Potential of Persistent Ectomycorrhizal Fungi in Fire Impacted Soil to Degrade Fluorinated Pollutants

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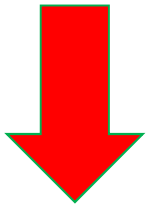
Over the last decades, the widespread use of **halogenated chemicals**, e.g., agriculture, pharmaceuticals, fire retardants, has increased

Most of these compounds **accumulate** in **soils**, sediments and water resources

However, their effect and fate in **natural environments** is still unclear

Most studies have been focused on **bacteria** capacity to degrade fluorinated compounds

Fungi, such as Ectomycorrhizal fungi – ECM, are often **neglected** as important **players in remediation processes**



Assessment of the potential contribution of ECM fungi as a rhizosphere remediation technology

Ectomycorrhizal fungi

Mutualistic associations between fungi and plants root

Enhancement of **root protection** against adverse conditions

- water deficiency
- extreme pH and temperatures
- heavy metal or toxin stresses

Photosynthetic compounds and other exudates via roots

Mycorrhizal Symbiosis, Smith and Read

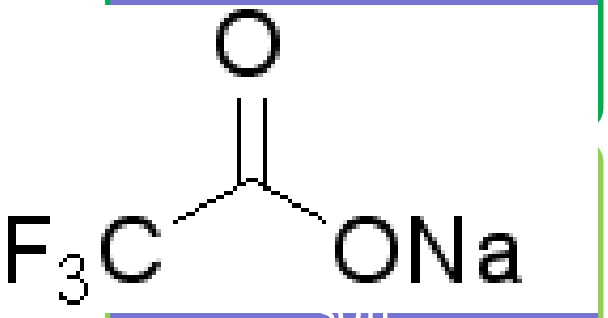
Play an important **role in nutrient cycling**, by degrading complex minerals or organic substances present in soil

The study …

Sodium trifluoroacetate (**TFA**) and Fluorophenol (**FP**)

TFA

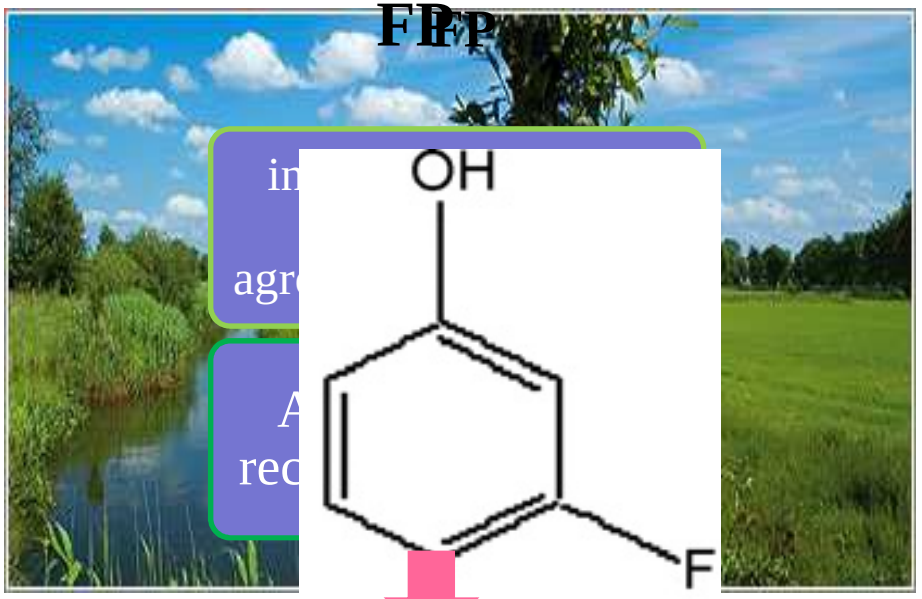
breakdown product



soil

Growth Inhibition test

FP



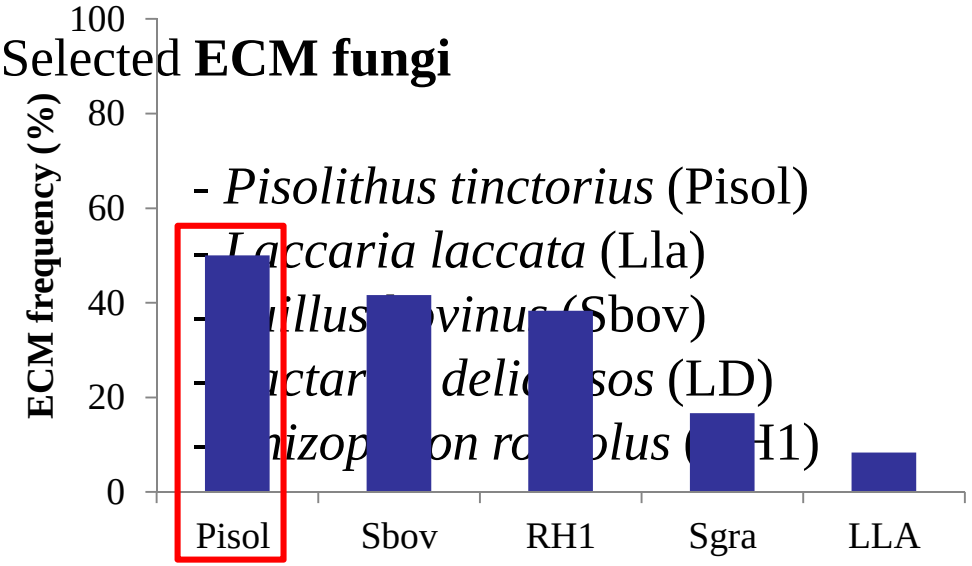
Degradation studies

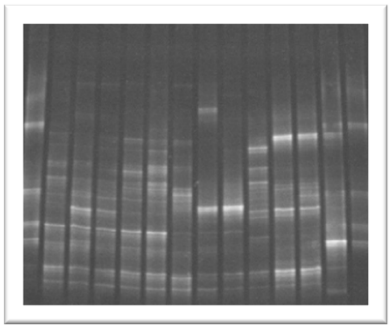
Growth Inhibition test

ECM screening

Persistence of **ECM fungal community** was monitored

Established ***Pinus pinaster*** inoculated with **selected ECM fungi** in Cabreira Mountain, Portugal





Pisolithus tinctorius **(Pisol)** was selected based on **persistence** on post fire forest soil

Growth Inhibition tests

Pisol mycelium growth was monitored after 30 days incubation, at 26ºC on Modified Melin Norkrans with 0.5 g/L of glucose

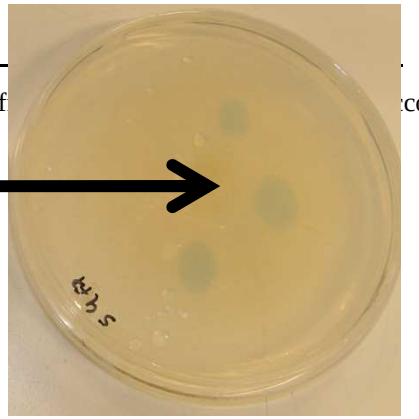
Possible expressed proteins were screened in plates, according to Gramss *et al.*, 1998

[TFA] (ppm)	Mycelium halo growth (mm)
CO	22.0 z
100	19.0 z
250	19.1 z
500	19.3 z
1000	

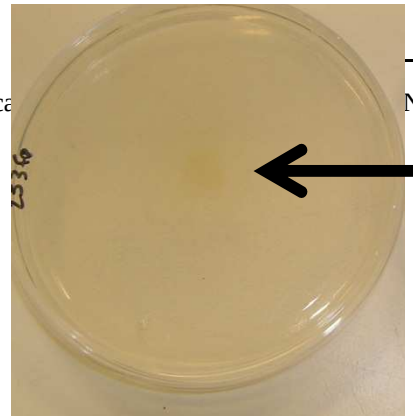
Columns marked with dif… …cording to Dunca… …N = 5

	Mycelium halo growth (mm)		
[FP] (ppm)	2FP	3FP	4FP
CO	38.0 d	38.0 d	38.0 d
5	18.0 a	14.0 a	9.4 a
10	25.2 b	25.6 b	12.0 b
25	25.0 b	30.0 c	15.0 c
		31.2 c	13.7 bc

Laccase and Peroxidase were **weakly expressed** after 72h



Laccase



Peroxidase

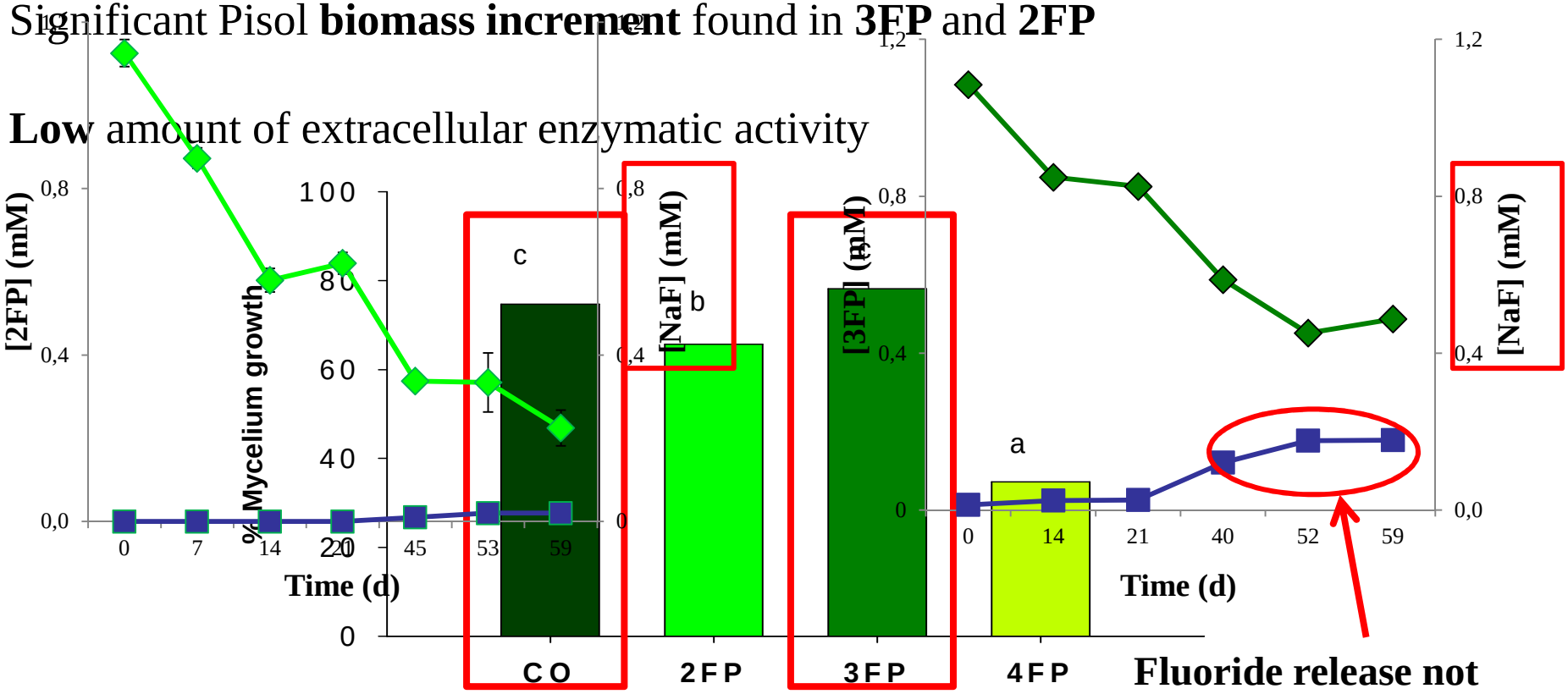
Gramss *et al*, 1998 *Mycol. Res.* 102 (1) : 67-72

Degradation of FP in liquid culture

Pisol was able to degrade aprox. **80% of 2 FP** and **60% of 3 FP** but did not degrade 4 FP

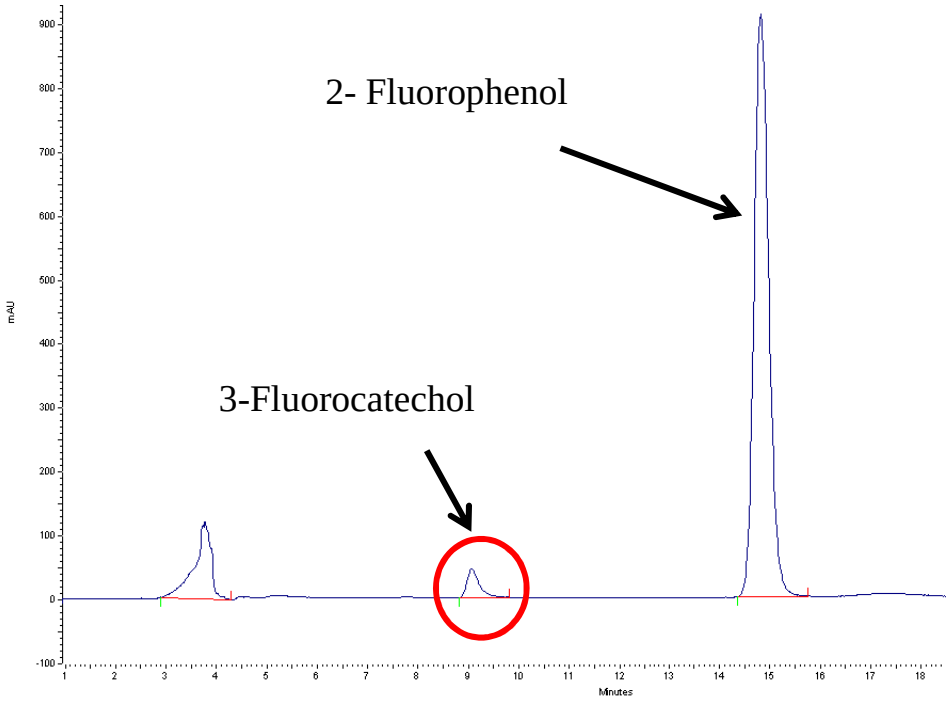
Significant Pisol **biomass increment** found in **3FP** and **2FP**

Low amount of extracellular enzymatic activity



MMN with 0,5g/L glucose; Columns marked with different letters differed significantly according to Duncan's Multiple Range test at $P < 0.05$; N = 5

Identification of degradation products

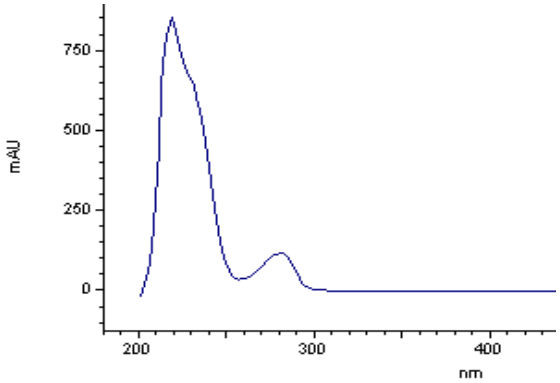


Mobile phase -Methanol:Water (40/60), reverse-phase C18 column; flow rate: 0,6mL/min

Degradation products in 2FP cultures were evaluated by **HPLC-DAD**

3-Fluorocatechol was identified as **intermediate metabolite**

Residual quantities of 3FC were found



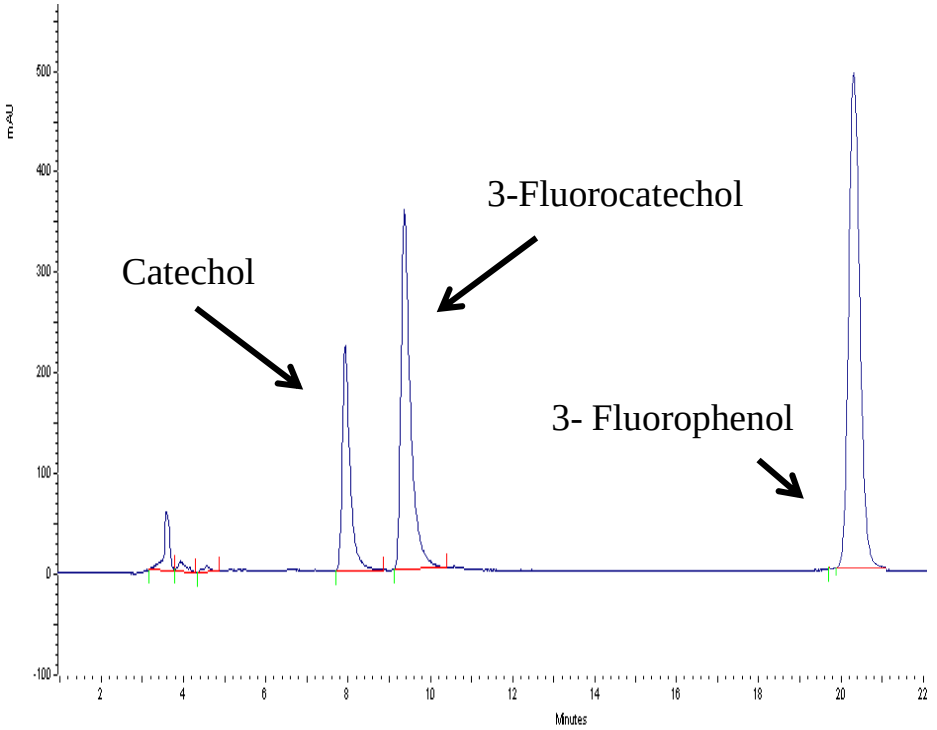
3-Fluorocatechol

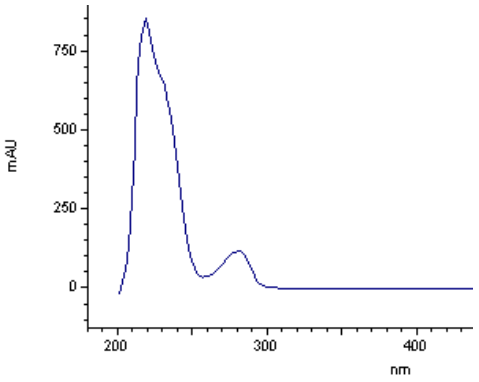
Accumulation of new intracellular product?

Identification of degradation products

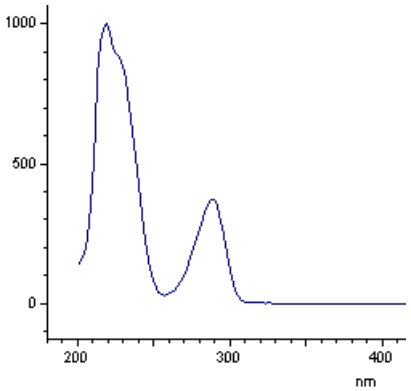
Two new metabolites in 3FP cultures were detected by HPLC-DAD

3-Fluorocatechol (**3FC**) and Catechol **(Cat)**





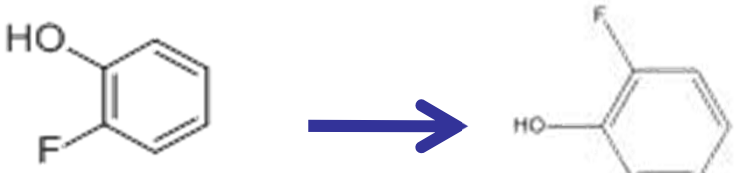
3-Fluorocatechol



Catechol

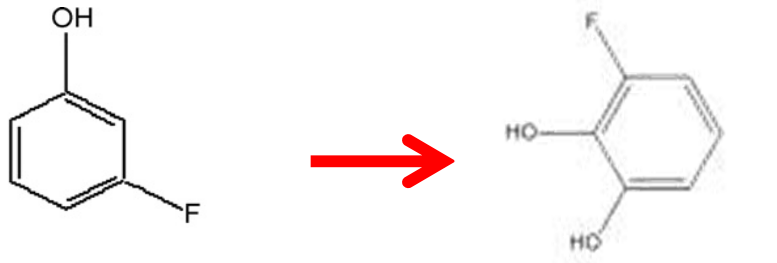
Accumulation of 3FC [10 µM] and Cat [6 µM] after **59 days** incubation

Proposed metabolic pathway ?



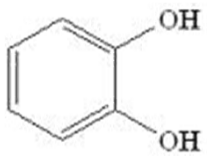
2-Fluorophenol

3-Fluorocatechol



3-Fluorophenol

3-Fluorocatechol



Catechol

Study results show that **Pisol** is able to **oxidase fluorophenols** onto other products

Catechol was only identified in 3FP degradation

Cell extracts will be screened for **enzymatic activity** (ie. Catechol 1,2-dioxygenase, Catechol 2,3-dioxygenase, others)

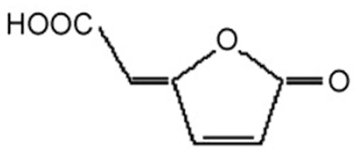
Some conclusions

Toxicity tests demonstrate the ability of ***P. tinctorius*** to grow on **fluorinated compounds**

- ECM fungi capacity to **tolerate** and grow on **TFA** and **FP**

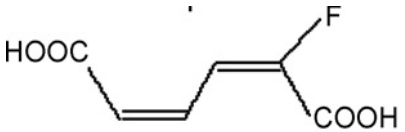
P. tinctorius is able to **degrade** mono-fluorophenol in liquid cultures

3-Fluorocatechol and **Catechol** were detected as **degradation metabolites**, but other possible **metabolic** products could also be produced at low quantities



Dienelactone

Hydroquinone



2-Fluoromuconate

ECM fungi as players on rhizosphere remediation technology

Thank you for your attention!

Acknowledgments

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