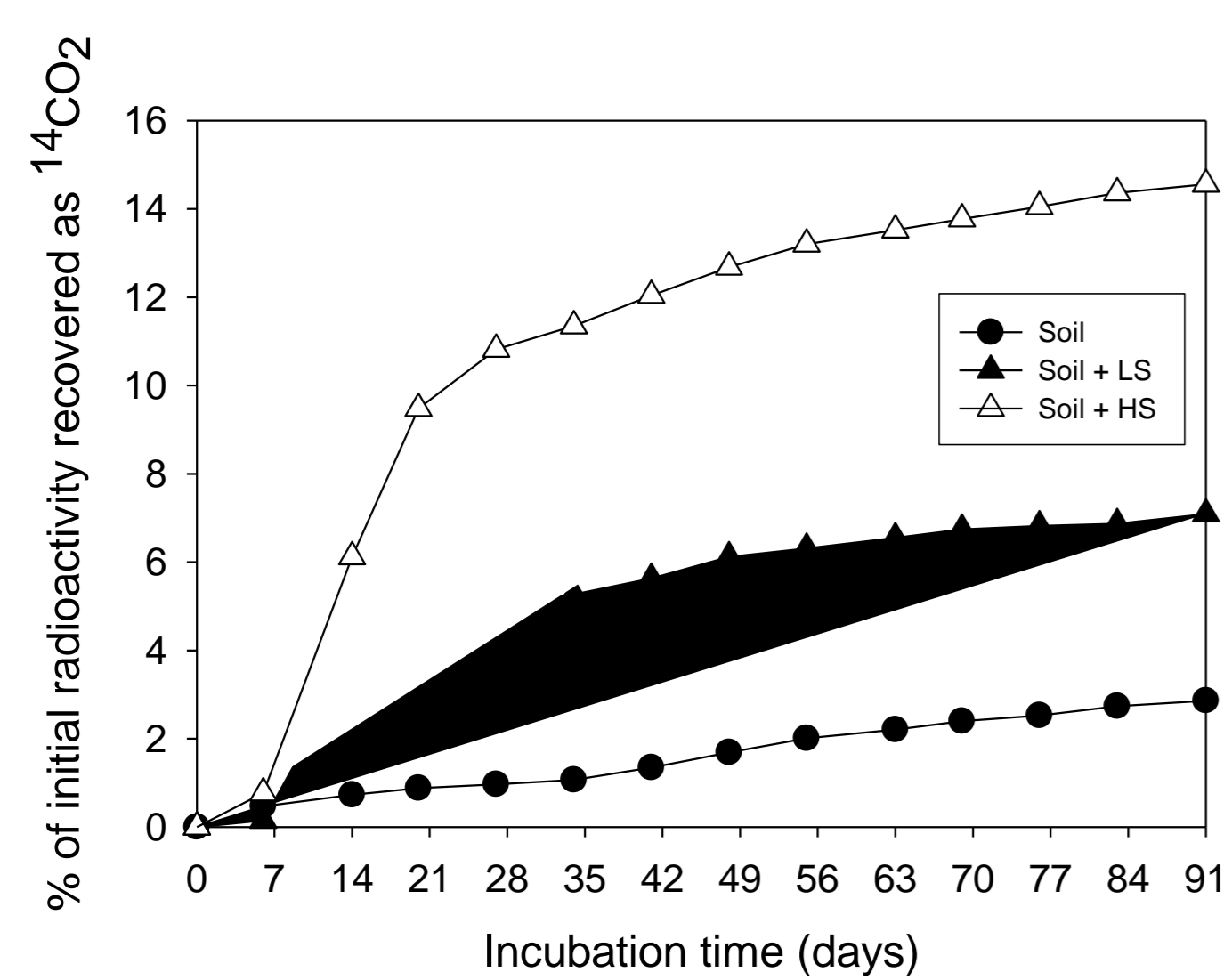
Rationale / objectives / strategy

- Natural and synthetic hormones enter in wastewater treatment plants, and accumulate in sewage sludge.
- The environmental risk due to these bioactive compounds remains to be determined.
- **Our aims are to determine the fate and the ecotoxicological impacts of steroid hormones, when they enter the soil ecosystem after spreading contaminated sludge on agricultural soils.**
- A **composted sludge** from urban origin (90000 equivalent inhabitants), spiked with ¹⁴C-estradiol, was applied at two doses onto an agricultural soil, and incubated in microcosms to assess the fate of the chemical.
- The effect of a mixture of natural and synthetic steroids was also checked on the microbial mineralization of the herbicide atrazine in soils.
- The mixture of hormones was used to assess possible impacts on pure fungal cultures.

Fate of ¹⁴C-estradiol in soil /sludge systems

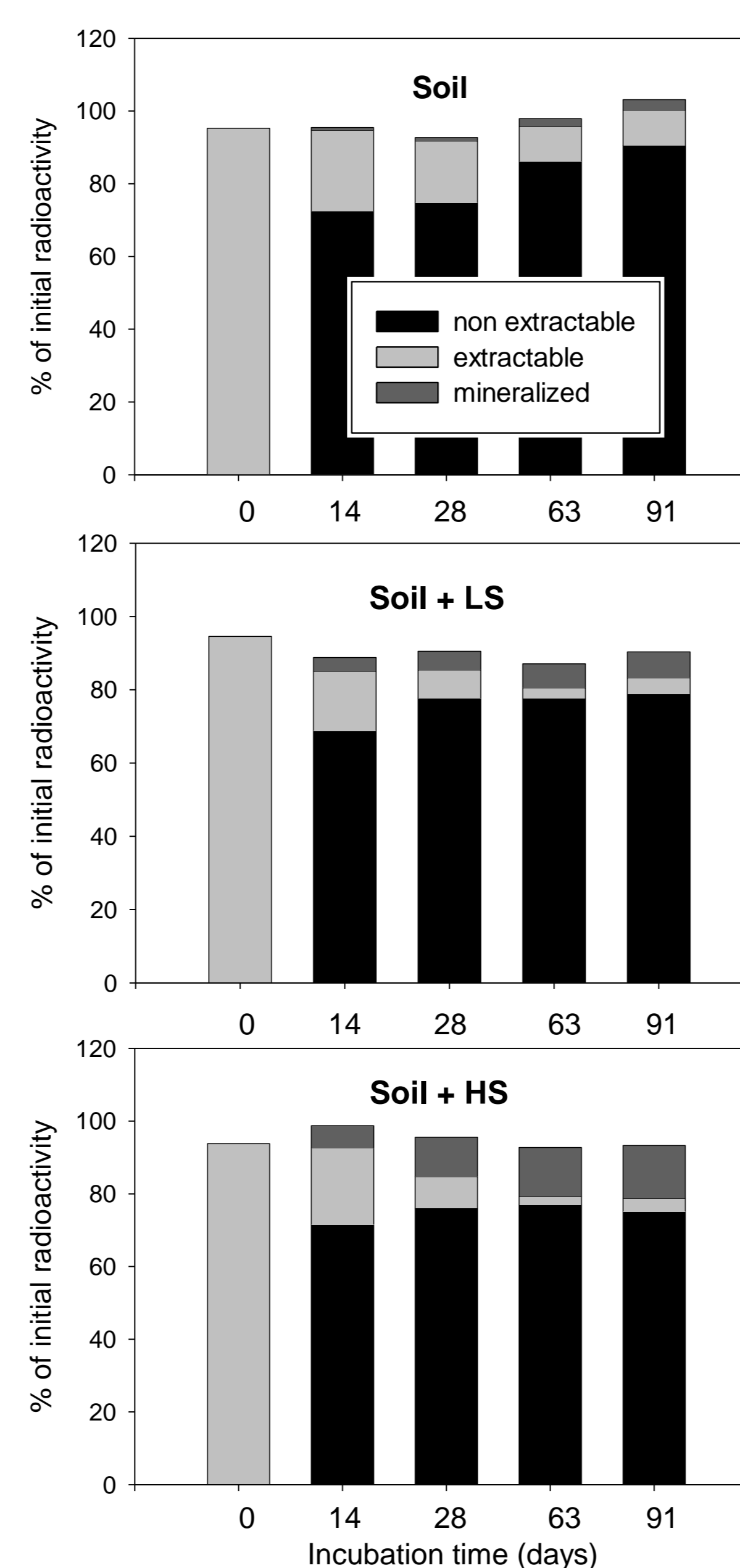
The soil was treated with ¹⁴C-estradiol at 75 μ g/kg, or low sludge (LS, corresponding to 3 T sludge / ha d.m.) spiked with estradiol to provide 75 μ g/kg soil, or high sludge (HS, 30 T sludge / ha d.m.) providing 750 μ g/kg soil.

Estradiol mineralization



- The mineralization of estradiol is low when the chemical is spread directly onto the soil. It is increased when provided by the sludge.
- The main part of the radioactivity is stabilized in the soil/sludge systems as bound residues. Estrone and estradiol are detected in equal amounts in the solvent extractable fraction.

Mass-balance analysis



Mobility of ¹⁴C

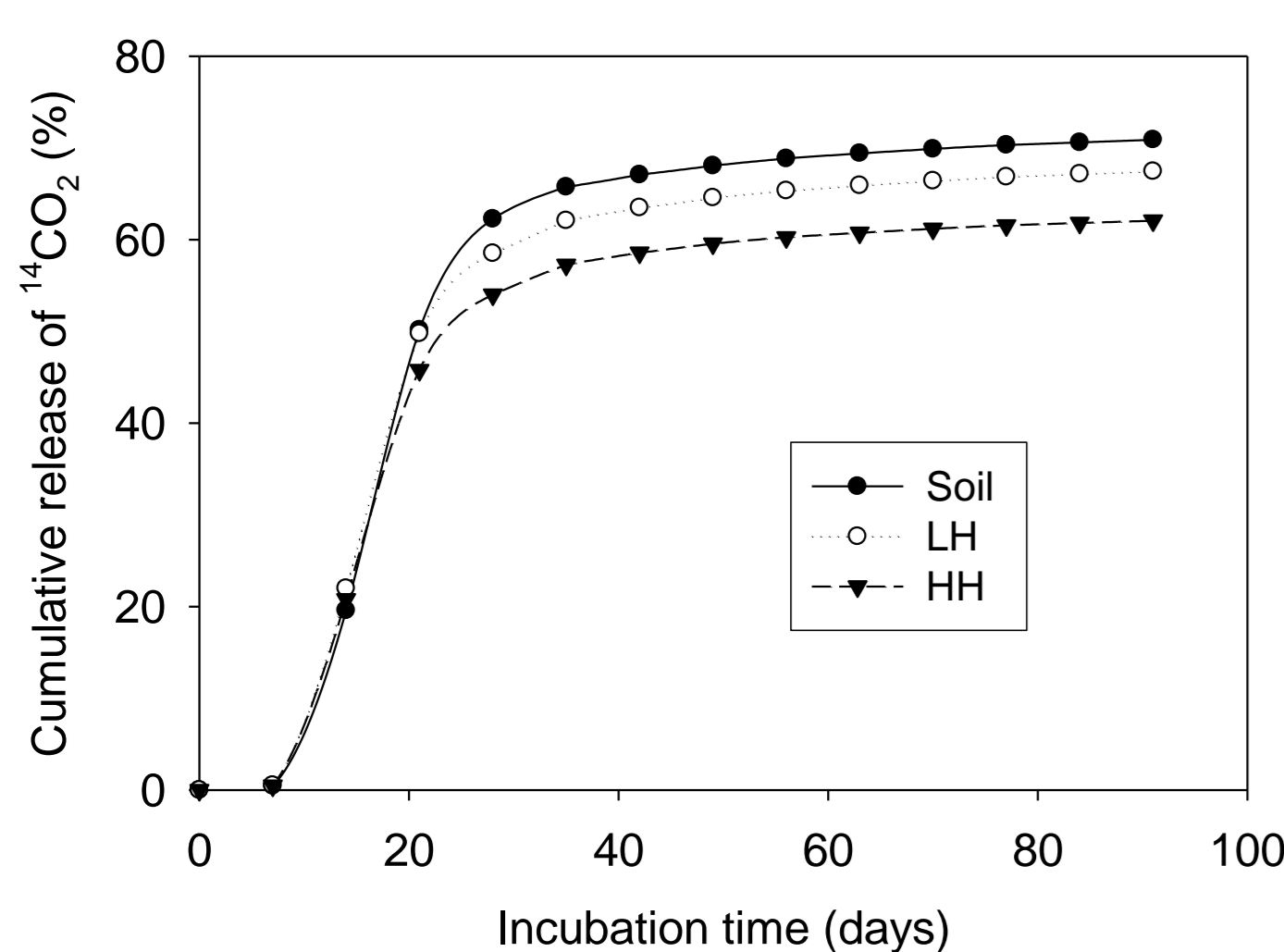
Rainfalls (equivalent to 20 mm water) were simulated after 28 and 91 days of incubation. Solvent extractable radioactivity in the two soil layers and water soluble radioactivity in leachates were then determined.

Compartment	% of initial radioactivity					
	28 days event			91 days event		
	Soil	LS	HS	Soil	LS	HS
0-10 cm layer	13.18	3.76	6.27	7.10	1.54	2.63
10-20 cm layer	3.80	3.97	2.39	2.68	2.88	1.46
leachate	0.02	0.10	0.03	0.04	0.05	0.01

- Rainfall events result in the mobility of the 14-carbon through the soil to leachates. Very low amounts of 14-carbon are detected in the leachates.

Impacts on microbial activity

The effects were assessed through the microbial transformation of the herbicide atrazine. A mixture of estradiol, estrone, estriol and ethynyl-estradiol was applied on soil columns (10 x 5.5 cm \varnothing previously treated with labelled atrazine, 1.5 kg/ha) after 15 days of incubation. Each hormone was provided at 1.35 (LH) or 6.73 (HH) mg/kg soil.



- Even at high concentration, the mixture of hormones do not affect the mineralization of atrazine.

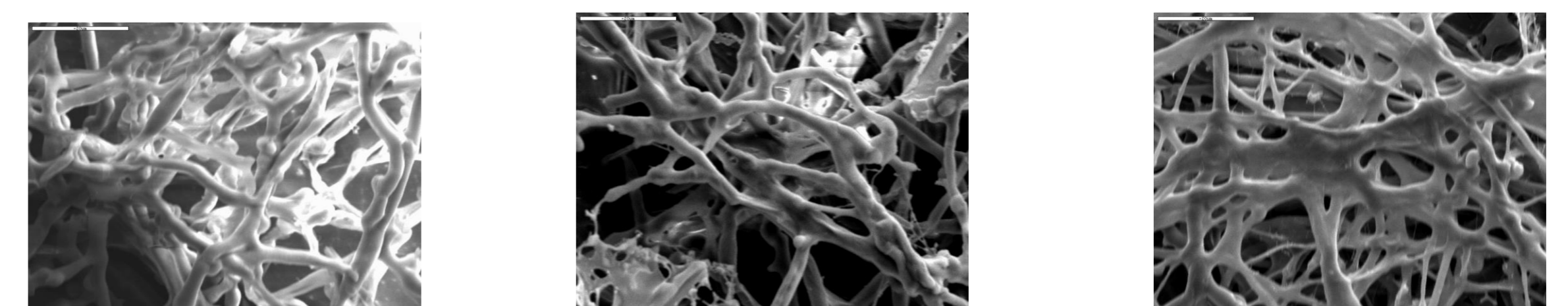
Impacts on filamentous fungi

The effects on the growth, the reproduction and the fungal enzymatic activity were assessed by treating pure cultures of filamentous fungi with the mixture of estradiol, estrone, estriol and ethynyl-estradiol. Each hormone was provided from 10 ng to 100 μ g/L culture medium.

- The growth of *Trametes versicolor*, *Phanerochaete chrysosporium*, *Fusarium solani*, *Fusarium oxysporum* and *Cunninghamella elegans* is not affected. The sporulation is affected, with maximal inhibitions or stimulations noticed at 1 μ g/L according to the strain. Laccases are not induced by the hormones.

The effects on fungal morphology were assessed by SEM after treating pure cultures of *Trametes versicolor* with each hormone provided at 1 mg/L.

Control estrone estriol



- Morphological defects of the fungus are noticed according to the chemical after 4 days of exposure.

Conclusions

- Estradiol applied to the soil by contaminated sewage sludge is mainly stabilized as bound residues.
- We were unable to evidence negative effects of the hormones (at environmental concentrations) on the microbial and fungal compartments.

Acknowledgements: The present study has been supported by the INSU Programme ECODYN (03CV117). It is intended to determine the dynamics and the ecotoxicological impacts of steroid hormones contained in urban wastewaters, sewage sludge and treated waters, on aquatic and soil ecosystems. We thanks N. Wolff for performing SEM studies.