Exploring ligninolytic fungi capabilities for pharmaceuticals detoxification in water effluents

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INTRODUCTION

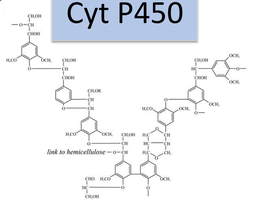
Statement of the Problem

- Pharmaceuticals consumption rise means contamination rise
- Conventional wastewater treatment plants are not efficient at pharmaceuticals removal

White rot fungi metabolism advantatges for bioremediation

Induced by stress → ▭ [pollutant]

Intracellular enzymes: Cyt P450



Extracellular enzymes: MnP, VP, LiP, Laccase → ▭ toxicity

Low substrate specificity

OBJECTIVES

Master Thesis specific objectives

Development and validation of HPLC-HRMS analytical method

Identification of TPs in water and fungal pellet

Master Thesis main goals

1- Ocurrence of selected pollutants in mycoremediation samples

2- Charactherization of TPs from mycoremediation

Creation of a spectral library of TPs

Identification of degradation causes of TPs detected

MATERIALS & METHODS

Experimental Design

Production of fungal pellet + Culture medium triplicates

Aerobic incubation

		Inoculation with fungus (*T. versicolor / I. lacteus / G. lucidum*)		
		ALIVE FUNGUS	HEAT-INACTIVATED	NO INOCULUM
Spiking with 1000 ppm of pollutants	YES	Experimental batch	Killed Control	Abiotic Control
	NO	Alive Control	N.A.	N.A.

Sampling (0 days / 7 days) → Centrifugation (Liquid phase (LP) / Solid phase (SP)) + Lyophilisation → Reconstitution on water (LP) / Solvent extraction (SP)

Analysis Method

HPLC-LTQ Orbitrap Velos (Thermo) Xcalibur software

HPLC → IT → HRMS → Data treatment

Column C18

Gradient elution

NH_4Fo/ACN **ESI (+)**

NH_4Ac/ACN **ESI (-)**

Acquisition
MS^1 Full Scan

MS^2 Data-dependent Scan

Manual
Xcalibur software

Automatic
Exact Finder software + Spectral home-made library of 173 compounds

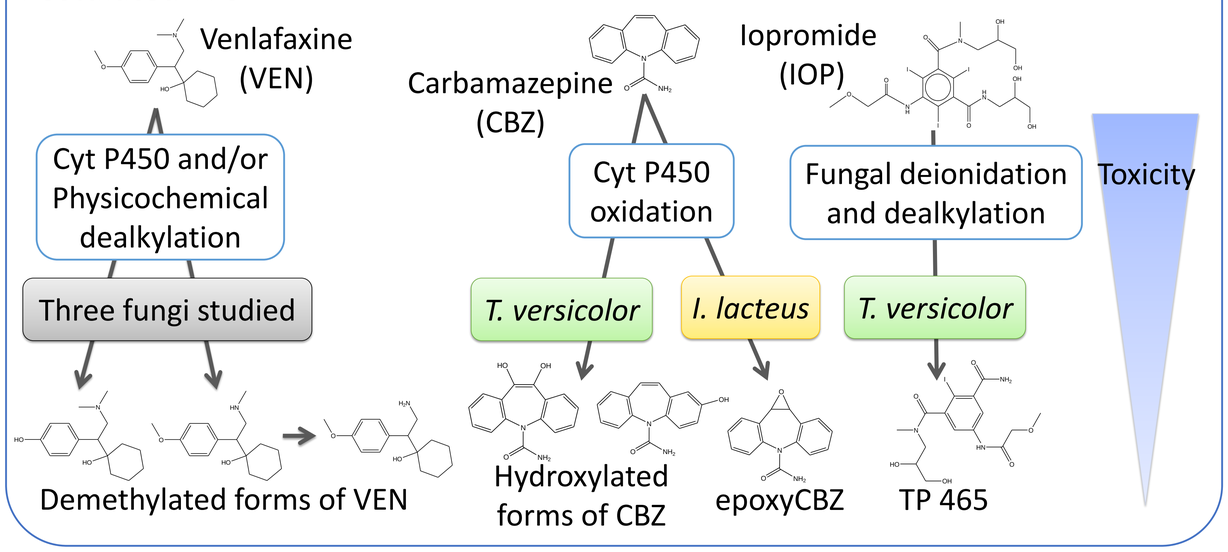
RESULTS

Method validation: UNE-EN-657/2002

Mass accuracy

Accuracy	Precision	Uncertainty
✓ Bias<±5ppm	✓ $RSD_{intraday}$<10ppm ✓ $RSD_{interday}$<10ppm ✓ RSD_T<10ppm	✓ u_{RSD}<10ppm ✓ u_{Bias}<10ppm ✓ U_{exp}<10ppm

Postulated TPs



CONCLUSIONS

Occurrence of selected pollutants

TPs toxicity < Parental compounds toxicity

Detoxification of water by mycoremediation similar to conventional waste water treatment

Sorption onto fungal pellet ≈ Sorption onto conventional sludge

Characterization of TPs

Specificity of method analysis (mass accuracy) + Physicochemical data from literature (t_R, pK_{OW})

Accurate TPs tentative identification

Degradation pathways

T. versicolor ≠ *I. lacteus* ≠ *G. lucidum*

Biological + Physicochemical

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H2PHARMA project (Fungi, algae and bacteria in pharmaceuticals degradation)

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