University of Thessaly
Department of Biochemistry & Biotechnology

Degradation and adsorption of terbuthylazine and chlorpyrifos in various biomix substrates with local interest

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Biobeds status in Greece

Research on biobeds just starting in Greece

- Select substrates relevant for Greek agriculture
- Evaluate these substrates as biomix substrates for use in pilot biobeds in Greece

  - Laboratory studies

  - Semi-field studies

  - Field studies

Evaluation of possible biomix substrates

- Soil
- Soil + Compost (50:50 w/w)
- Soil + Compost (75:25 w/w)
- Soil + Compost + Straw (50:25:25 w/w)

Compost

Origin: Composted remains from cotton flower and seed

C/N = 300

Organic matter content = 45%

Soil

Soil texture: Clay loam (sand 38%, silt 25%, clay 37%)

pH: 7.8

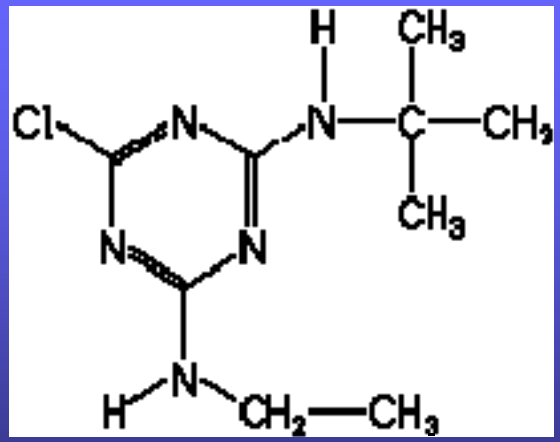
Organic matter content: 1.5%

Why this biomix substrate (compost) ?

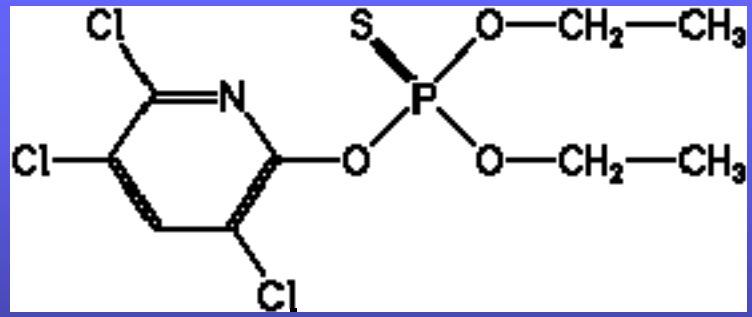
- Easily available at high amounts
- Negligible or no cost
- Their biodegradation and adsorption potential regarding pesticides has not been evaluated so far

Pesticides studied

- **Terbuthylazine:** Post-emergence herbicide used mainly in corn as a replacement for atrazine
- **Chlorpyrifos:** Organophosphorus insecticide used mainly in orchards



Terbuthylazine (TA)



Chlorpyrifos

Why these pesticides?

- High – dose pesticides
- Very popular in Greece and other EU member-states
- Commonly found in groundwater (terbuthylazine) and surface water systems (chlorpyrifos) adjacent to intensively cultivated agricultural areas

Aim of the study

To identify the degradation and adsorption behaviour of certain pesticides on biomix substrates which are candidates for use in pilot biobed systems in Greece

Degradation study

- 4 biomix substrates
- Pesticides were applied at two dose levels

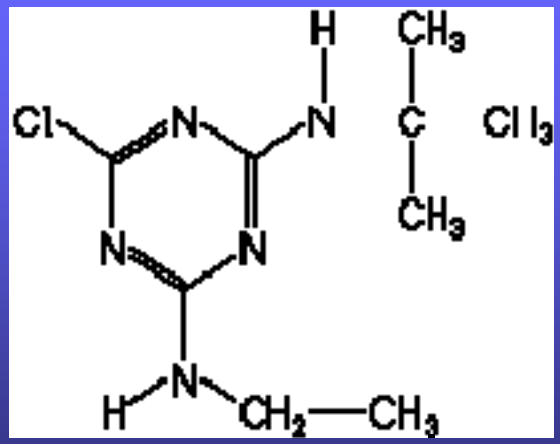
  - Low dose: 4 and 2 mg kg^{-1} for TA and chlorpyrifos (in progress…)

  - High dose: 100 and 50 mg kg^{-1} for TA and chlorpyrifos

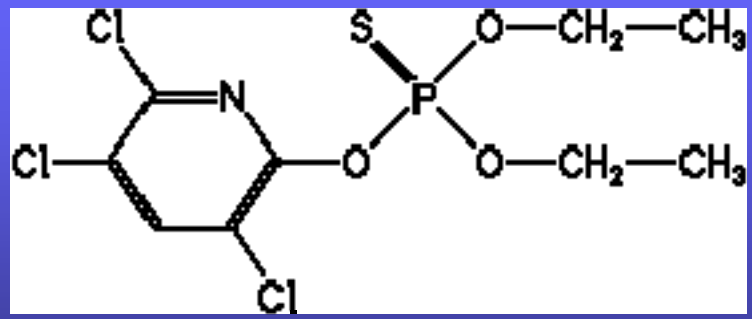
- Incubation for 100 days at 25ºC, moisture content adjusted to 40% Water Holding Capacity

Pesticide metabolism and analysis

- Degradation of TA and **formation of OH–TA and Desethyl-TA (DETA)** were determined via HPLC – UV analysis (in progress….)
- Degradation of Chlorpyrifos and **formation of Trichloropyridinyl (TCP)** were determined via HPLC -



Terbuthylazine (TA)

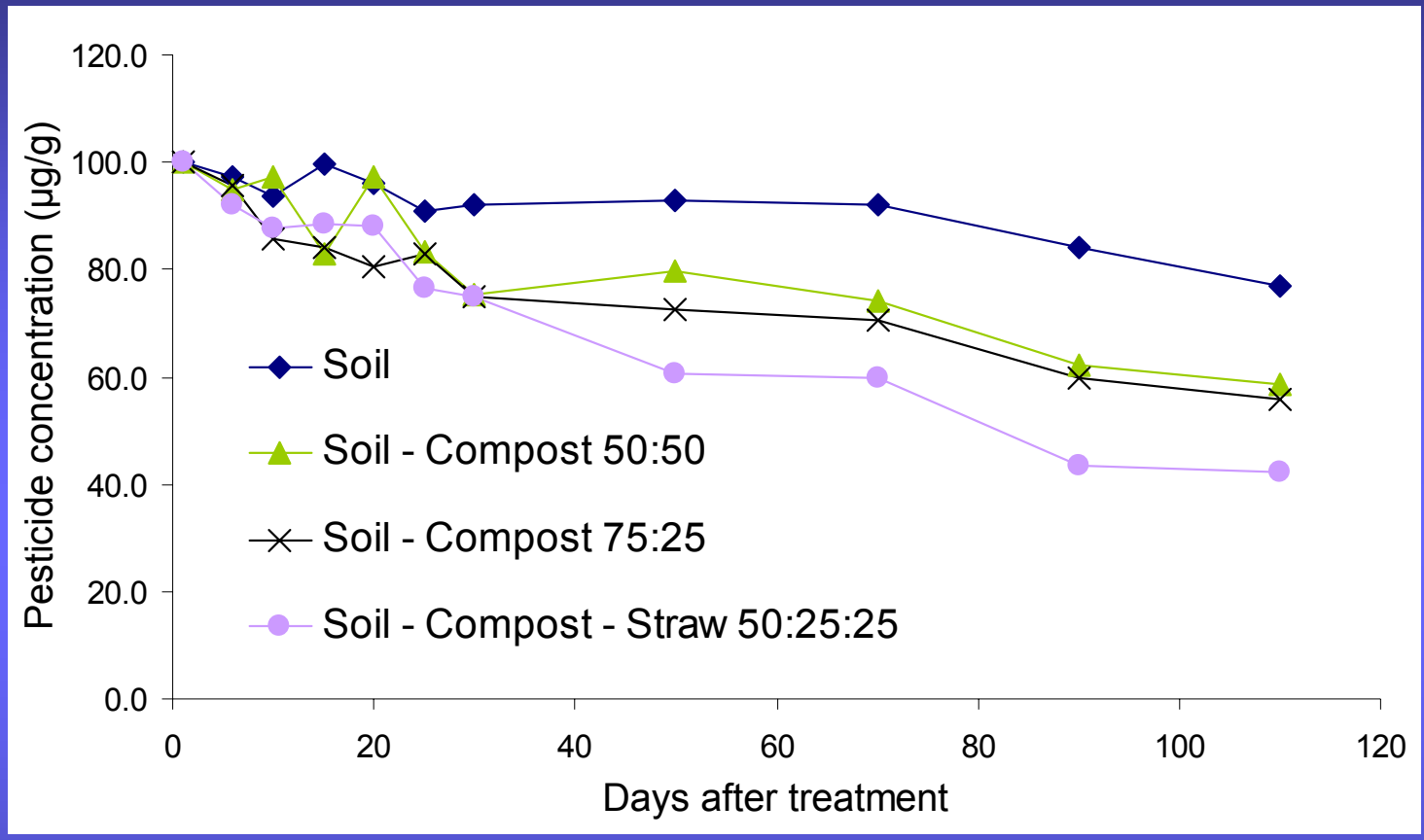


Chlorpyrifos

Degradation studies

Terbuthylazine

Degradation of terbuthylazine – High Dose



Degradation terbuthylazine

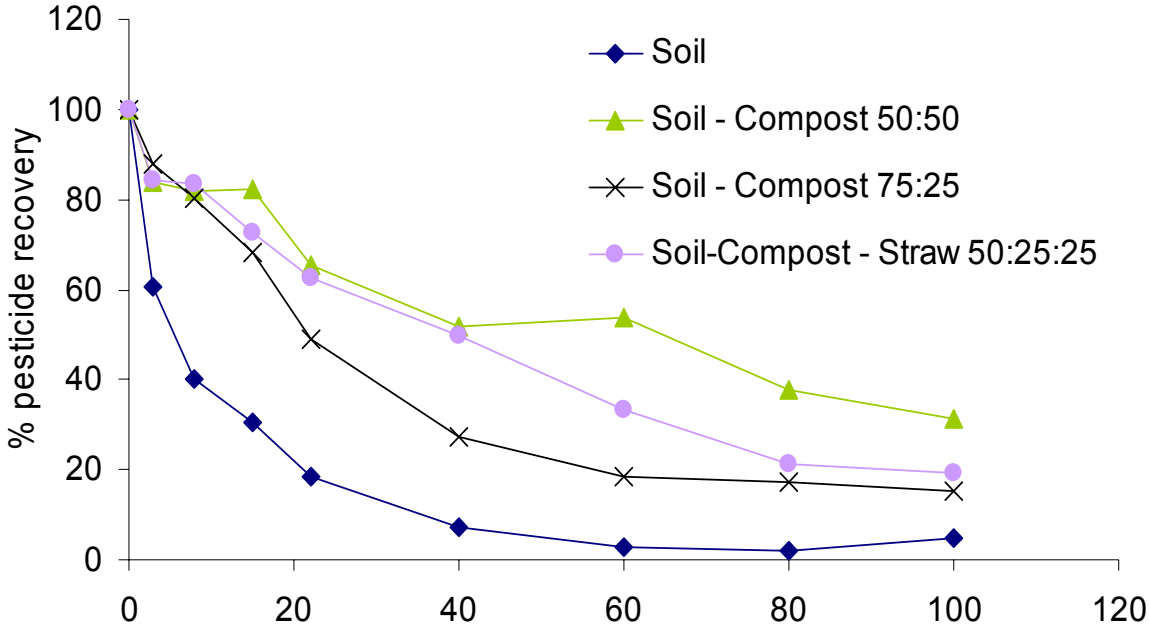
Degradation of terbuthylazine followed first-order kinetics and $T_{1/2}$ were calculated by $T_{1/2} = ln2/K_{deg}$

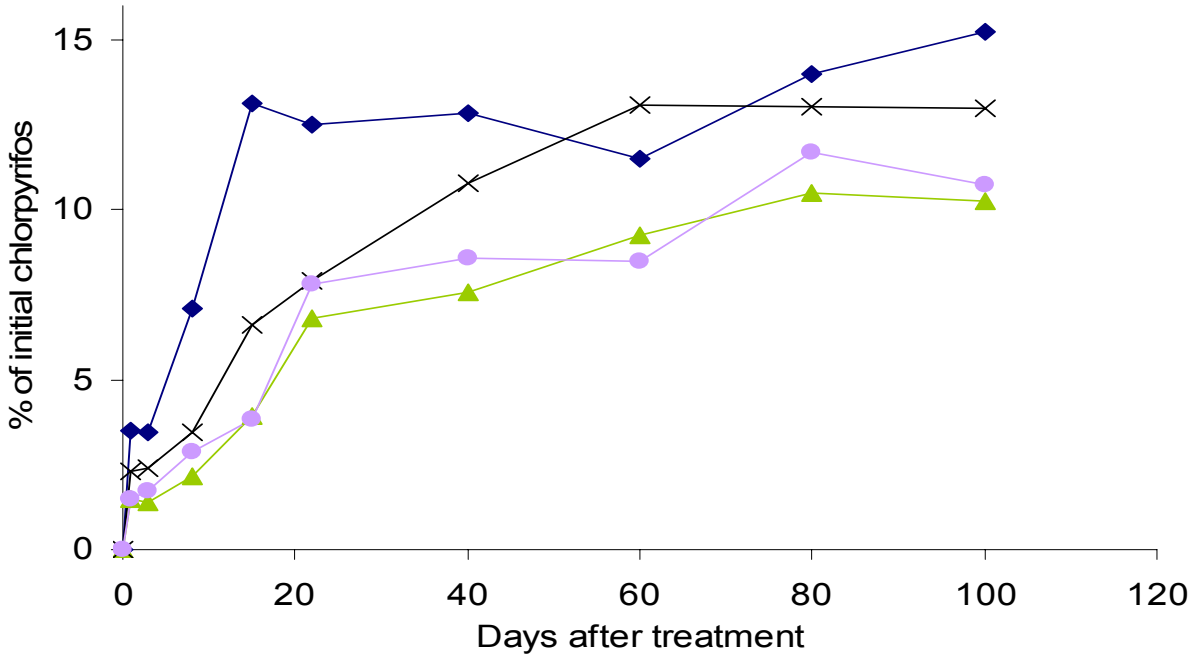
Treatment	$T_{1/2}$ High Dose
Soil	329.0
Soil-Compost (50:50, w:w)	133.1
Soil-Compost (75:25, w:w)	150.5
Soil-Compost-Straw (50:25:25,w:w:w)	88.8

Summary for terbuthylazine transformation

- Low degradation rates of TA in all substrates tested (high dose rate 100 µg/g)
- The slowest degradation of TA was evident in the soil
- The higher the contribution of soil in the biomix the slowest the degradation of TA
- Addition of straw in the biomix significantly accelerated degradation of TA

Degradation and metabolism studies - Chlorpyrifos





Degradation of Chlorpyrifos

Formation of TCP

High Dose (50 mg/kg)

Degradation Chlorpyrifos

Degradation of chlorpyrifos followed first-order kinetics and $T_{1/2}$ were calculated by $T_{1/2} = ln2/K_{deg}$

Treatment	$T_{1/2}$ (d) High Dose
Soil	19.7
Soil:Compost (50:50, w:w)	64.7
Soil:Compost (75:25, w:w)	33.9
Soil:Compost:Straw (50:25:25,w:w:w)	39.3

Summarize for chlorpyrifos transformation

- Chlorpyrifos is partially transformed into TCP in all matrices
- Fastest degradation of chlorpyrifos in the soil matrix
- The higher the contribution of compost in the biomix the slower the degradation of chlorpyrifos

Adsorption studies

Adsorption study

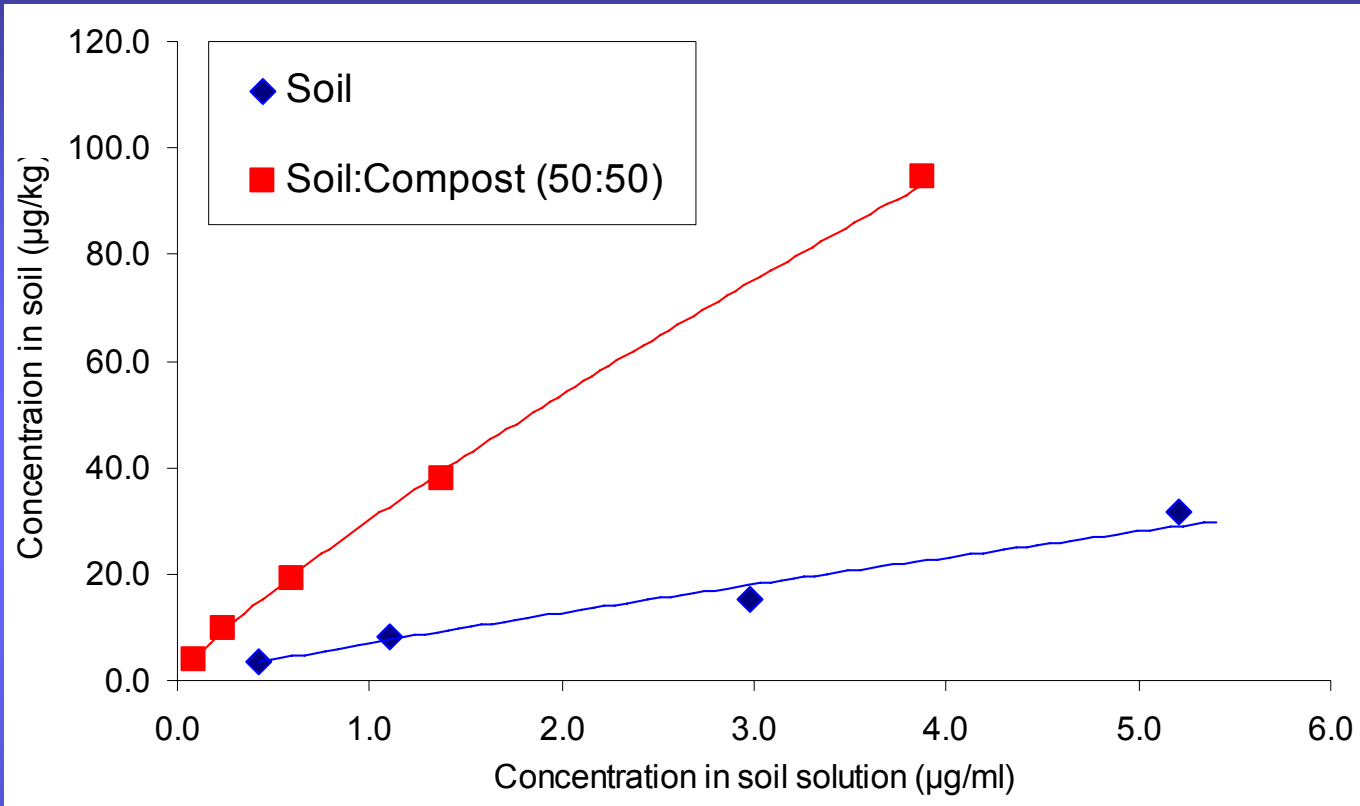
- Batch equilibration method
- Pesticide adsorption measured only in two substrates

  - soil

  - soil – compost (50/50 w/w)

- 10 g air-dried solid substrate shaken with 20 ml 0.01M $CaCl_2$ containing 2, 5, 10, 20 and 50 mg/L of the pesticide
- Equilibration was obtained after 5 h

Adsorption isotherms for Terbuthylazine



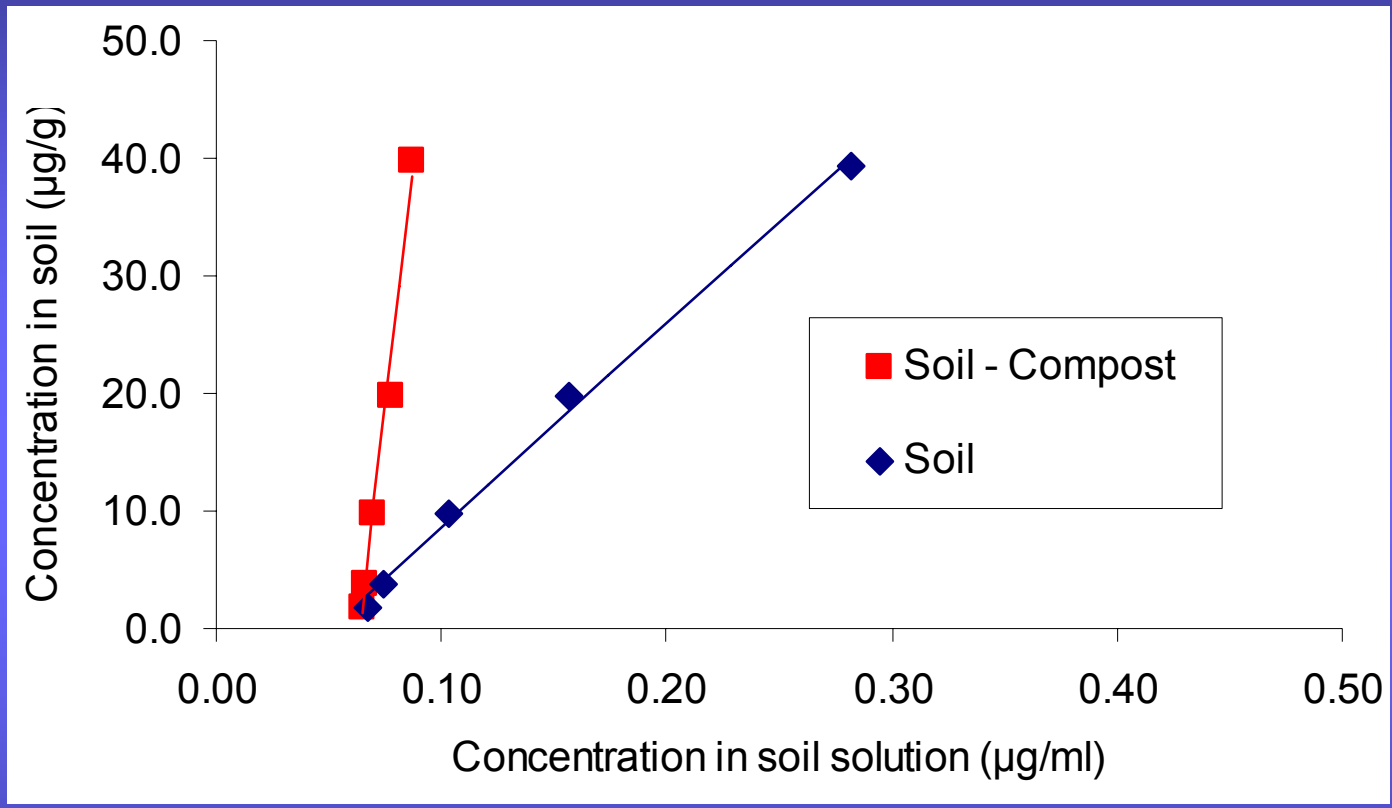
Adsorption of terbuthylazine was better described (r^2>0.98) by the Freundlich equation $Cs = Kf * Cw^N$

Kf values for Terbuthylazine

Treatments	K_f (g/ml)	N
Soil	7.04	0.83
Soil:Compost (50:50)	30.0	0.86

The high organic matter content of the compost (45%) compared to soil significantly increases the affinity of terbuthylazine for adsorption

Adsorption isotherms of Chlorpyrifos



Adsorption of chlorpyrifos was better described (r^2>0.99) with linear regression

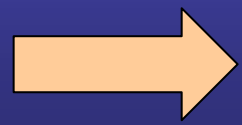
Kd values of Chlorpyrifos

Treatments	K_d (g/ml)
Soil	124.2
Soil:Compost (50:50)	417.9

Kd was calculated using a reference concentration of 1 µg/ml in soil solution

To summarize…..

- Very slow degradation of terbuthylazine in soil alone
- Addition of compost and straw accelerated degradation of terbuthylazine although its adsorption affinity was 3x higher in the compost-soil (50/50)……. dominant effect of the compost microbial community ?
- The higher the contribution of compost in the biomix the slower the degradation of chlorpyrifos due to increasing adsorption of the more hydrophobic chlorpyrifos in the compost – amended biomix substrates…..dominant effect of the compost organic matter surfaces for sorption?)

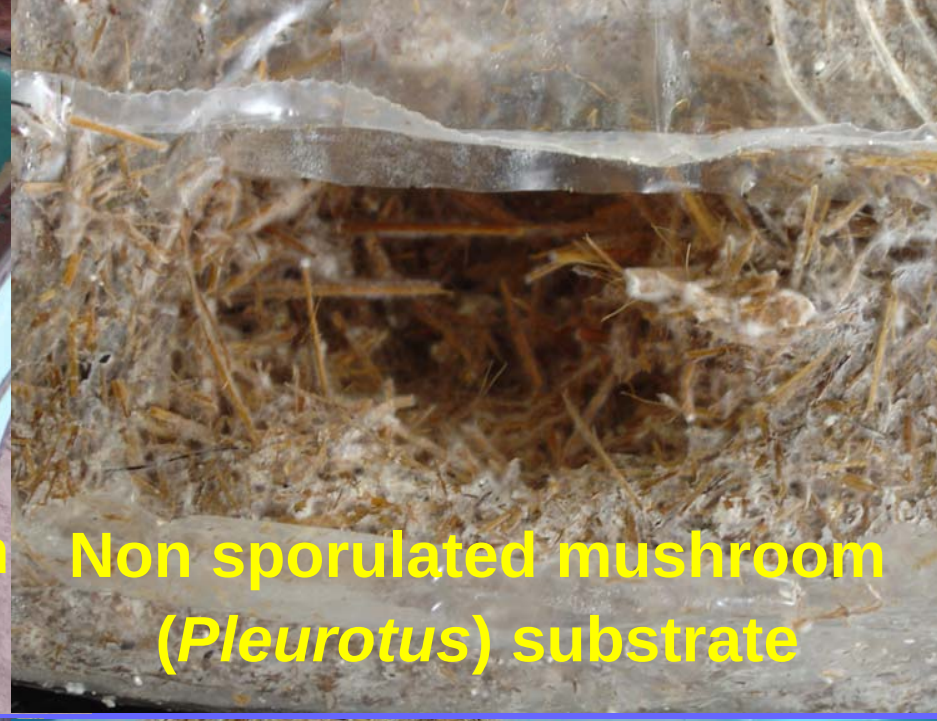


Overall, the soil – compost – straw biomix provided the most promising degrading potential although a better picture will be obtained after the completion of the low dose study

How do we continue?

- Evaluate further substrates for potential use in biobeds (relevant for Greek agriculture)
  - Composted olive leaves
  - Spent mushroom substrate for *Agaricus* and *Pleurotus*
  - Non sporulated mushroom substrate for *Pleurotus*
  - Composted residues of cotton crop
- Assessment of the most promising substrates in packed column experiments
- Evaluation of the efficacy and operation of pilot on-farm biobeds


Composted residues of cotton crop, seed etc


Non sporulated mushroom (*Pleurotus*) substrate


Composted olive tree leaves


Spent mushroom (*Pleurotus*) substrate

How are we going to do this?

- An MSc student is currently working on the preliminary evaluation of biomix substrates (Konsantina Kravariti)
- A PhD student has just started working on the application of biobeds in Greece (Vagelis Karanasios)
- A preliminary financial support is expected by the Hellenic Ministry for the Environment, Physical Planning and Public Works for six months (Jan – Jun 2008) with the option of extension for another six months or more