Project "Dioxins : contamination and biodegradation"

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Introduction

✓ Aims

- Deepen the knowledge on dioxins degradation in grounds
- Find a "soft" cleanup technique for dioxins contaminated grounds

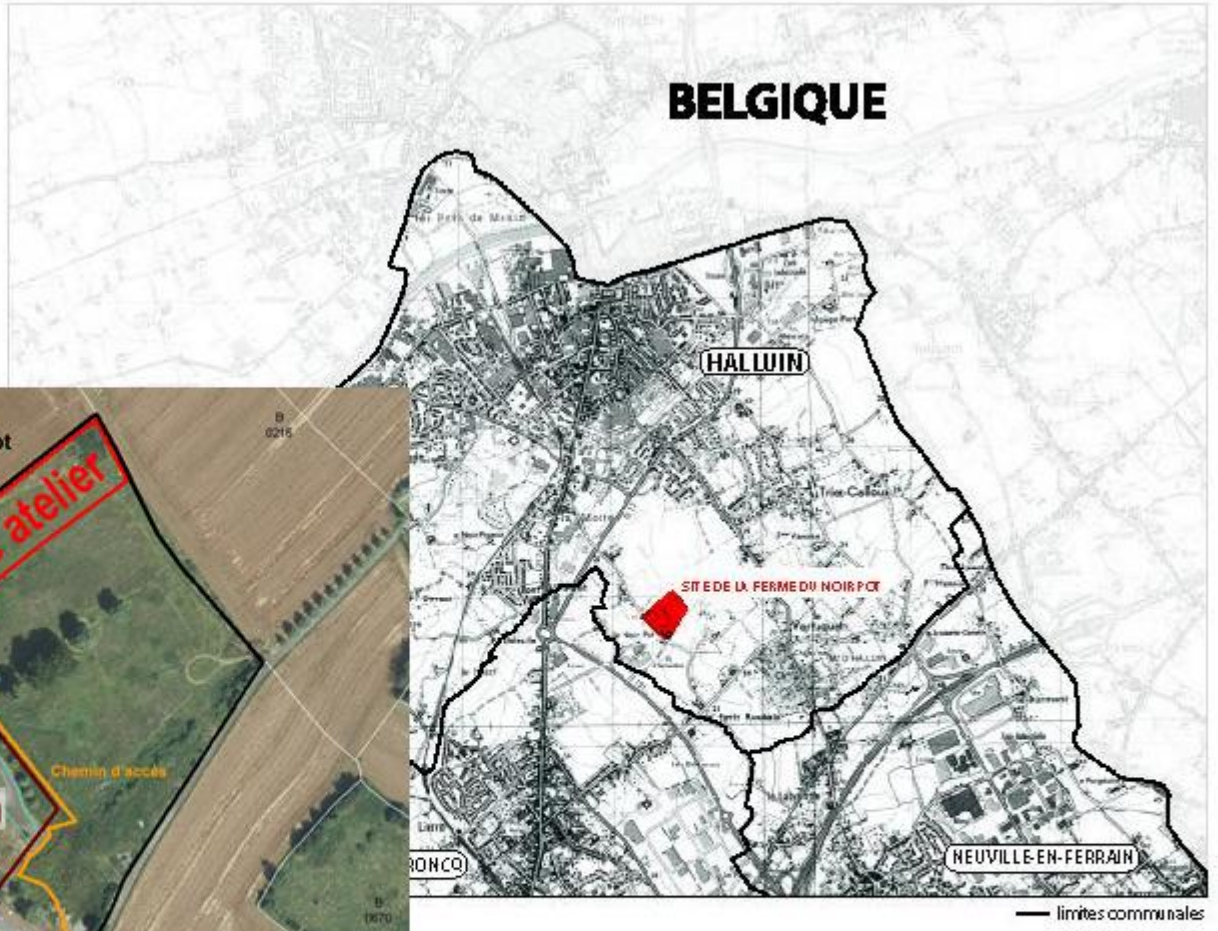
✓ Stakes

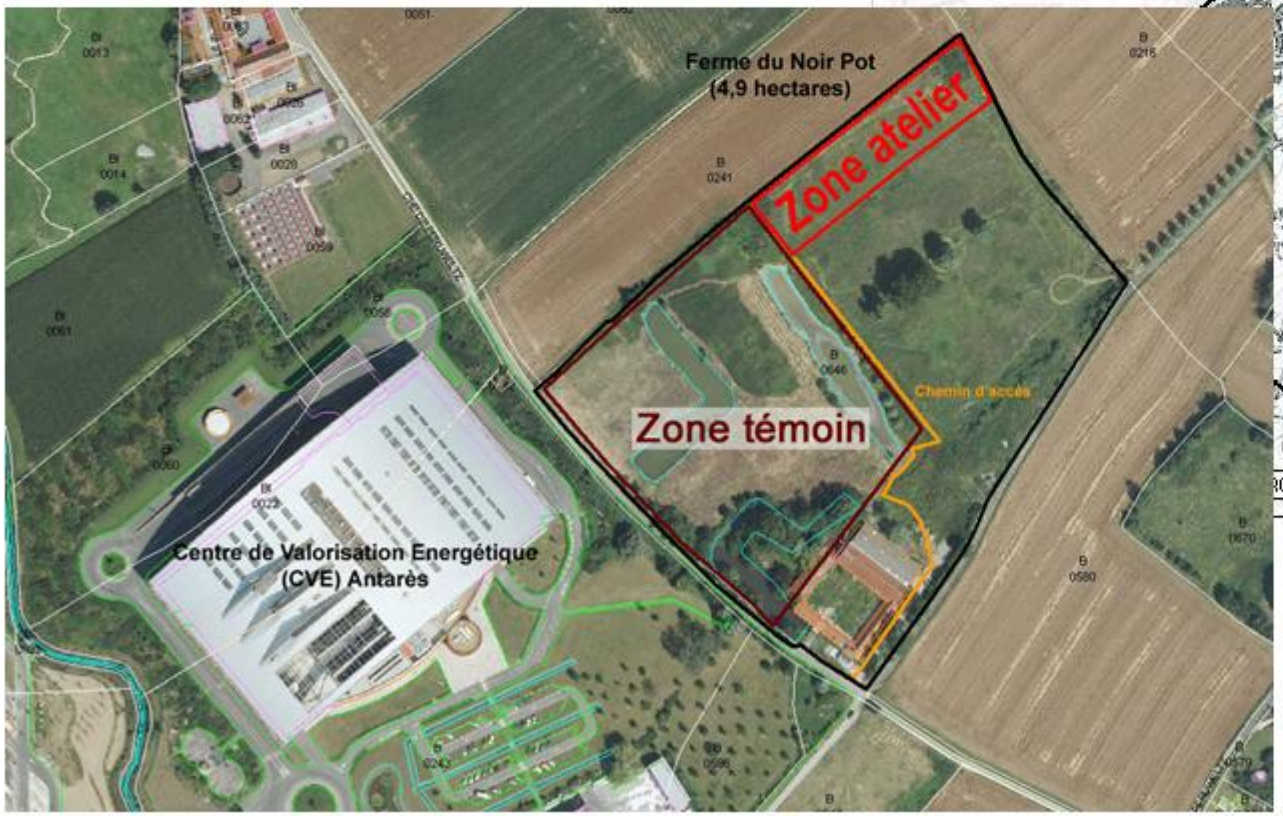
- Find natural spaces and/or quality agriculture
- Intend to produce in short circuit (truck farming, breeding) on Halluin
- Be able to "transpose" the cleanup technique into other contaminated zones

✓ Approach

- Use the degradation potential of the indigenous fungal biodiversity
- Experiments in laboratory and *in situ* – access to an experiment ground (3500 m^2)
- Three parts :
    - Evaluation of the dioxins contamination of vegetables
    - Development of biocompatible methods of remediation of dioxins polluted soils coupling an advanced chemical oxidation process to biodegradation by soil saprotrophic fungi
    - Role of arbuscular mycorrhizae in the phytoremediation of dioxins/furans soils


Halluin
Pas-de-Calais
62
Nord
59
Nord-Pas-de-Calais


BELGIQUE
HALLUIN
SITE DE LA FERME DU NOIR POT
NEUVILLE-EN-FERRAIN
limites communales


Ferme du Noir Pot
(4,9 hectares)
Zone atelier
Zone témoin
Chemin d'accès
Centre de Valorisation Energétique
(CVE) Antarès

Partners

ulco Université du Littoral Côte d'Opale Université Lille Nord de France – Pôle de Recherche et d'Enseignement Supérieur	UCEiV – Unité de Chimie Environnementale et Interactions sur le Vivant **Equipe Mycologie de Dunkerque**	Catherine RAFIN Etienne VEIGNIE
	UCEiV – Unité de Chimie Environnementale et Interactions sur le Vivant **Equipe Mycologie de Calais**	Anissa LOUNES HADJ SAHRAOUI Joël FONTAINE
INRA – Institut National de la Recherche Agronomique	**LAS d'Arras**	Antoine RICHARD Giovanni CARIA
	Unité Infosol d'Orléans	Nicolas SABY
Halluin 3R – Recherche • Réseau • Requalification	**Association Halluin 3R (Réseau, Recherche, Requalification)**	Annabelle CARLIER, coordinatrice du projet « Dioxines »

Part 1. Development of biocompatible methods of remediation of dioxins polluted soils, coupling an advanced chemical oxidation process to biodegradation by soil saprotrophic fungi

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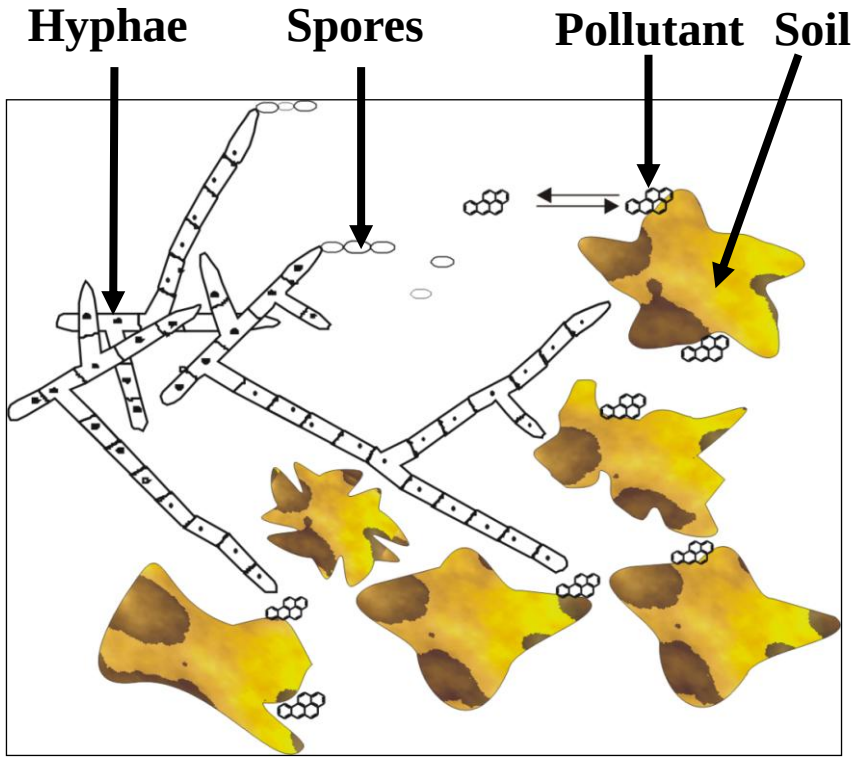
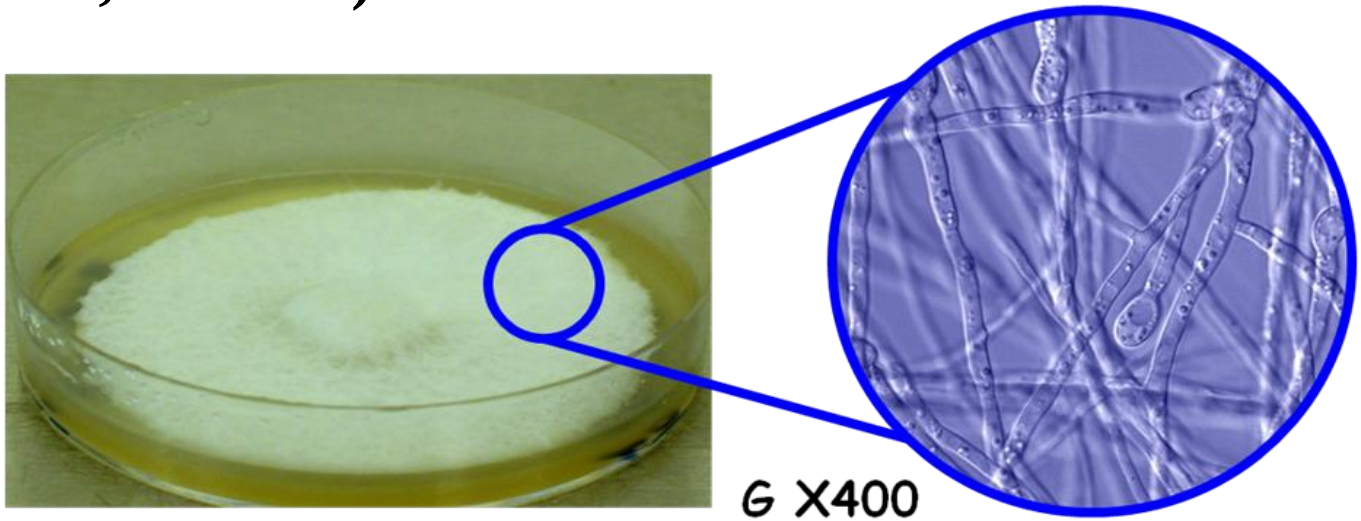
Saprotrophic telluric fungi



- ✓ Eukaryotes
- ✓ Ubiquitous in terrestrial ecosystems
- ✓ Filamentous branching growth habit
- ✓ Chemoheterotrophic metabolism
- ✓ Reproduction: sexual and/or asexual (spores)
- ✓ Diversity of fungal communities: free-living, symbiotic, pathogens
- ✓ Interactions with other partners (bacteria, plants, fauna…)

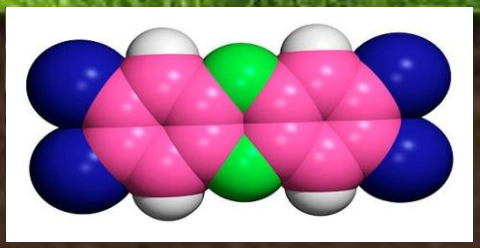


Fusarium solani

Aims of the project

Sources

Transfert



Persistant Organic Pollutants POPs : Dioxins, PAHs

Modified Starch

Soil saprotrophic fungi

- more soluble
- used as reactive matrix

Chemical oxidation

Biological oxidation

Isolation of saprotrophic fungi from polluted soil samples

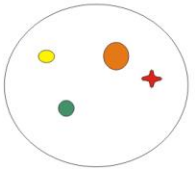
Echantillons de sols pollués

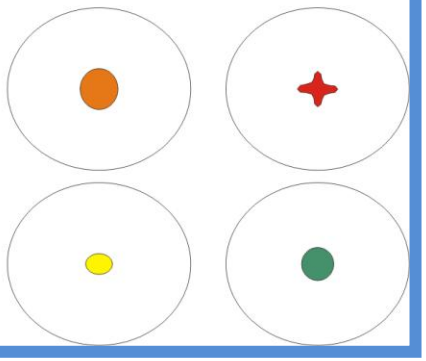
1

Isolement de champignons

2

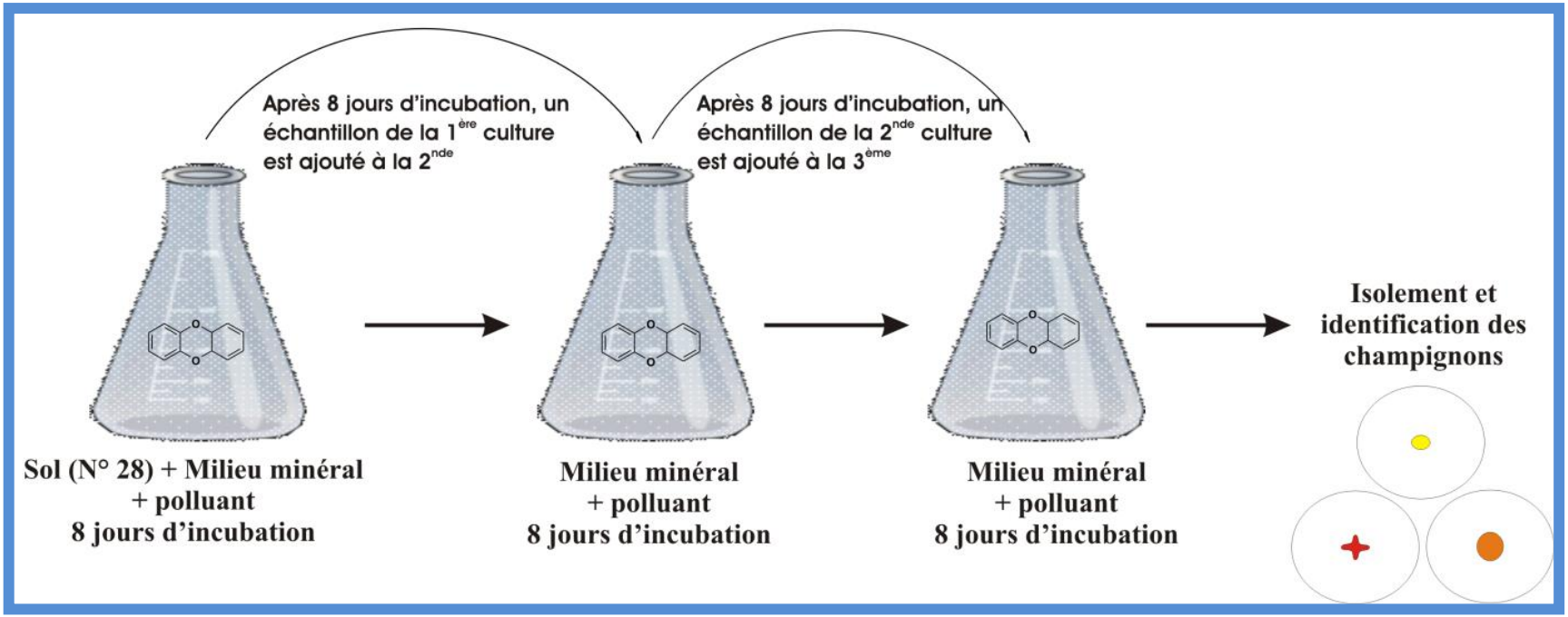
Identification des champignons

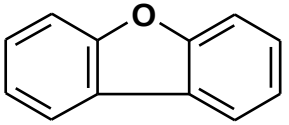
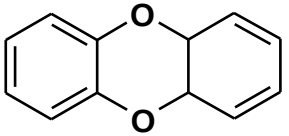




Saprotrophic fungi isolated from dioxins polluted soil (300 ng/kg soil)	Quantitative	Qualitative
	9 10^3 CFU per gram of soil	9 isolates

Soil enrichment method with dibenzodioxin



	Fungal growth in presence of	Fungal diversity
Dioxins polluted soil (300 ng/kg soil) enriched with	**Dibenzofuran (4,6 g/l)** 	**1 isolate**
	Dibenzodioxin (5,1 g/l) 	**3 isolates**

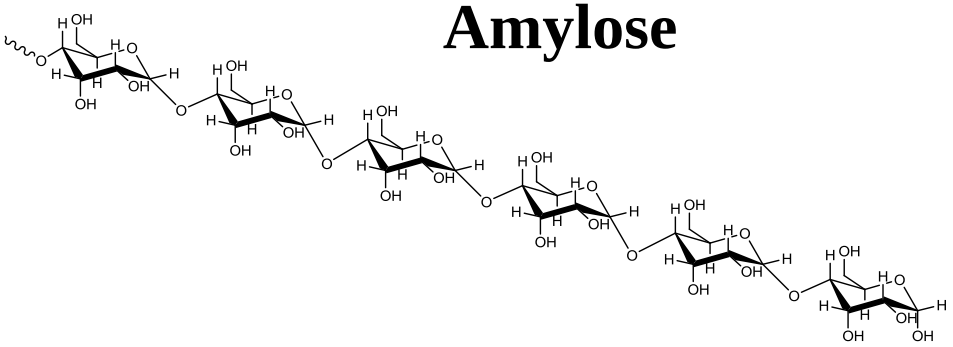
Starch structure

Carbohydrate consisting of a large number of D-glucose units joined by glycosidic bonds.

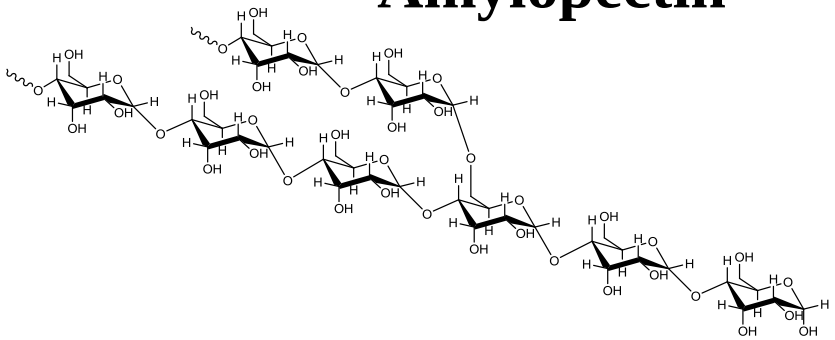
Two types of molecules:

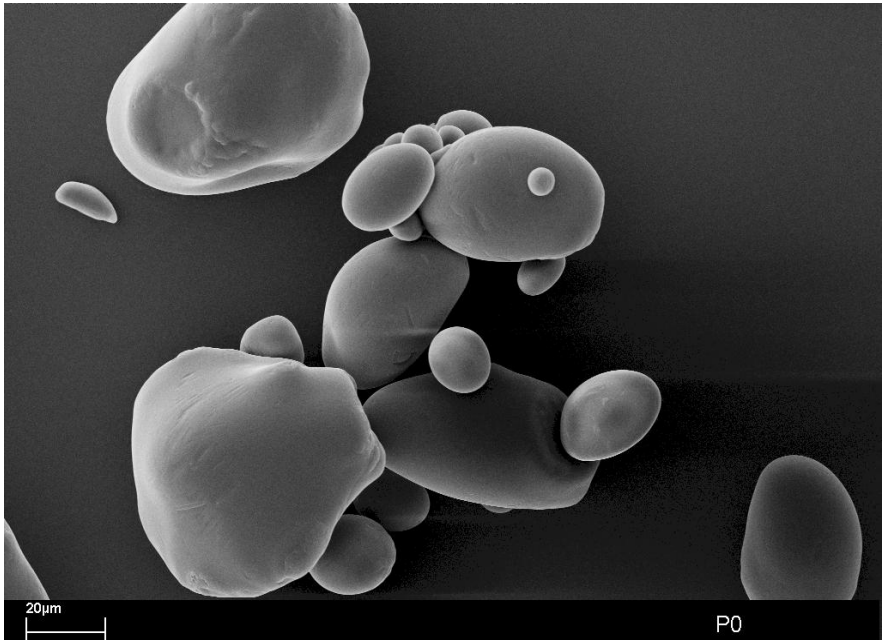
- ✓ **the linear and helical amylose**
- ✓ **the branched amylopectin**

Amylose



Amylopectin

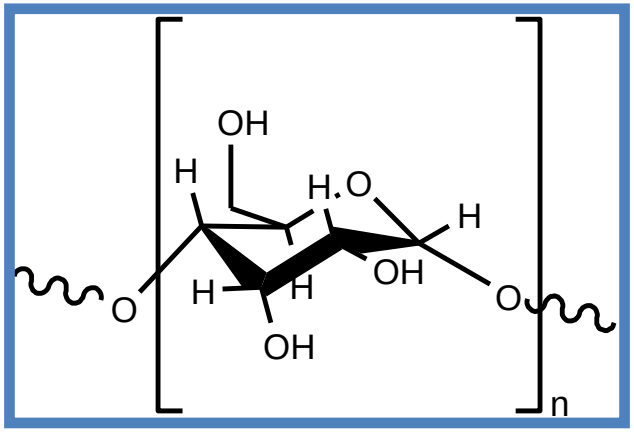




A-M Dospinescu-Rosu. (2011). *Synthèse et étude d'amidons modifiés pour le développement de procédés d'oxydation du benzo[a]pyrène, un modèle de polluant organique persistant.* Thèse de doctorat, ULCO/Bacau.

Starch advantages

- ✓ Abundant
- ✓ Renewable
- ✓ Costless
- ✓ Biodegradable
- ✓ High chemical reactivity



Anhydroglucose unit of starch

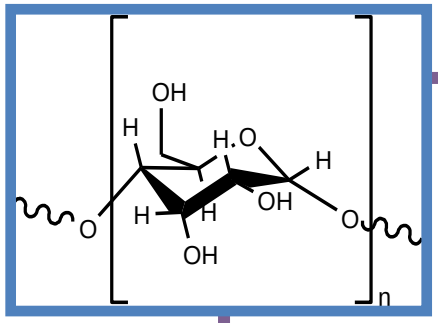
Synthesis of modified starches

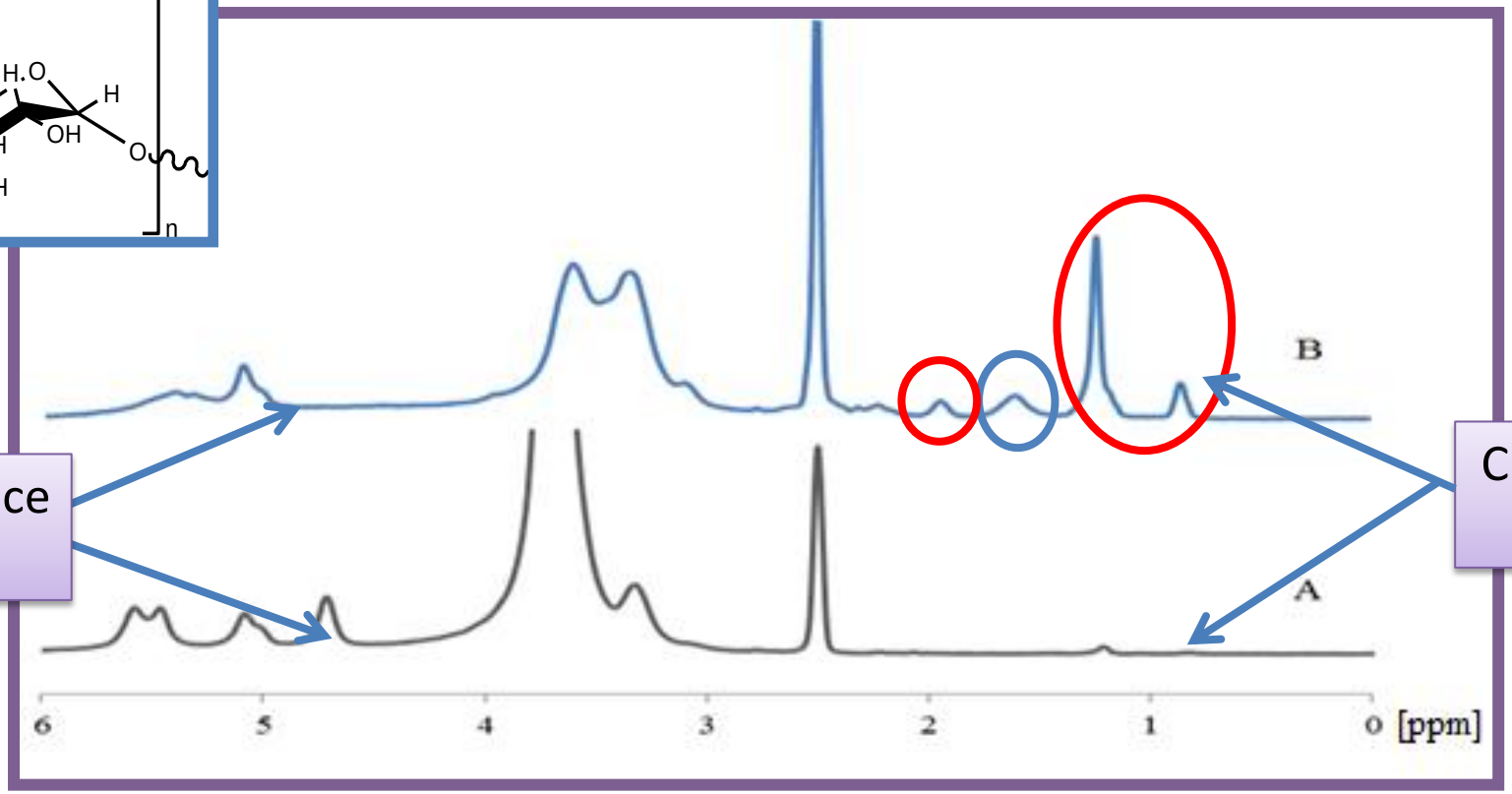
Modification of native starches by adding alkyl groups

- ➢ **Chemical structures characterization of the obtained products by ^{1}H NMR and infrared spectroscopy**
- ➢ **To enhance POPs solubility**
- ➢ **To enhance water solubility of starch (0,35 g/L)**

Results

^{1}H NMR spectrum of native gelatinized maize starch (A) and of bialkylated maize starch (B)



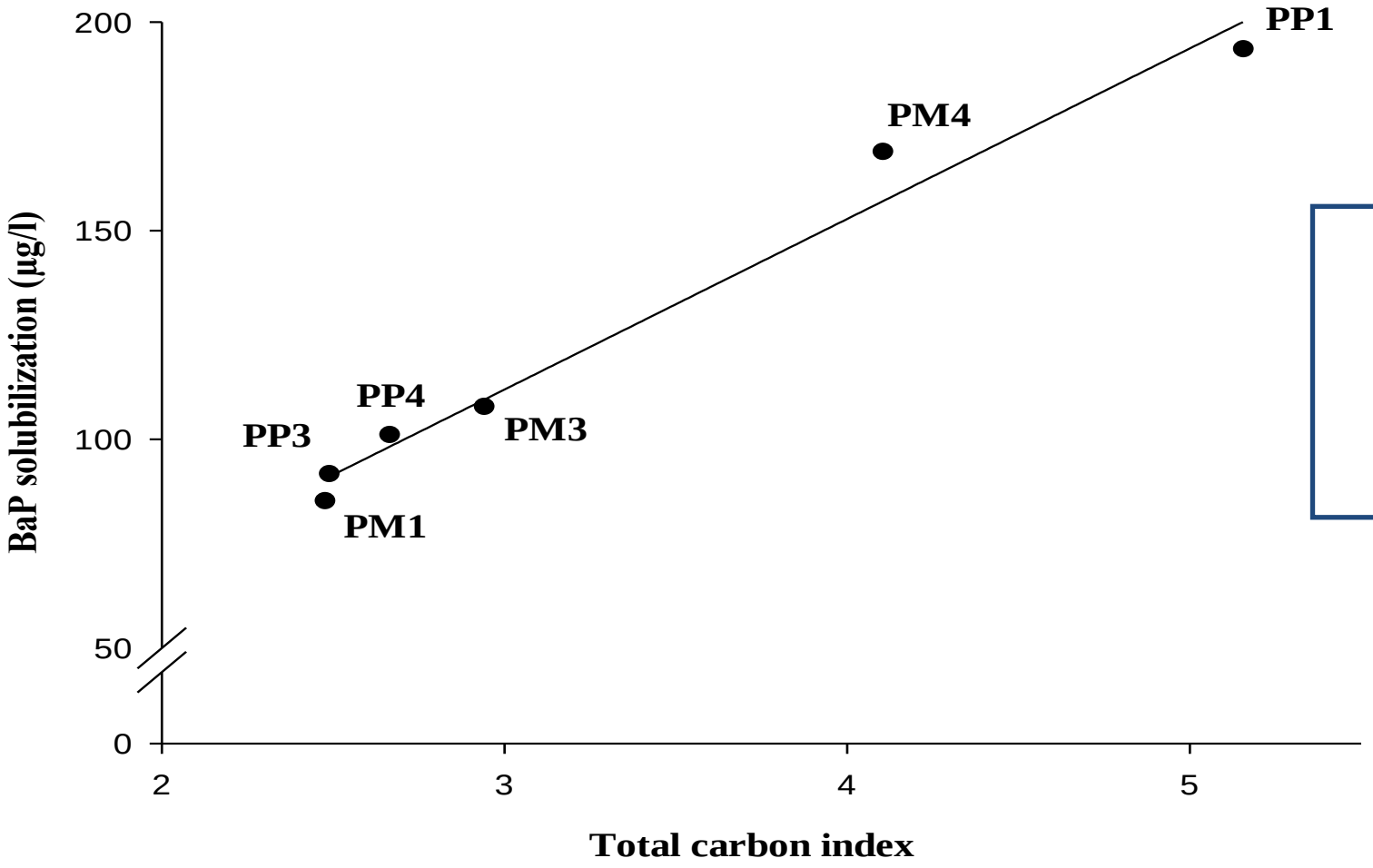


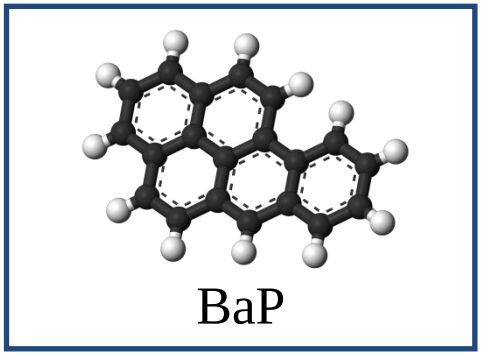
Disappearance peaks

Characteristic peaks

MS (the average number of substituents per mole of OH-AGU) and carbon index calculation

Results


200
150
100
50
0
2
3
4
5
BaP solubilization (µg/l)
Total carbon index
PP1
PM4
PP4
PM3
PP3
PM1


BaP

Conclusion et perspectives

✓ **Exploration of the saprotrophic telluric fungi biodiversity**

✓ **Obtention of a right balance between hydrophobic character of starch derivatives and starch aqueous solubility for enhancing dioxins solubility and bioavailability**

✓ **Developing an innovative method of dioxins degradation by coupling, simultaneously or sequentially, chemical oxidation (i.e. Fenton reaction) to biological oxidation process by fungi**

Such a method could accelerate degradation processes while preserving as much as possible the integrity of the structure and functions of the soil

Part 2. Role of arbuscular mycorrhizae in the phytoremediation of dioxins/furans soils

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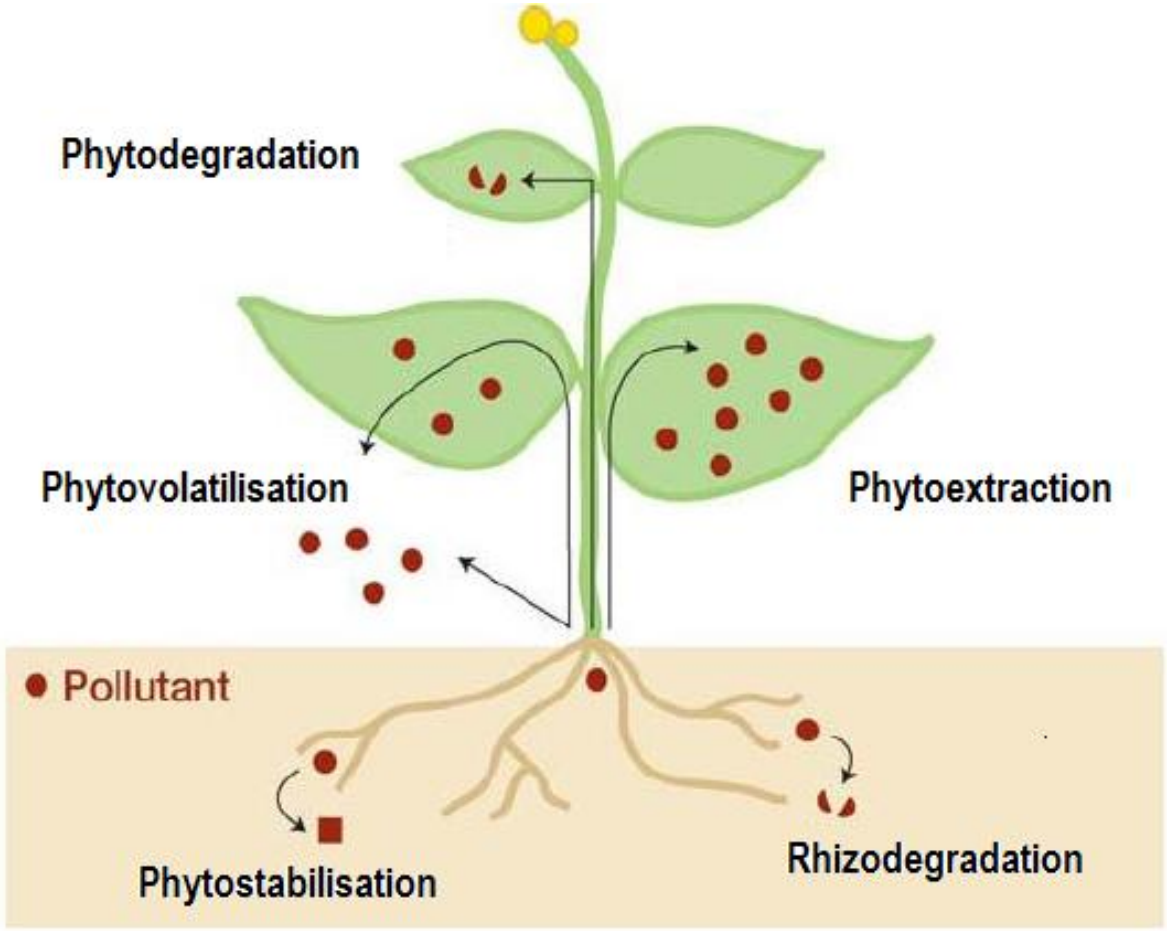
The phytoremediation : Use of the natural abilities of plants and their associated microbiota to eliminate, contain or render less toxic environmental contaminants.

Degradation or transformation of organic pollutants by plants.

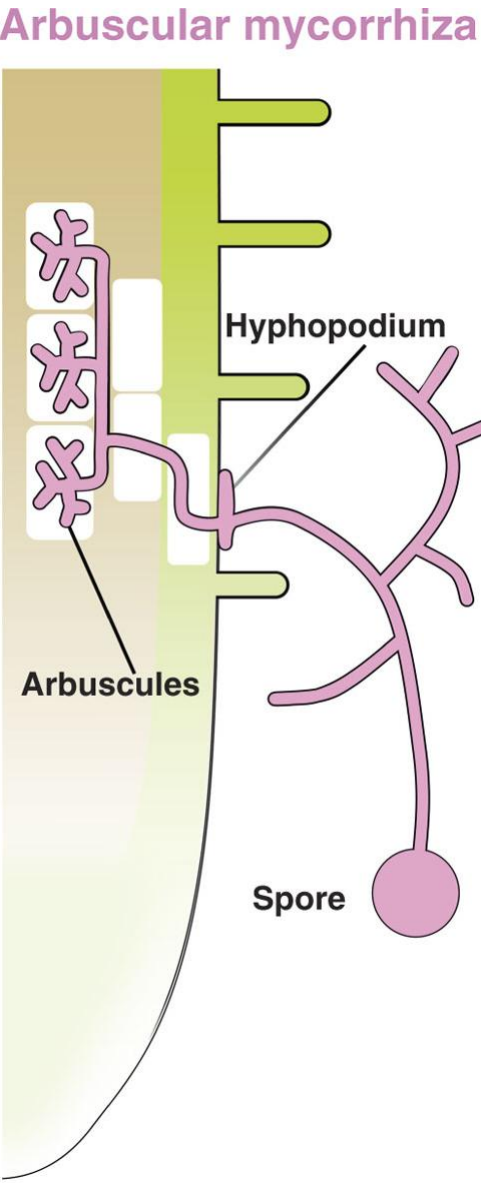


Advantages :

- Low cost when compared to other more conventional methods
- Ecological
- Suitable for large surfaces
- Well adapted to soils with low pollutant levels
- Easy to implement
- High public acceptance

Degradation or transformation of organic pollutants by microorganisms.

AMF assisted phytoremediation :



Arbuscular mycorrhizal fungi (AMF) benefits:

- Improve plant growth
- Increase the plant tolerance to environmental stresses
- Enhance pollutant dissipation

(Affect physico-chemical proprieties of the rhizosphere and stimulate telluric microbial degrading activities)

Mycorrhizal plants enhance dissipation of organic pollutants

Organic pollutants	Mycorrhizal plants	Degradation rates	Experiment duration	References
PAH	Clover, Ryegrass	42-66 %	16 weeks	Joner et al., 2001
	Alfalfa	57-86 %	13 weeks	Liu et al., 2004
	Leek	43-88 %	12 weeks	Liu and Dalpé, 2004
	Clover, Ryegrass	33-65 %	4 weeks	Chiapusio et al., 2007
	Alfalfa	88-98 %	10 weeks	Gao et al., 2011
PCB	Alfalfa	12 %	16 weeks	Teng et al., 2010
Dioxins/Furans	No studies available			

AIMS OF OUR PROJECT :

To Study the arbuscular mycorrhizae role in the phytoremediation of dioxins/furans polluted soils

I

To establish an inventory of the mycorrhization on the experimental plot

***in situ* experiment**

II

To study the impact of dioxins/furans on the development of both symbiotic partners

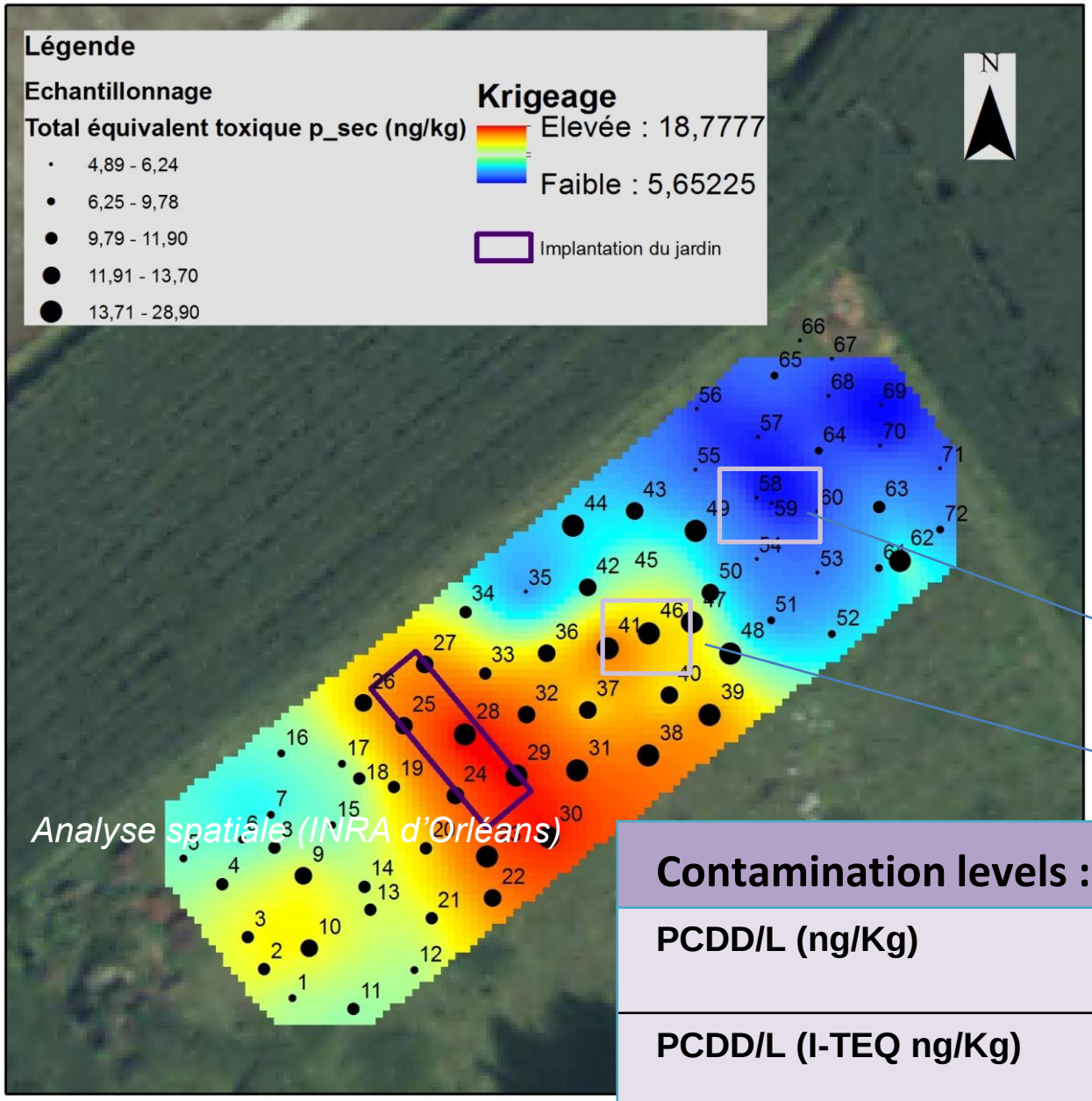
***in vitro* experiment**

III

To study dioxins/furans dissipation potential of the mycorrhizal symbiosis

Pot experiment

Experimental site: Two sampling areas

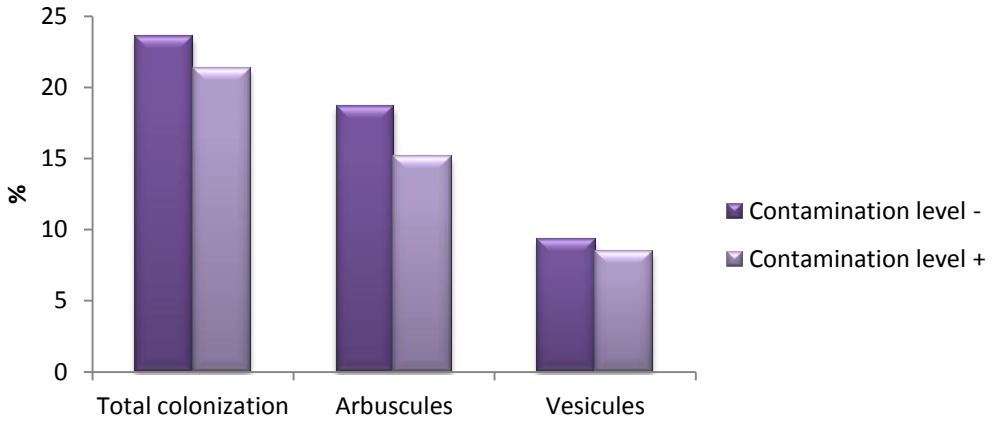


Analyse spatiale (INRA d'Orléans)

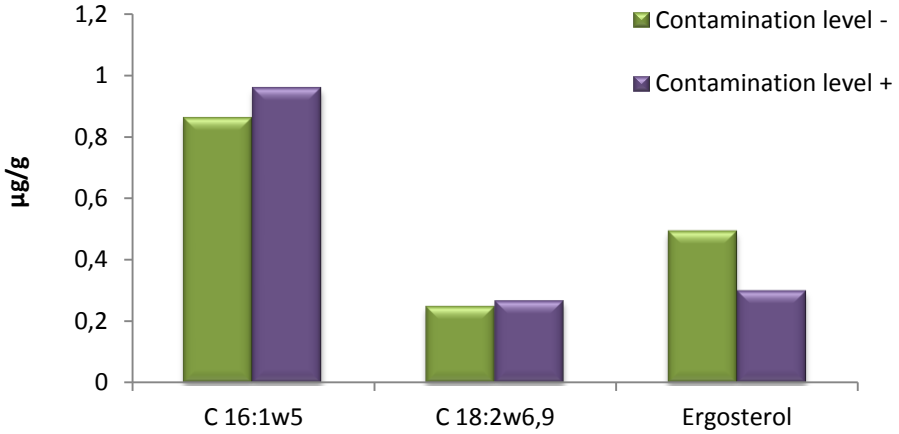
Contamination levels :	+	-
PCDD/L (ng/Kg)	**200**	**58**
PCDD/L (I-TEQ ng/Kg)	**16**	**5**

I. inventory of the mycorrhization on the experimental plot

Mycorrhizal colonization on the experimental plot



Fungal specific lipid markers

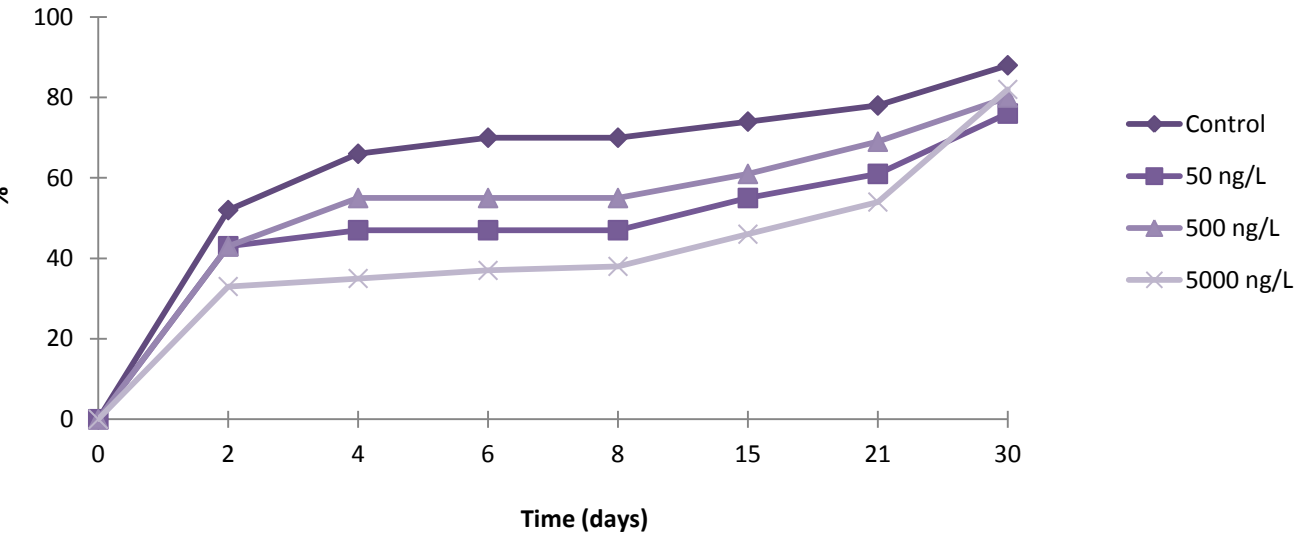


	Glomus constrictum	*Glomus geosporum*	*Glomus mosseae*	*Total*
Contamination level -	37	187	140	363
Contamination level +	0	230	70	300

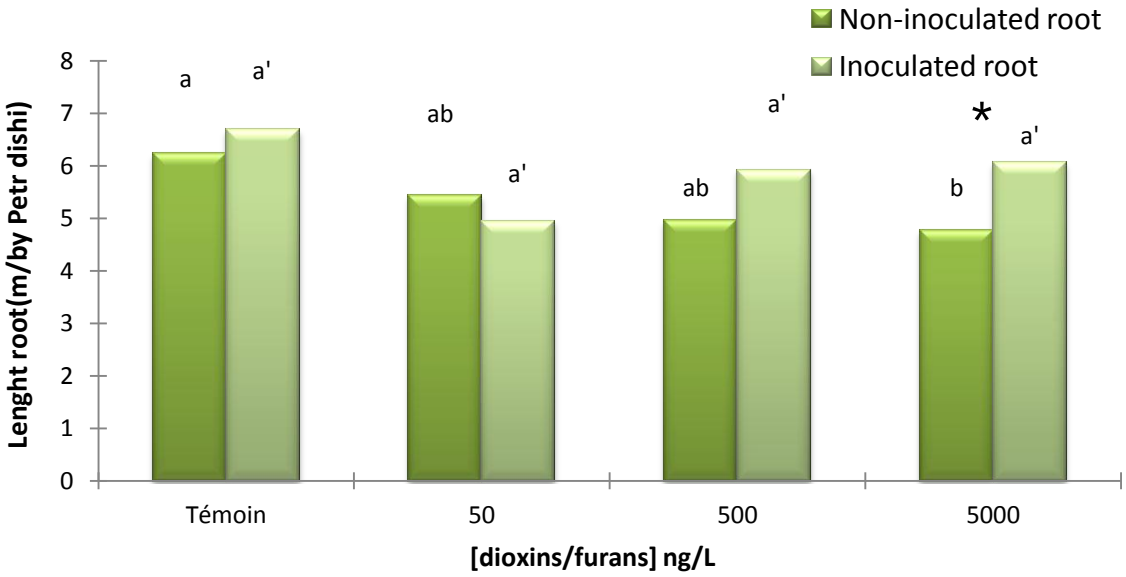
(collaboration with Dr Y. Dalpé, CRECO, Ottawa)

II. Impact of dioxins/furans mixture on the development of both symbiotic partners

Spore germination of *Rhizophagus irregularis*



Root elongation



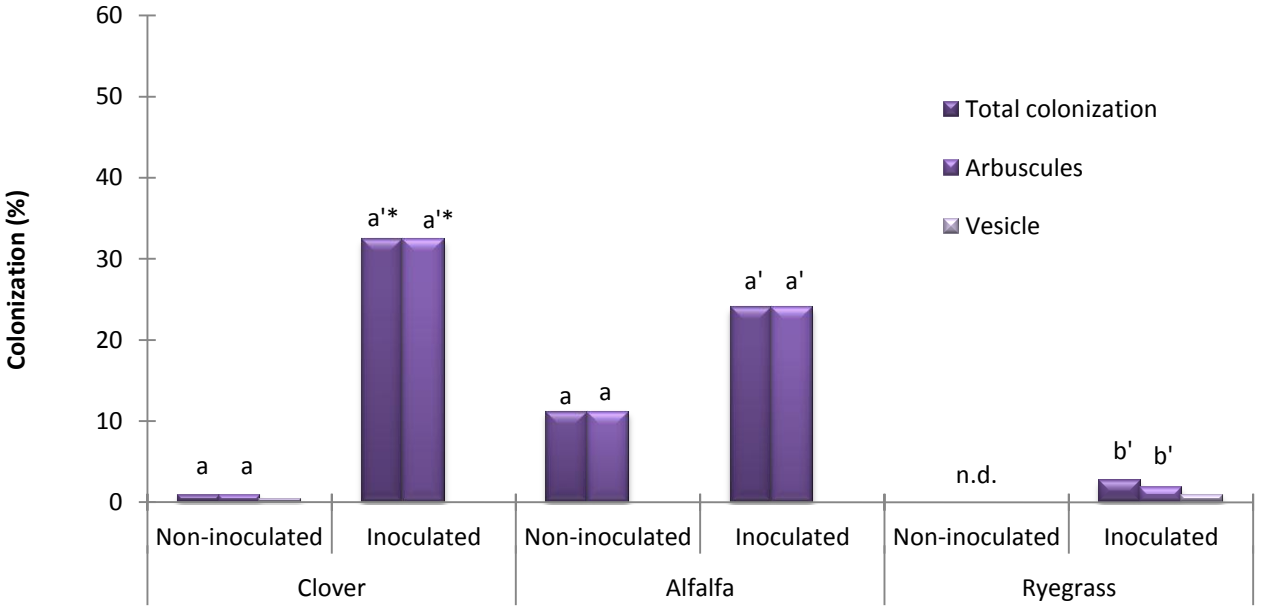
III. Dioxins/furans dissipation potential of the mycorrhizal symbiosis

Alfalfa **White clover** **Ryegrass** **Control without plants**

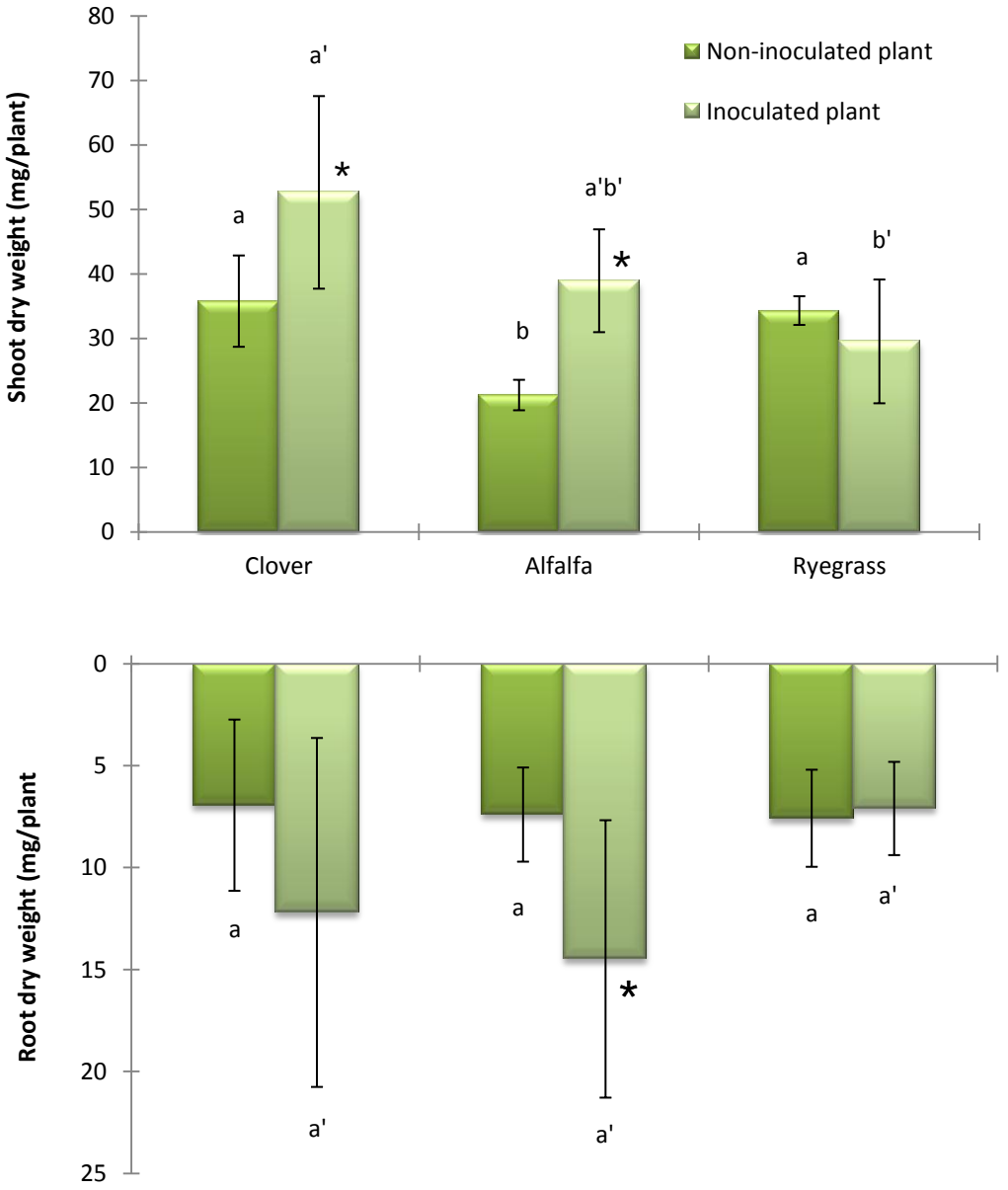
Inoculated or not by AMF (Solrize ®)

Experiment duration: 6 weeks

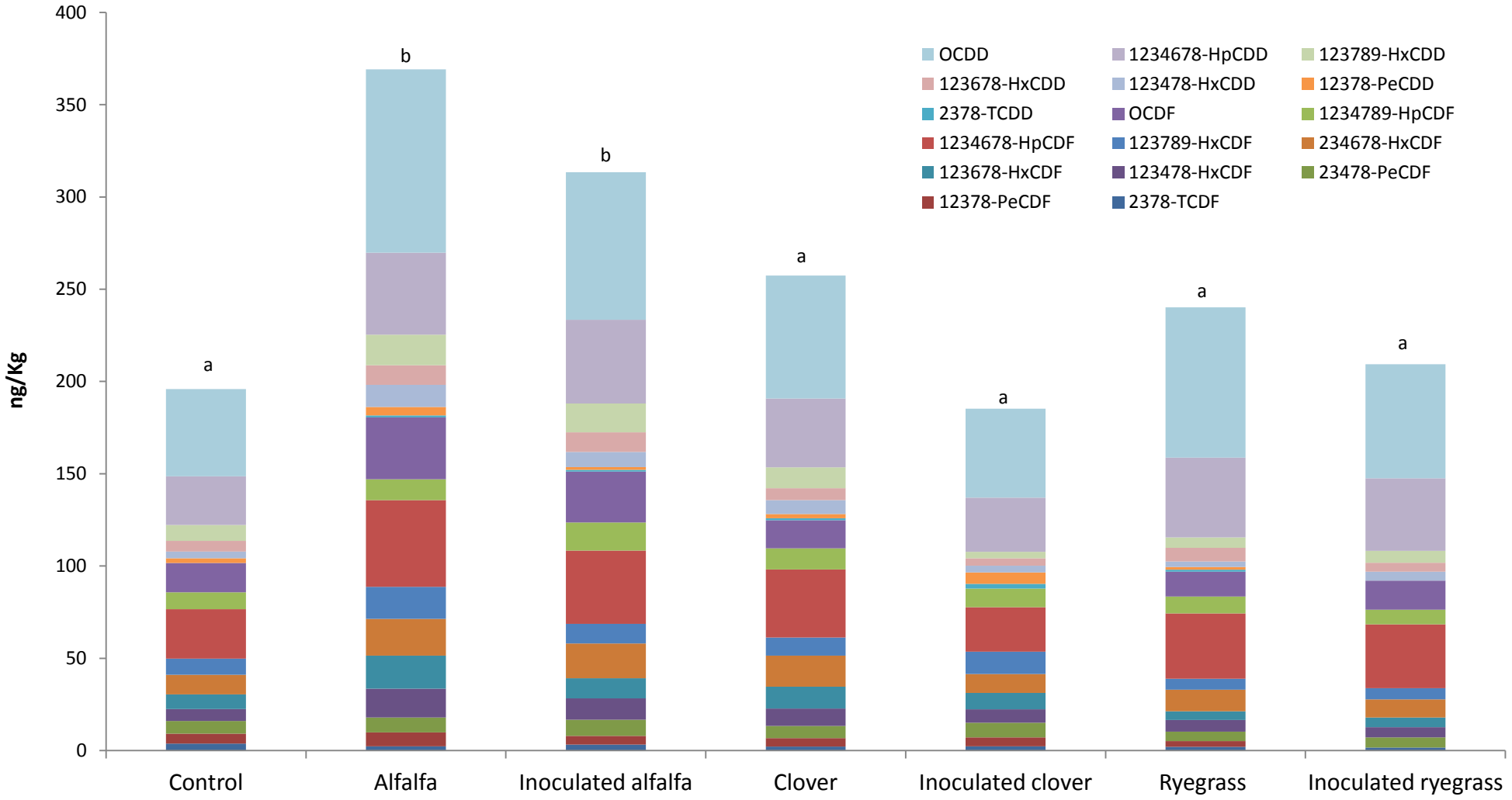
Mycorrhizal colonization



Effect of mycorrhizal inoculation on the plant growth



Residual dioxins/furans after 6 weeks of culture



Conclusion and perspectives

- Presence, survival and development of AMF in dioxins/furans polluted soil

- The mycorrhizal inoculation is, not only possible in dioxins/furans polluted soils but it improves plant growth

- The experimental duration (ie 6 weeks) was too short to observe the dioxins/furans dissipation

Extend the culture duration

Test another plant species

Use surfactant in order to increase the dioxins/furans bioavailability