

# SEARCHING FOR FUNGI IN VERMICOMPOSTS TO DEGRADE PERSISTENT PESTICIDE METABOLITES



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**XIV Symposium in Pesticide  
Chemistry *Piacenza, Italy***



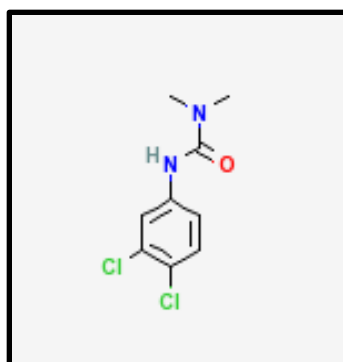
Two principal research line :

- Behaviour and degradation of pesticide.
- Management of agro-industrial wastes.

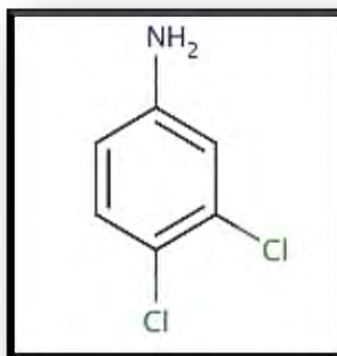


➔ Aromatic amines (AA) are widely used in the manufacturing of dyes, pharmaceuticals and **PESTICIDES**

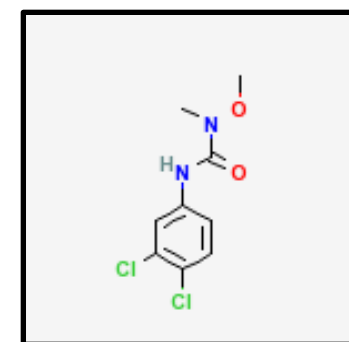
➔ 3,4-DCA is a common intermediate in the biodegradation of several pesticides.



Diuron

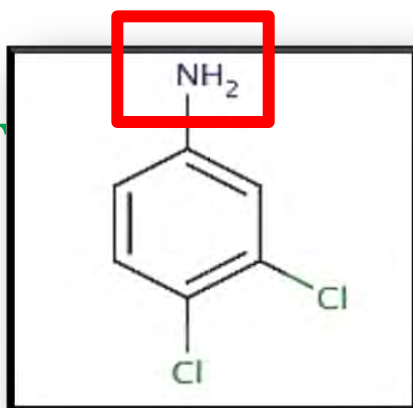


3,4 DCA



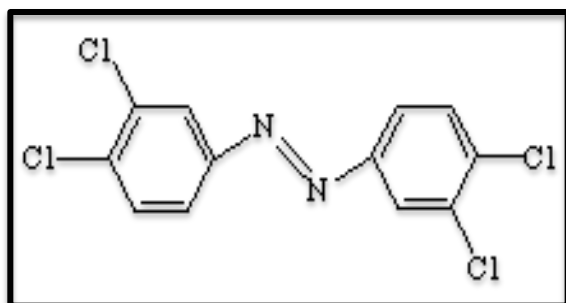
Linuron

➔ **3,4 DCA**  
**PERSISTENT**

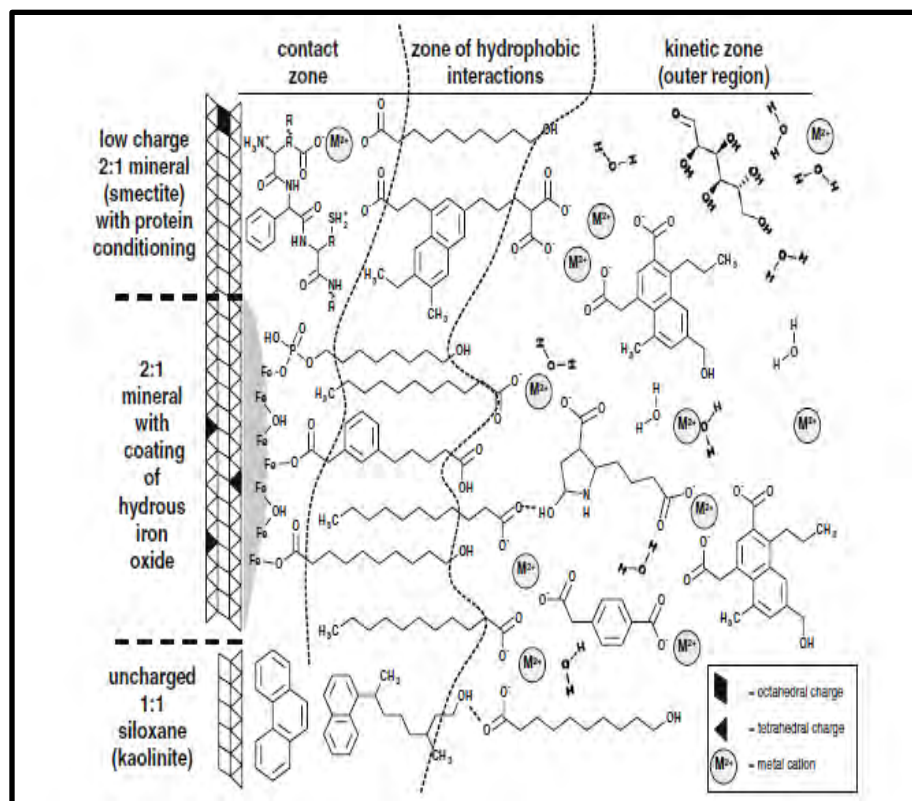


⊙ **Bound to humic acids or lignin polymers**  
**for large periods in soil and sediments**

⊙ **Condensation reactions**



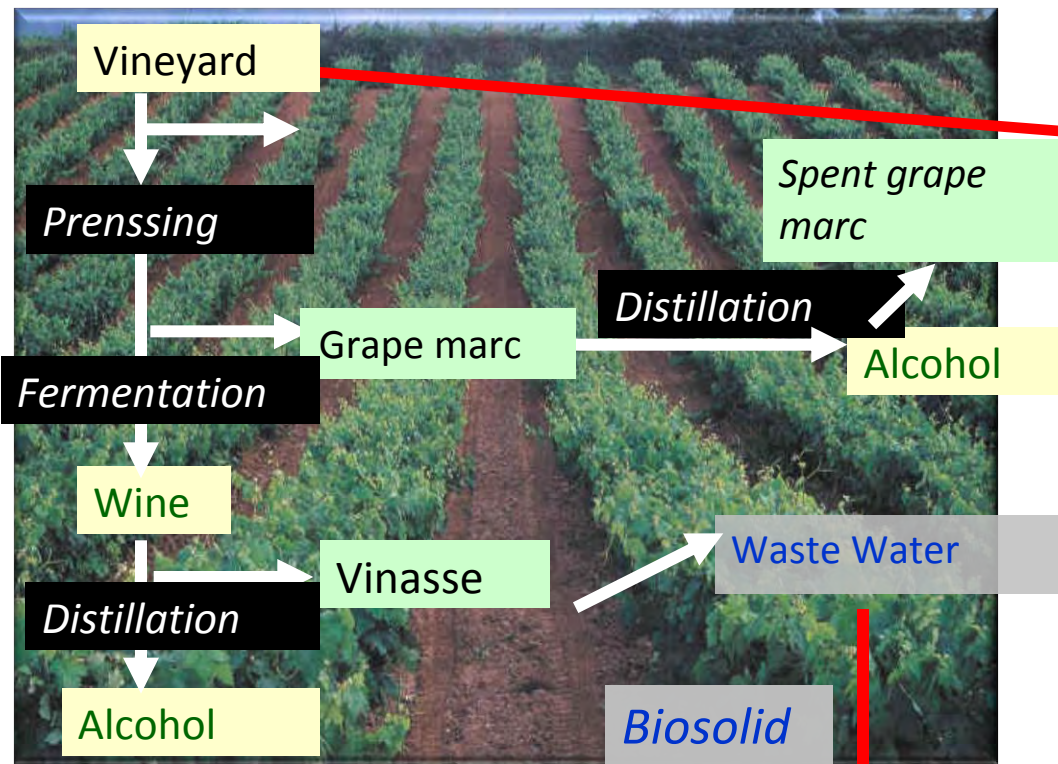
**3,3',4,4'-Tetrachloroazobenzene**  
**(TCAB)**



➔ **3,4 DCA TOXIC**

**Affect the soil microbial population and have genotoxic and cytotoxic potential**

# MANAGEMENT OF AGRO-INDUSTRIAL WASTES : Vermicomposting.



*Eisenia andrei*

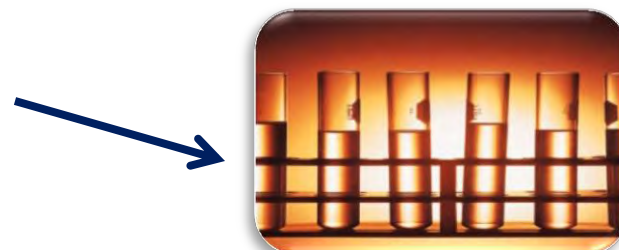
## **OBJECTIVES**

- ➡ **To isolate of fungi that often growth in wine organic substrate submitted to vermicomposting.**
- ➡ **To determinate the biodegradation rate of 3,4-DCA and the presence of metabolites.**

**conditioning stage vermicomposting**



**$10^{-1}$   $10^{-2}$   $10^{-5}$  dilutions in PDA agar**



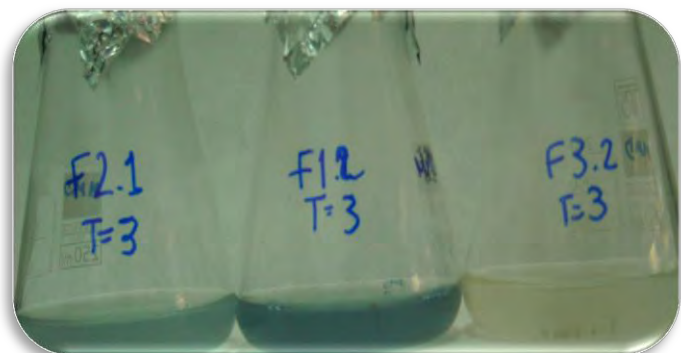
**Isolated fungi in Rose-Bengal agar**



**F1**



**F2**



**FUNGI GROWTH in MMFN  
42 day at 28°C and 110 rpm**



**HPLC-DAD  
SPME-GC/MS**



**$10 \text{ mg L}^{-1}$  3,4-DCA in  
MMFC and MMFN**



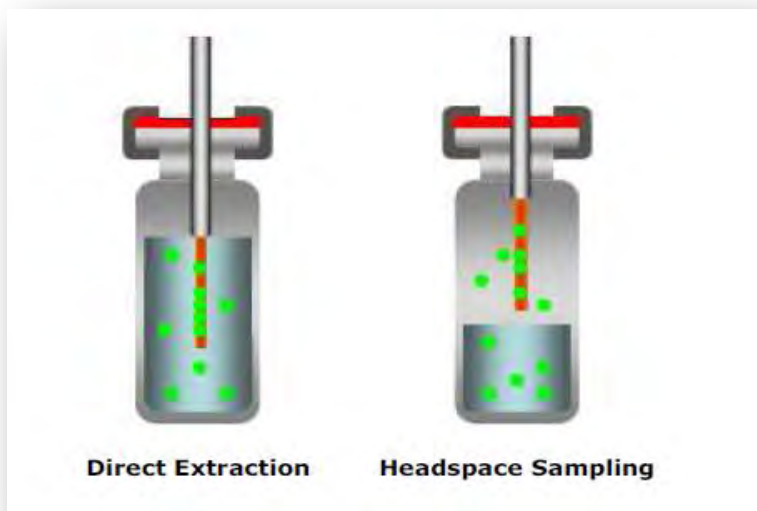
**F3**

**Molecular Identification: 18S RNA  
un-SSU-0817F  
un-SSU-1536R**

## **SOLID-PHASE MICROEXTRACTION (SPME)**

Advantages:

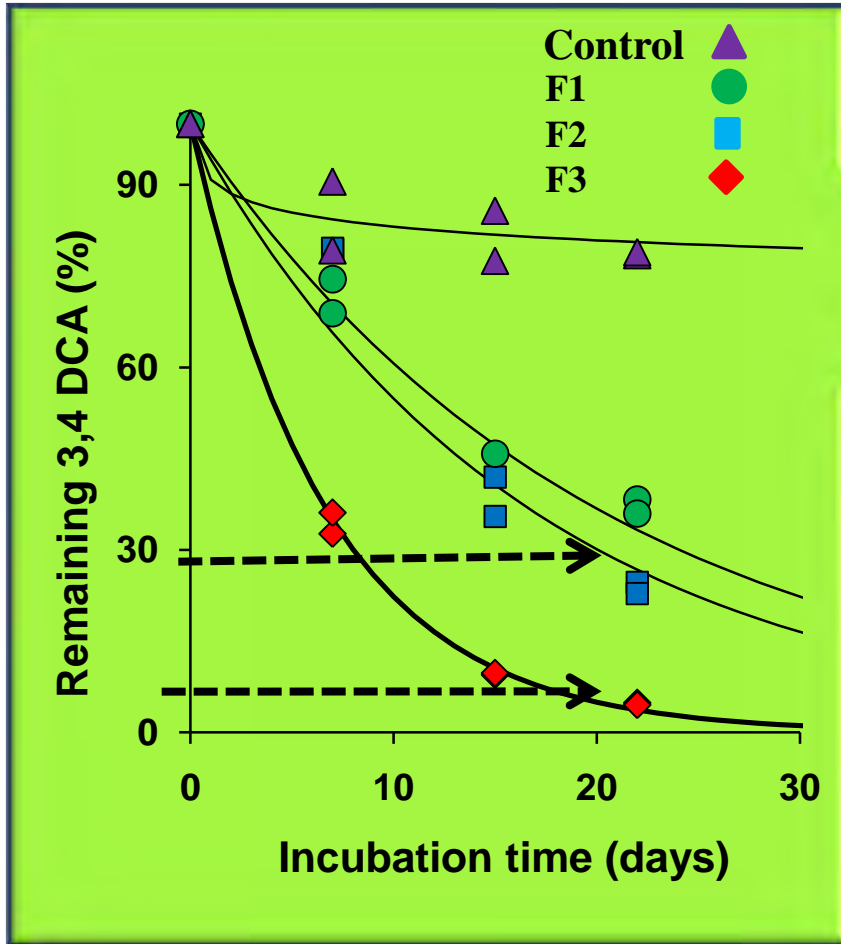
- ☑ Is a rapid sample preparation technique
- ☑ analyses using a single solvent-free step
- ☑ small sample volumes.



- ☑ Can be used in combination with GC-MS to identify the presence of metabolites in MMFN

# First-order degradation kinetics for 3,4-DCA

## by the three identified fungi

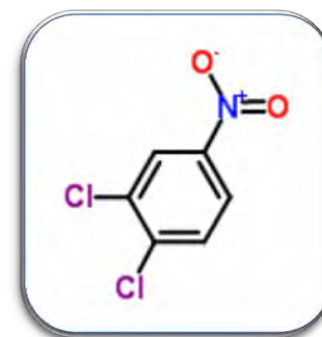
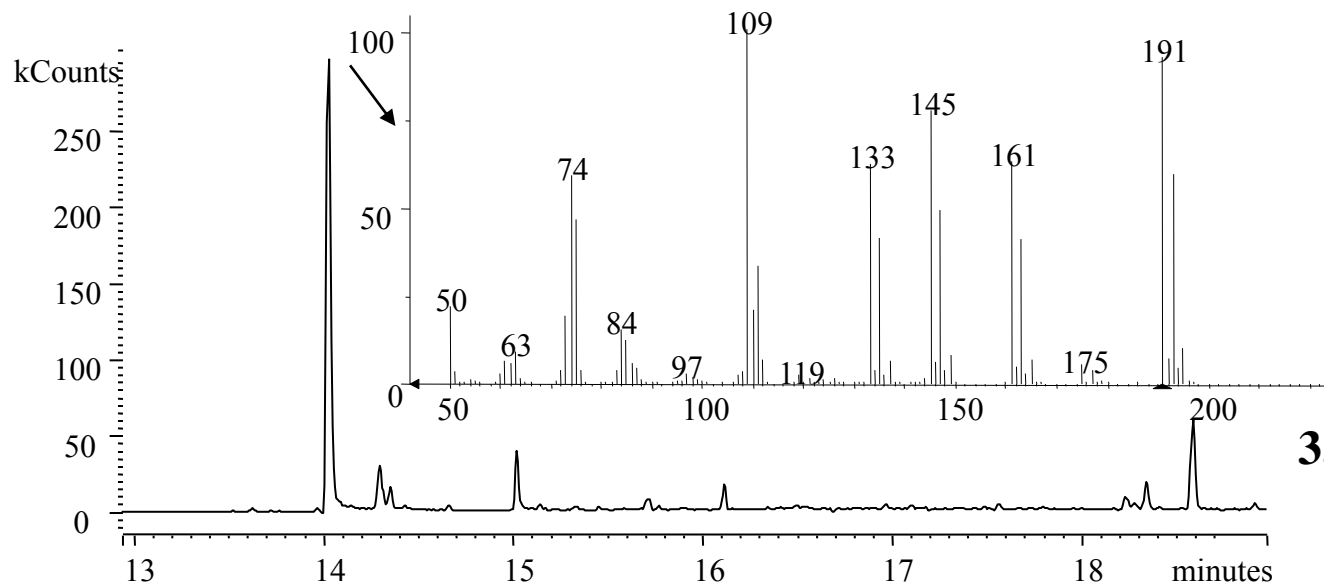


Fungal strain	$k \cdot 10^{-2}$ ( $d^{-1}$ )	$DT_{50}$ (d)	$R^2$
<i>Fusarium sp 1</i>	0.6	11.6	0.97
<i>Fusarium sp 2</i>	0.5	13.9	0.93
<i>Aspergillus niger</i>	1.5	4.6	0.99
Control (-)		$1.3 \times 10^8$	0.84

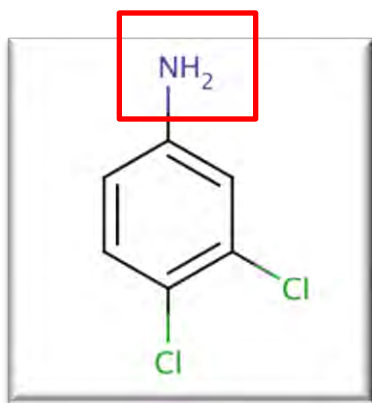
Degradation constant (k), Half-life dissipation time ( $DT_{50}$ ), determination coefficient ( $R^2$ )



**Metabolites identified**

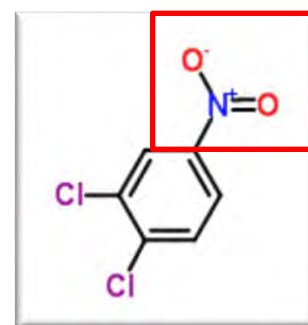


**3,4-Dichloronitrobenzene**

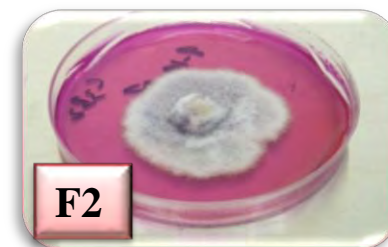


**3,4-DCA**

**Oxidation**



**3,4-DCBN**



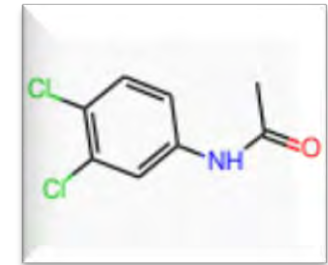
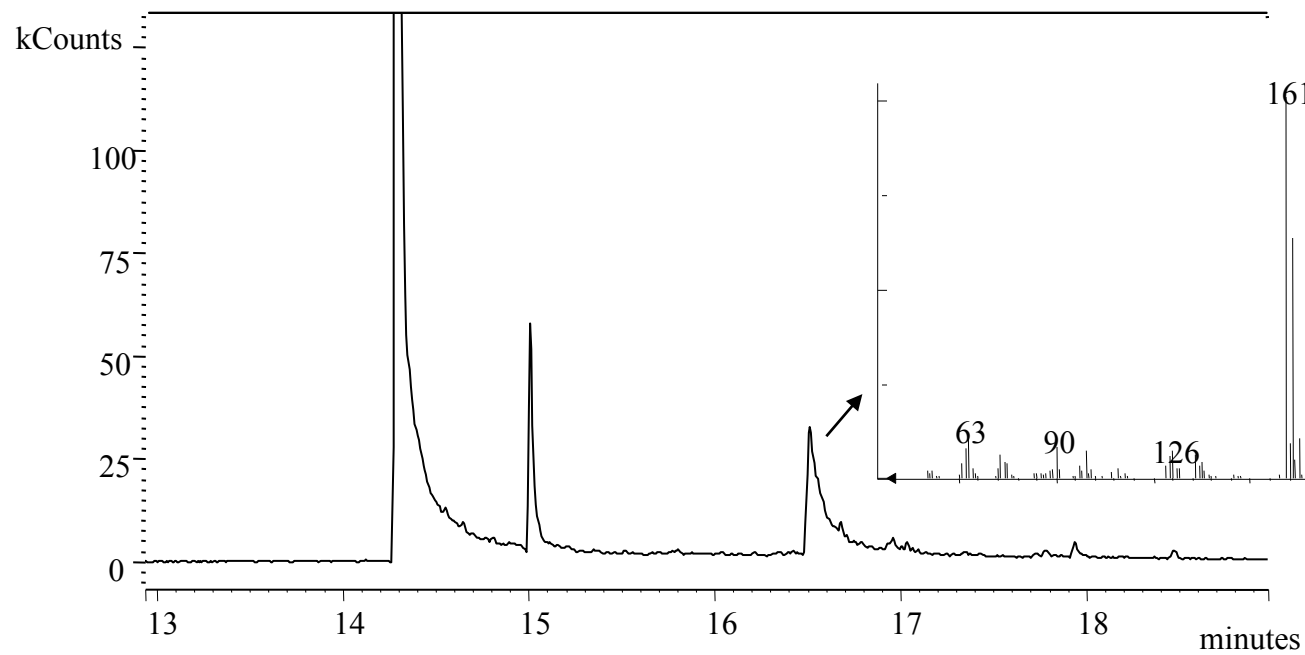
**F2**



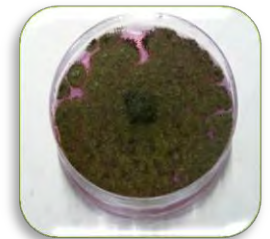
**F1**

*Fusarium sp*

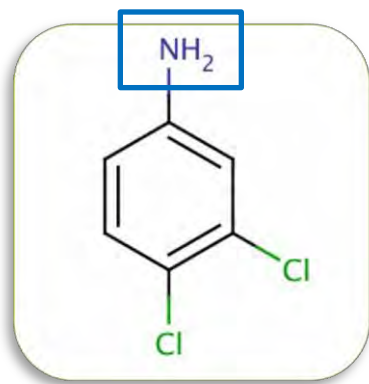
# RESULTS



**3,4-Dichloroacetanilide**

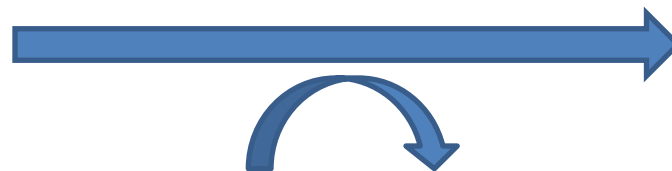


***A. niger***



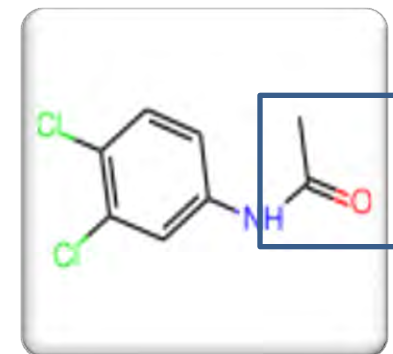
**3,4-DCA**

**N-Acetyltransferase  
(NAT)**



**AcCoA**

**CoA**



**3,4-DCAN**

(Martins *et al.*, 2009)

# CONCLUSION

- ➡ Fungi from vermicompost can constitute a detoxification pathway for toxic and persistent metabolites and the vermicompost could be used as a natural bead to maintain these beneficial fungi in bioremediation strategies.
- Further research has been done.

! THANK

YOU!

