

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

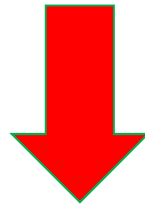
Most of these compounds **accumulate** in **soils**, sediments and water resources

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

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

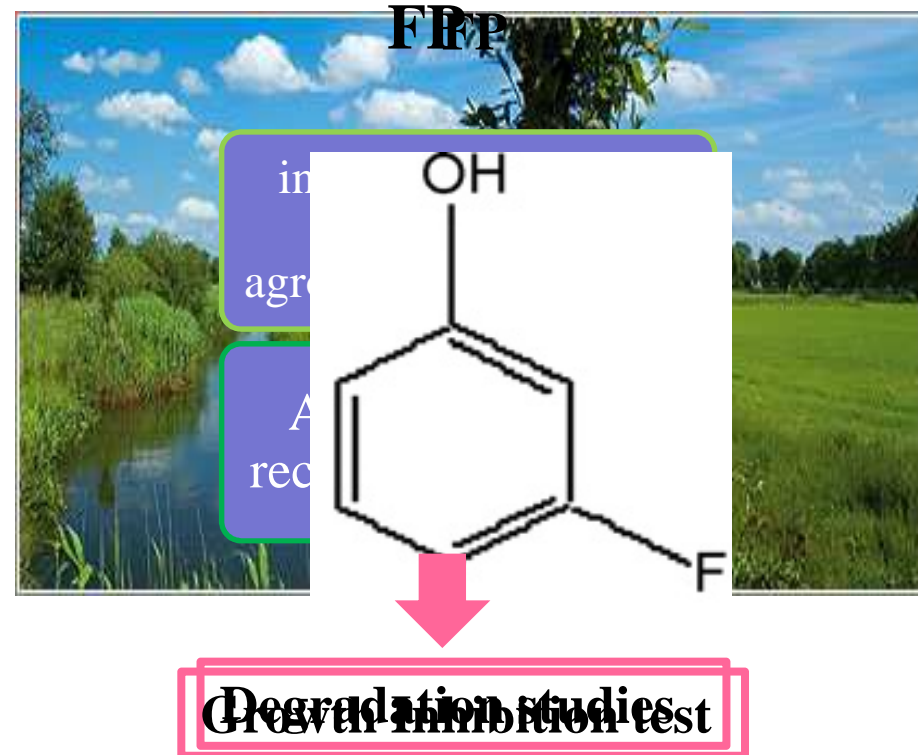
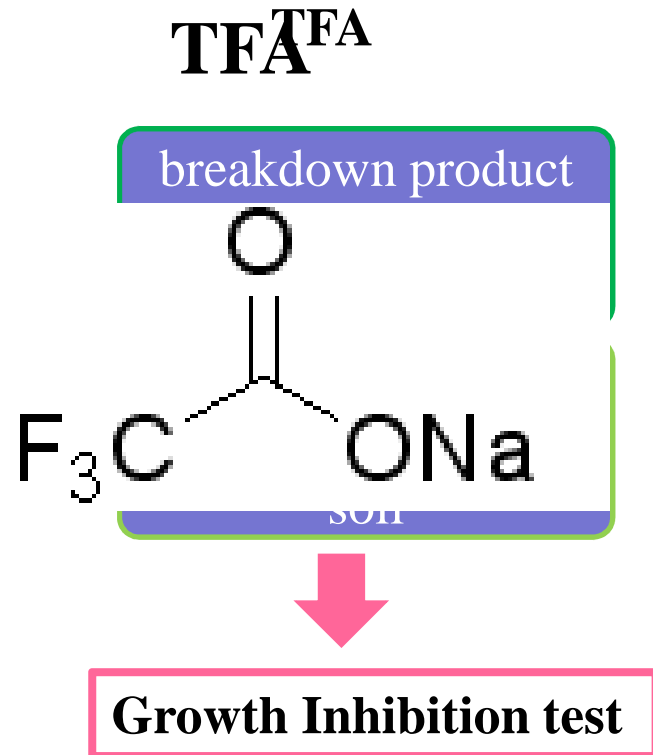


Mycorrhizal Symbiosis, Smith and Read



The study ...

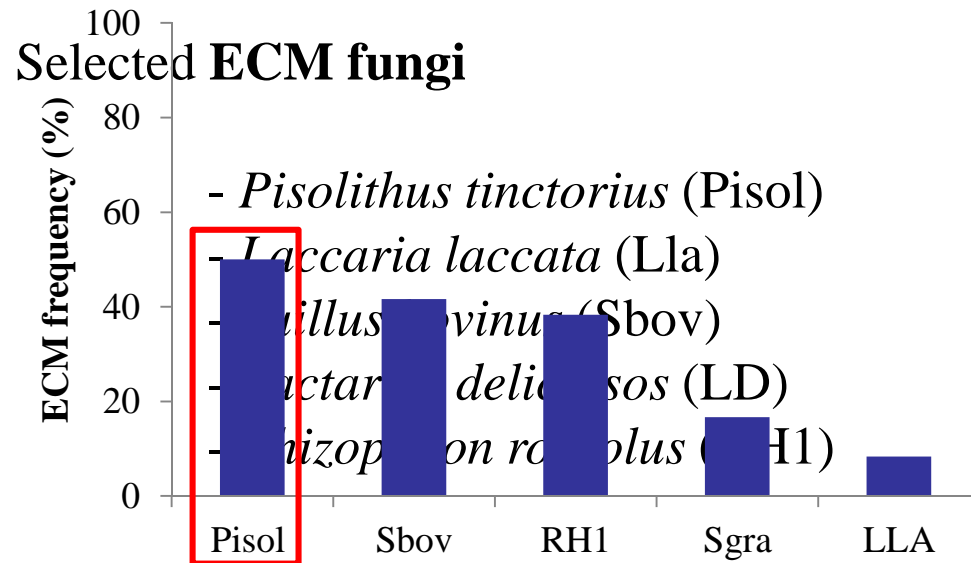
Sodium trifluoroacetate (**TFA**) and Fluorophenol (**FP**)



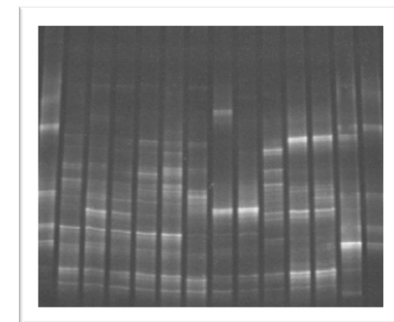
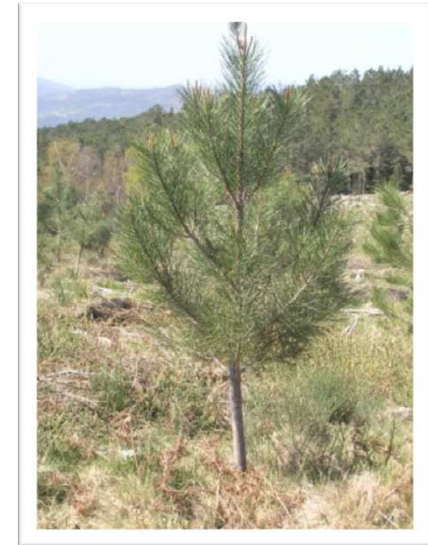
ECM screening

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



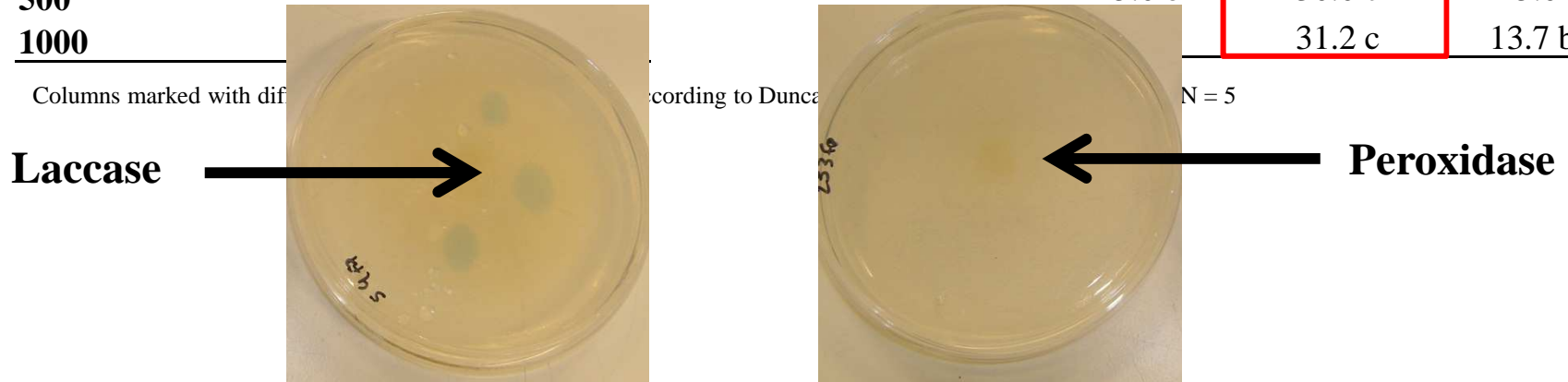
Growth Inhibition tests

Pisol mycelium growth was monitored after 30 days incubation, at 26°C 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) | [FP] (ppm) | 2FP | 3FP | 4FP |
|-------------|---------------------------|------------|--------|--------|---------|
| CO | 22.0 z | CO | 38.0 d | 38.0 d | 38.0 d |
| 100 | 19.1 z | 10 | 18.0 a | 14.0 a | 9.4 a |
| 250 | 19.3 z | 25 | 25.2 b | 25.6 b | 12.0 b |
| 500 | | | 25.0 b | 30.0 c | 15.0 c |
| 1000 | | | | 31.2 c | 13.7 bc |

Laccase and Peroxidase were weakly expressed after 72h



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



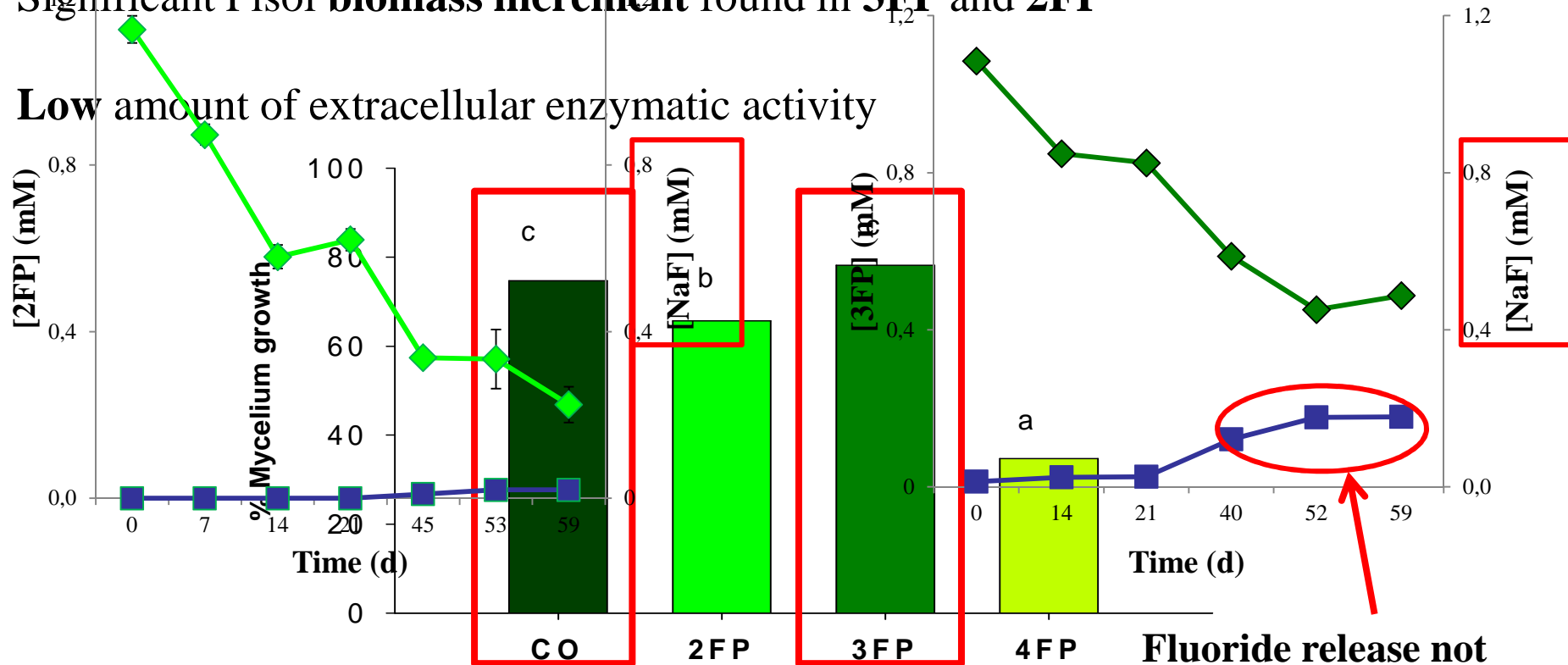
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Degradation of FP in liquid culture

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



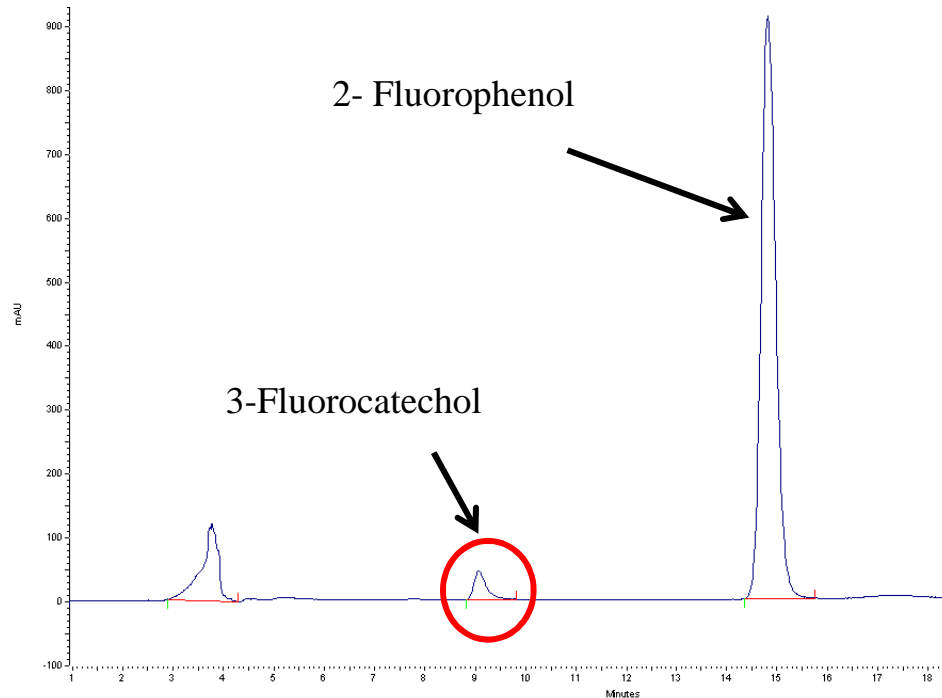
MMN with 0,5g/L glucose; Columns marked with different letters differed significantly according to Duncan's Multiple Range test at $P < 0.05$; $N = 5$

Fluoride release not stoichiometric



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Identification of degradation products

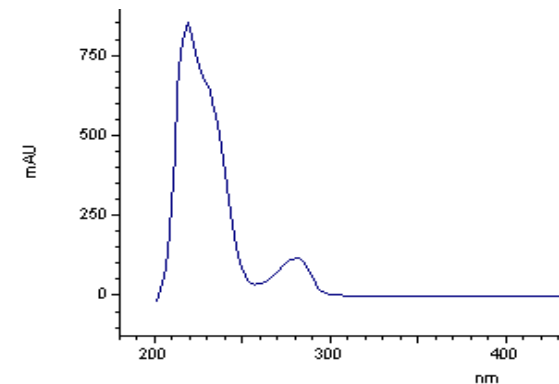


Mobile phase -Methanol:Water (40/60), reverse-phase C18 column; flow rate: 0,6mL/min

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

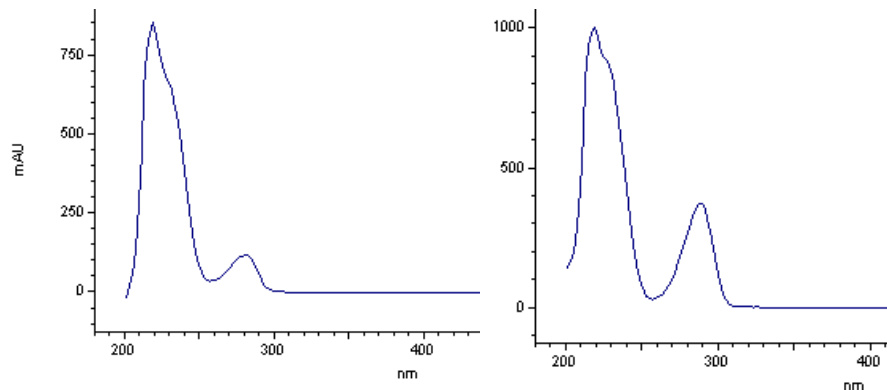
Accumulation of new intracellular product?



Identification of degradation products

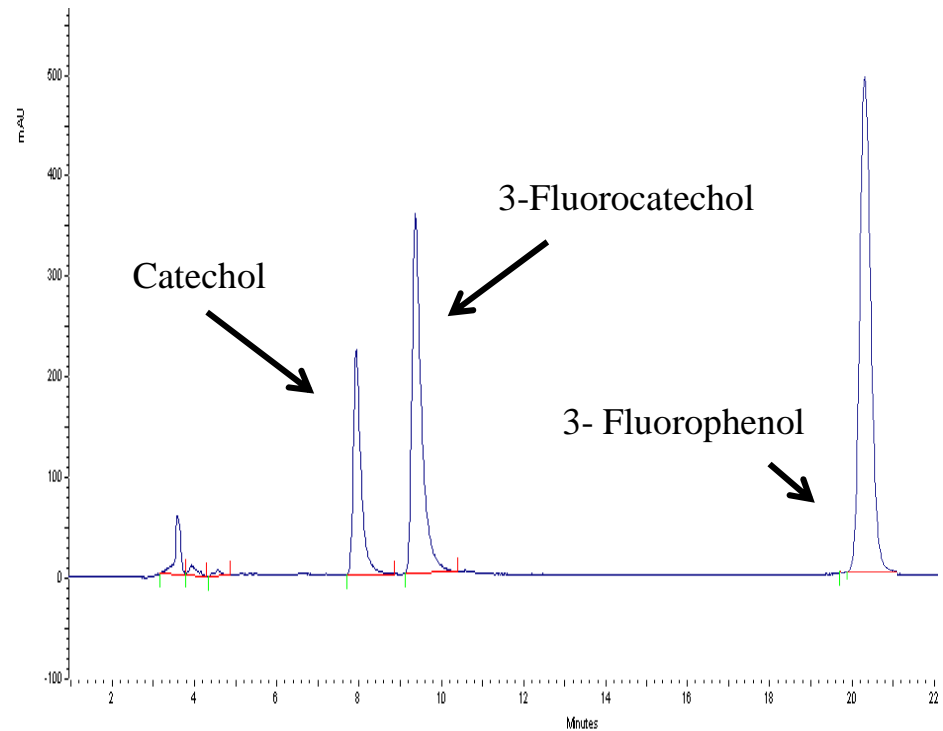
Two new metabolites in 3FP cultures were detected by HPLC-DAD

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



3-Fluorocatechol

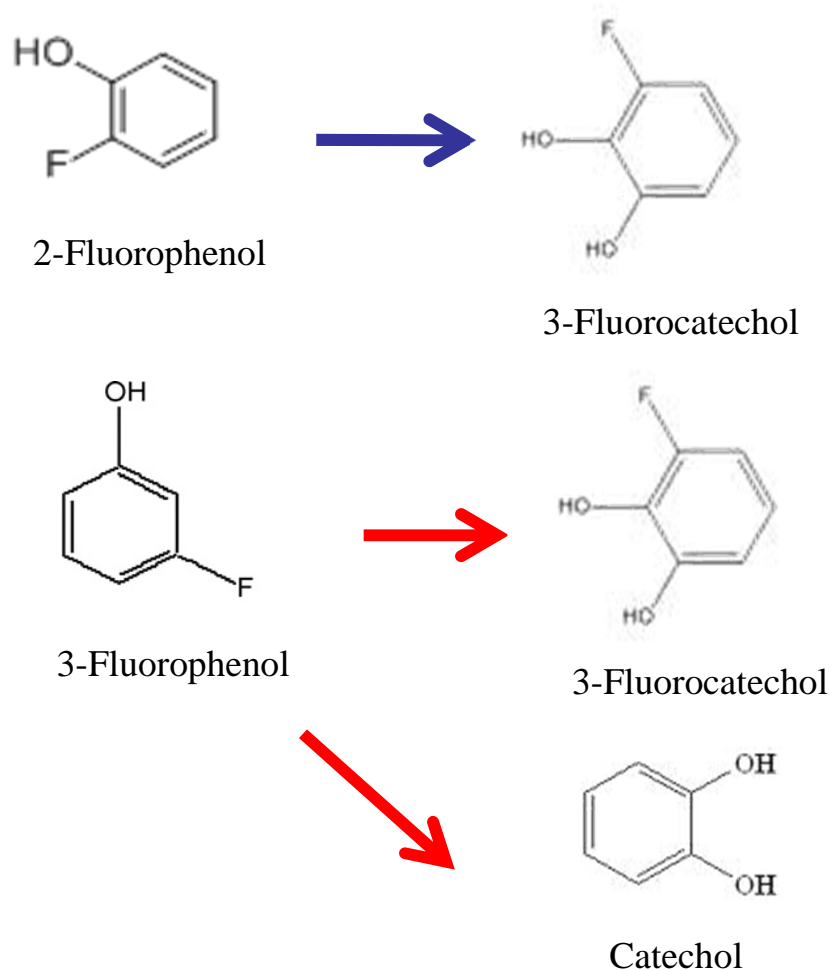
Catechol



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



Proposed metabolic pathway ?



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)



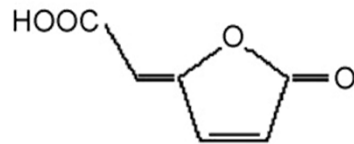
Some conclusions

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

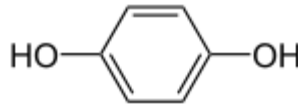
- ECM fungi capacity to **tolerate** and grow on **TFA** and **FP**

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

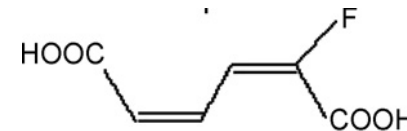
3-Fluorocatechol and **Catechol** were detected as **degradation metabolites**, but other possible **metabolic** products could also be produced at low quantities



Dienelactone



Hydroquinone

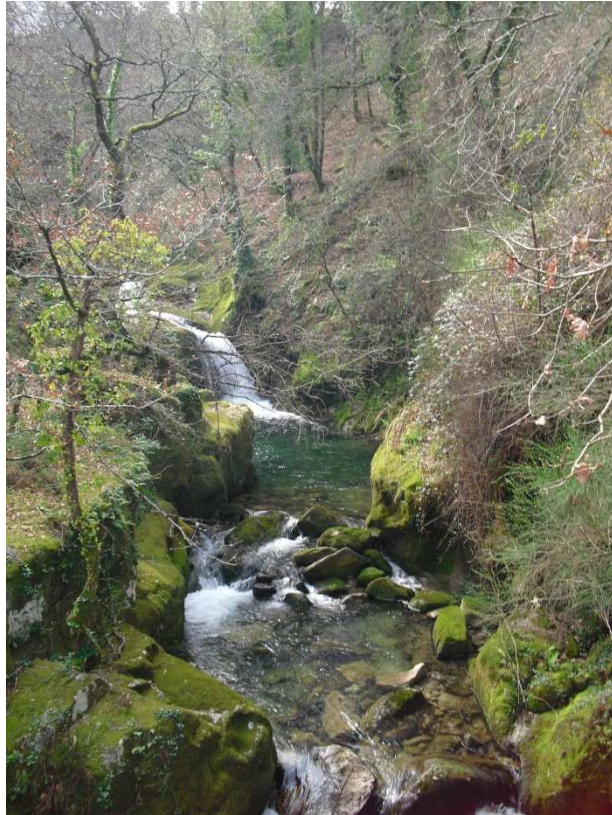


2-Fluoromuconate

ECM fungi as players on rhizosphere remediation technology



Thank you for your attention!



Acknowledgments

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