BIOREMEDIATION OF CONTAMINATED SOIL: A STRATEGY BASED ON FUNGI AND BACTERIA

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RemTech Expo 2018 (19, 20, 21 Settembre) FerraraFiere
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Fidenza, Emilia Romagna, Italy

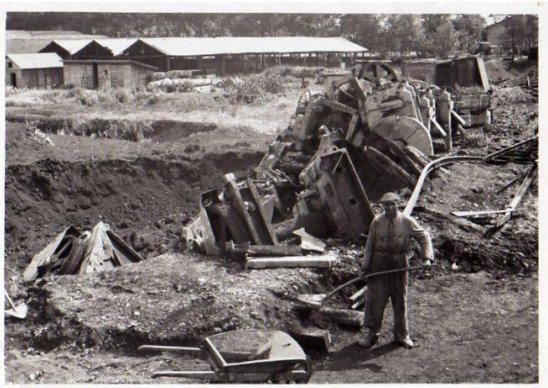
Site of National Interest (SIN)

1888

Tar distillery

Lead tetraethyl production

Sulfuric acid and phosphate fertilizers production



1945

Bombarded during Second World War

2003

Starting disposal of industrial installations and soil remediation by Fidenza Municipality using traditional techniques

LIFE-BIOREST

2016

On-going activities foresee the soil excavation from the polluted area, followed by *in-situ* remediation.

An adjacent area is used to built up biopiles where bio-attenuation processes take place.

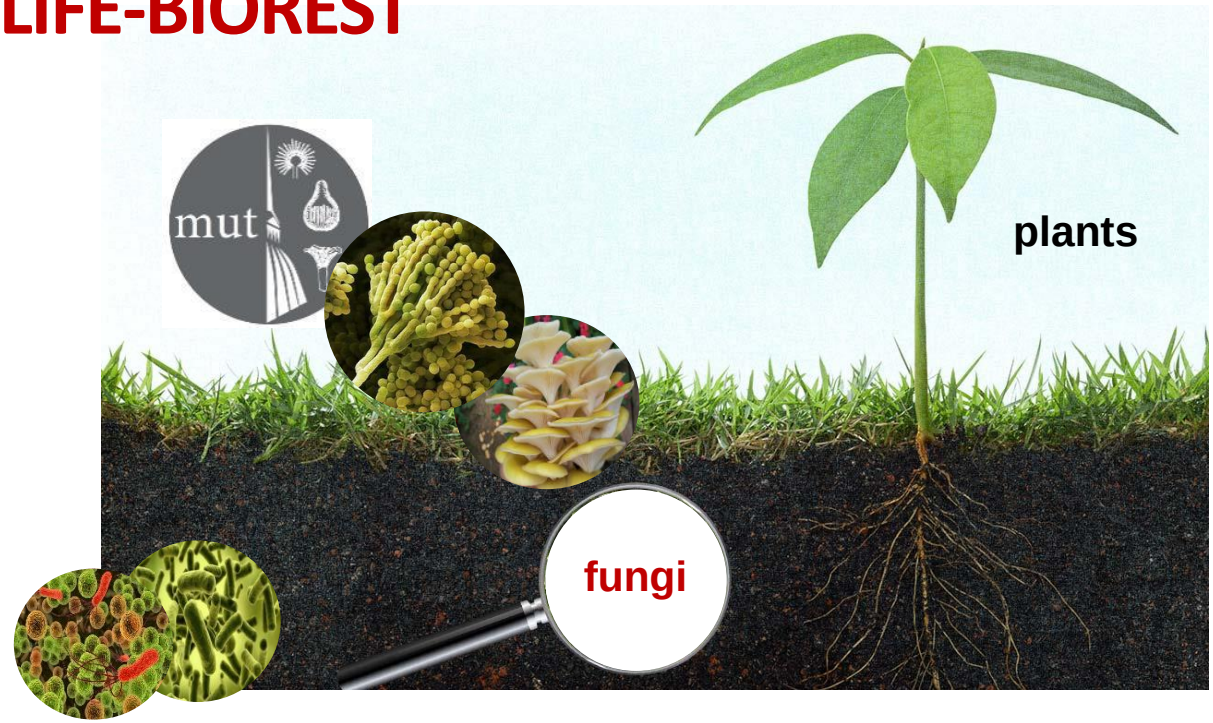
Degradation yields are low (industrial use of the soil) and the process is time consuming (6-9 months).

There is room for improvement of the process.

LIFE-BIOREST

Selection of microorganisms adapted to this peculiar ecological niche

Aims of LIFE-BIOREST

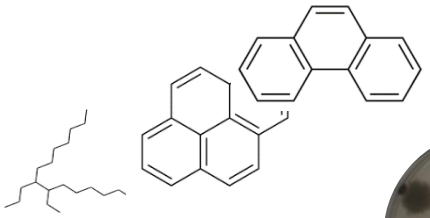


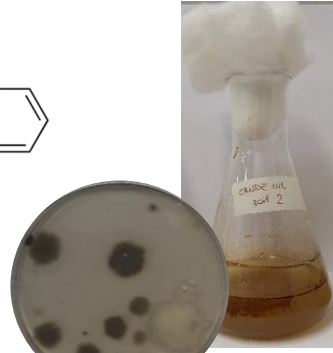
… for urban and residential uses

RESTORE ECOLOGICAL FUNCTIONS

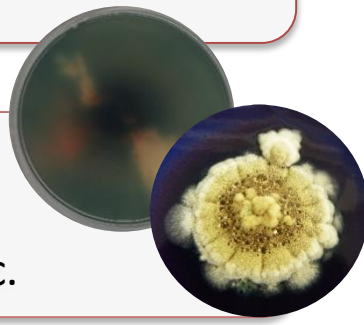
OF THE CONTAMINATED LAND

LIFE-BIOREST Outline





- **Which are the microorganisms adapted to this ecological niche?**



- **Which are the best performing strains?**
- Degradation skills, production of enzymes, biosurfactants, etc.

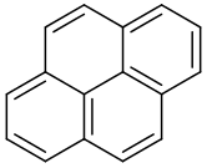
- **Will be the microorganisms effective in real conditions?**
- Degradation assessment: microcosm, mesocosm and *in situ* biopile

Microorganisms isolation

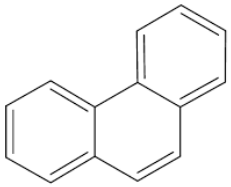
Selective pressure posed by the use of:

PAHs

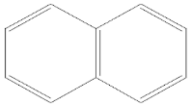
Pyrene

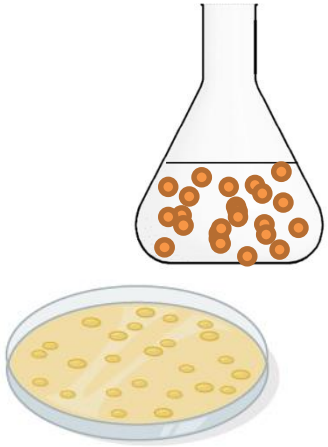


Phenanthrene

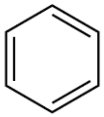


Naphthalene

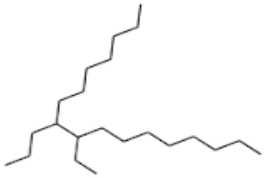




BTEX → Benzene



alkanes → Paraffin oil

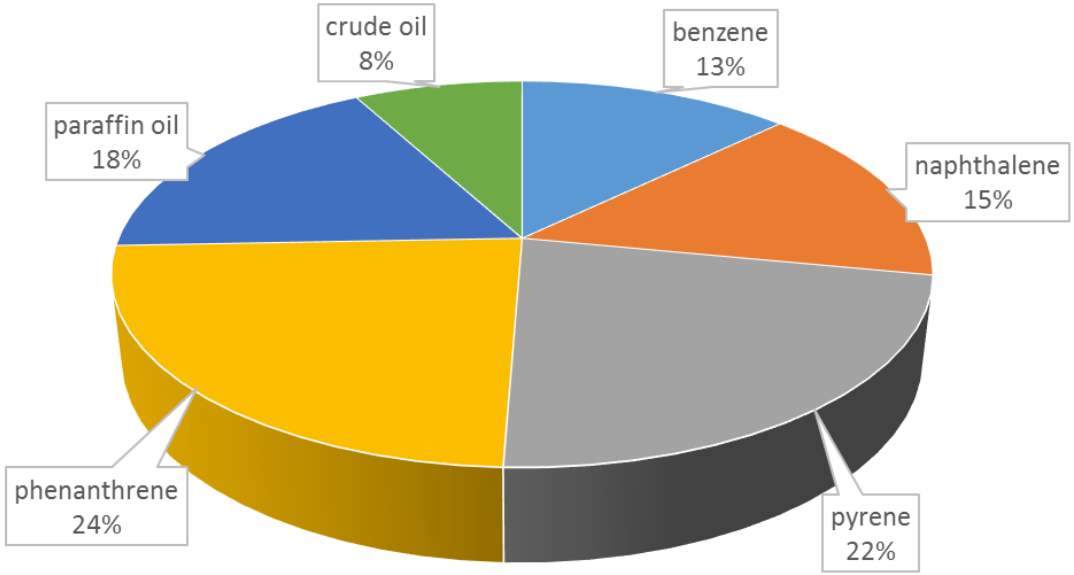


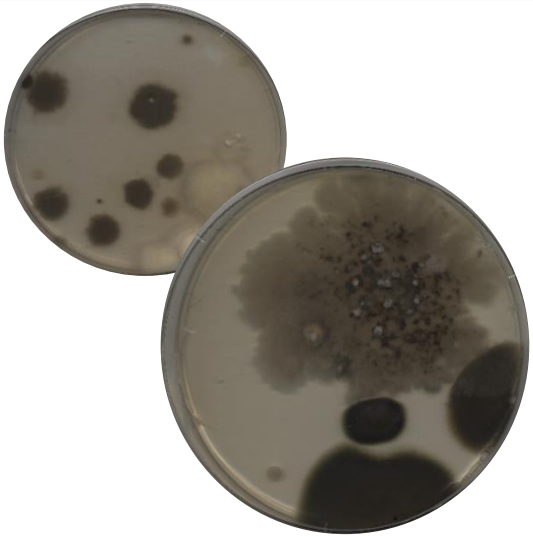
aliphatic and aromatic compounds → Crude oil extracted from the soil

Solid Screening

high biodiversity: 162 isolated fungi, belonging to 24 genera and 51 taxa

Percentage of strains isolated from all the cultivation conditions





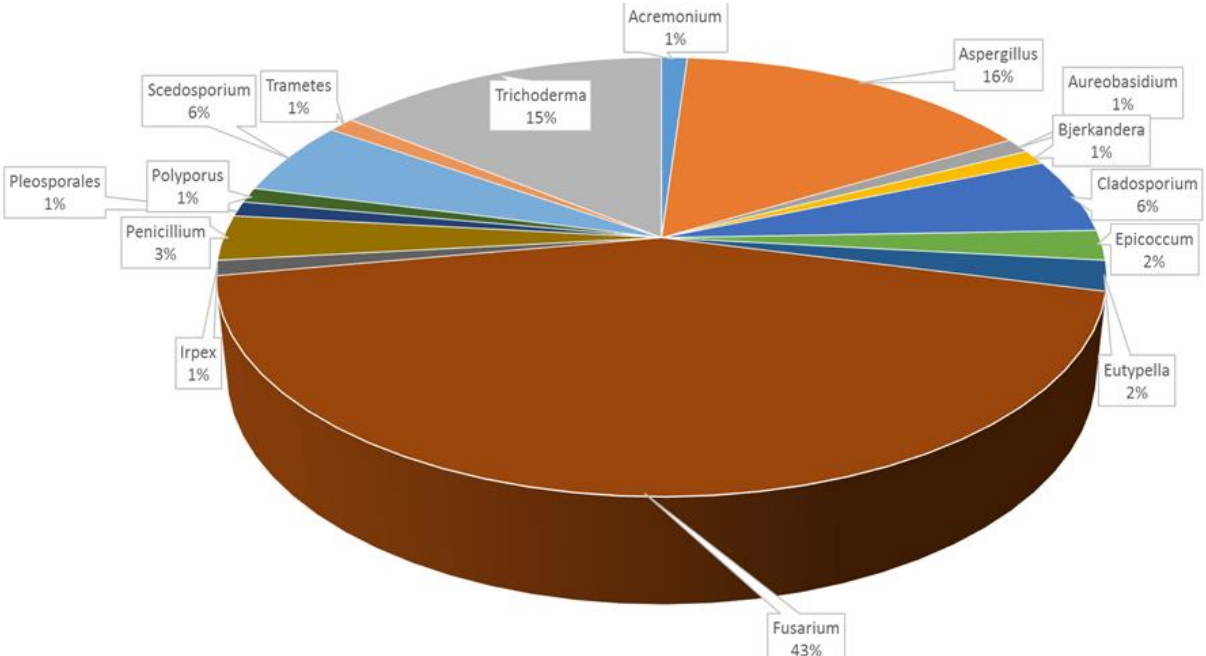
PAHs are good substrates, whereas fewer strains were isolated from the oil and benzene, due probably to the toxicity or the low availability (e.g. evaporation).

crude oil → **benzene/naphthalene** → **paraffin oil** → **pyrene/phenanthrene**

Liquid Enrichment

Different fungal communities developed according to **pollutants** as **sole carbon source** and the soil depth.

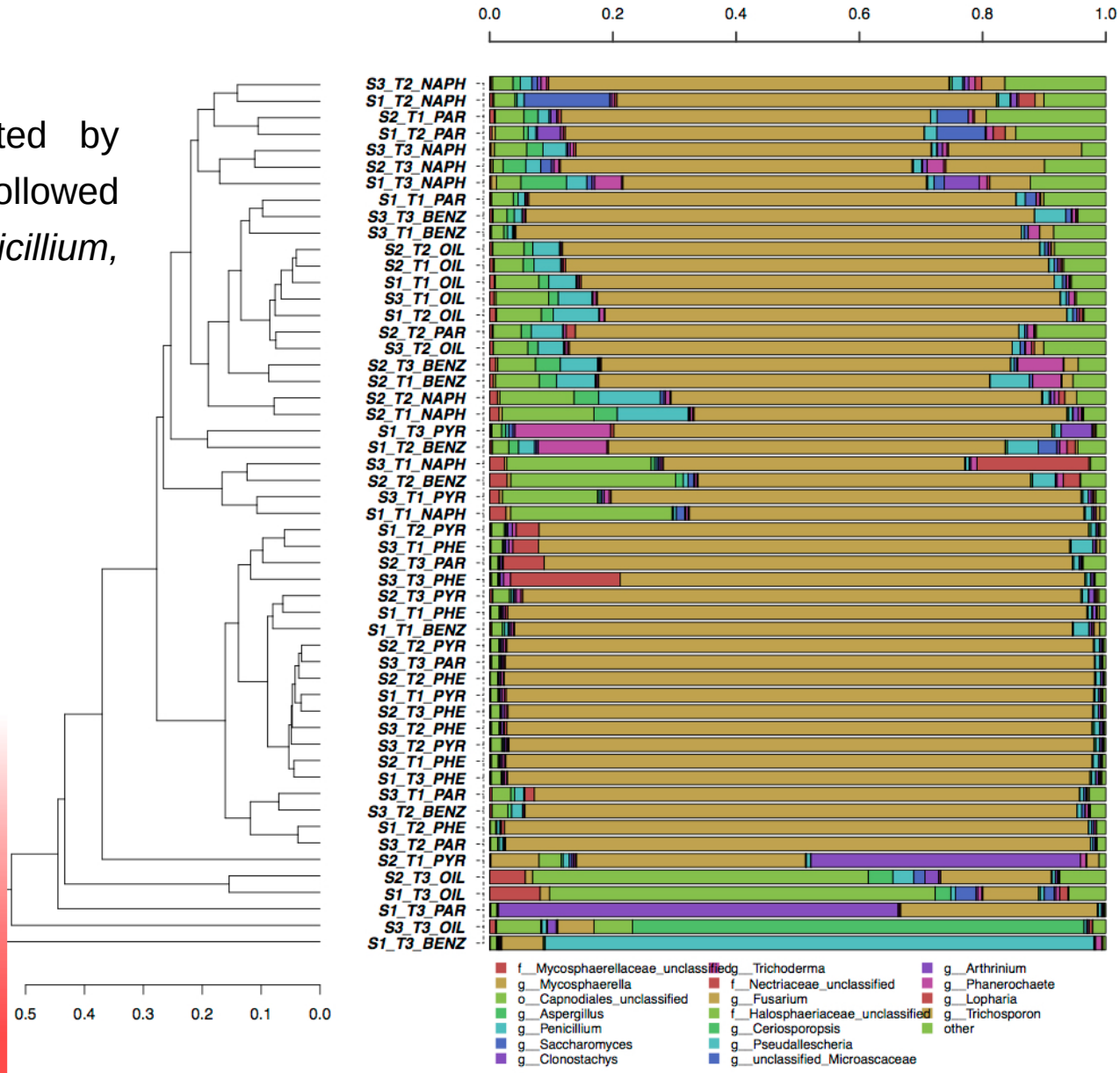
91 isolated fungi, belonging to 19 genera and 31 taxa

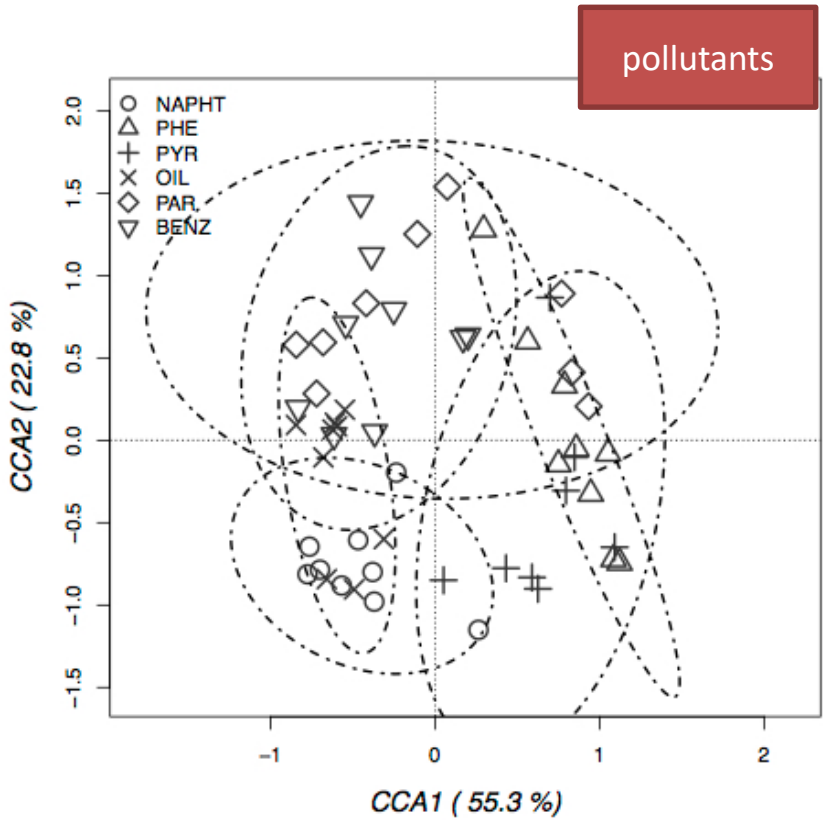


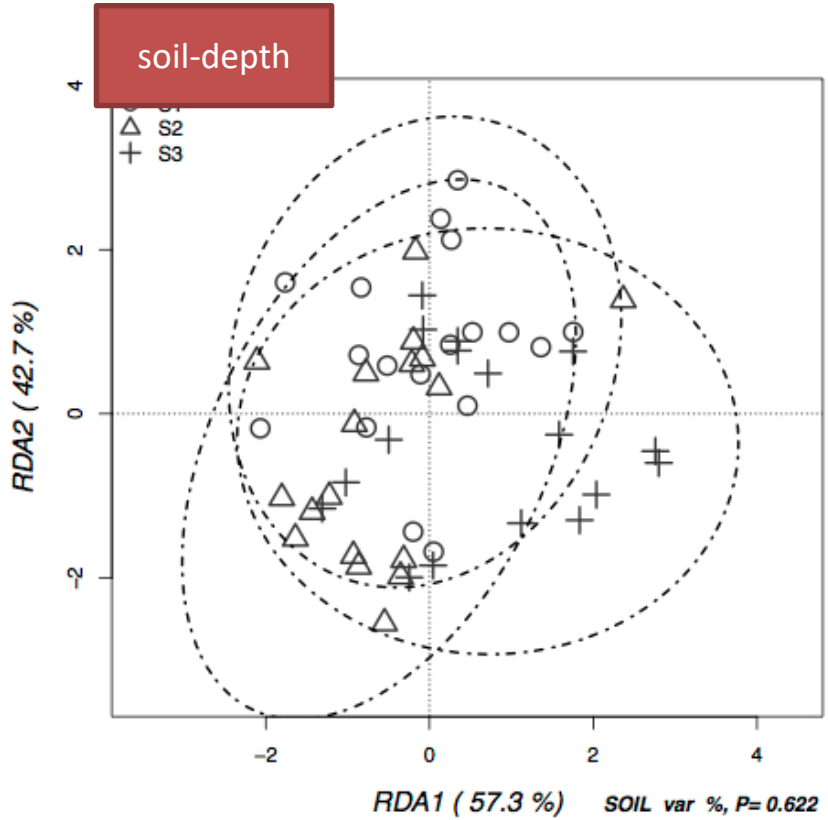
Fusarium, Trichoderma and *Aspergillus* are the most abundant genera

Molecular analyses of microbial community

All samples were dominated by *Fusarium* (more than 80%), followed by *Aspergillus, Penicillium, Trichoderma* and *Arthrinium.*



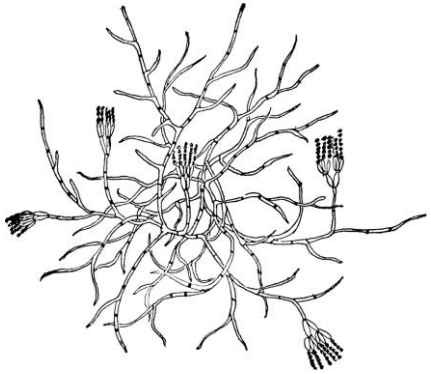


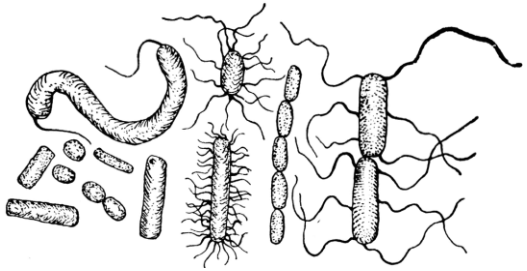


Only the pollutant factor was significant. The groups formed by soil-depth and time overlapped.

- The pollutants significantly affected the fungal community.
- PAHs (pyrene and phenanthrene) grouped separately from oil or the volatile naphthalene.

Isolation from different cultural techniques…



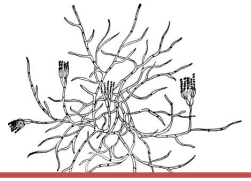


	Fungi	Bacteria
Total count	54 isolates	37 isolates
Solid Screening	164 isolates	125 isolates
Liquid Enrichment	91 isolates	94 isolates

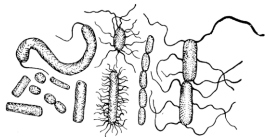
565 isolates

309 isolates

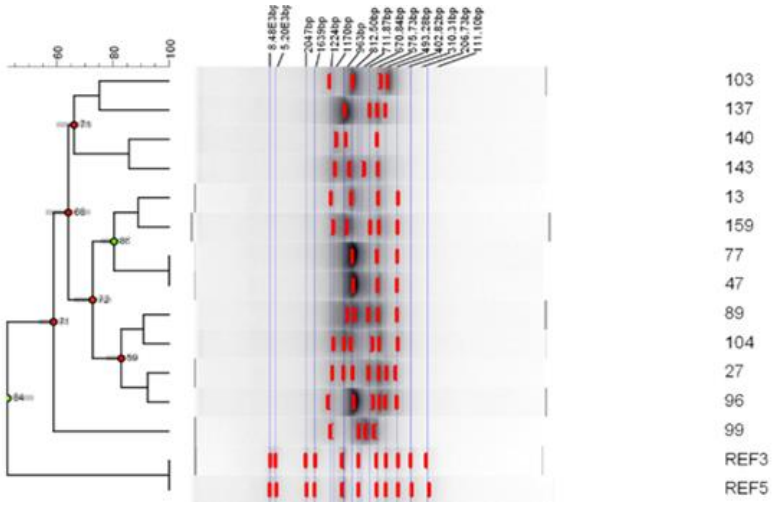
256 isolates

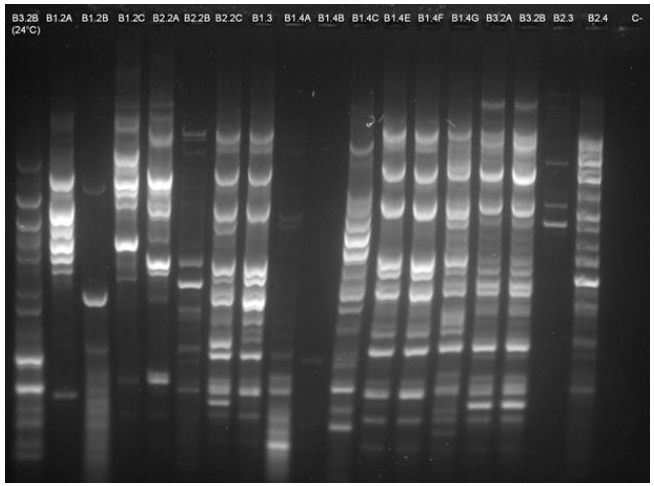


Fungi



Bacteria

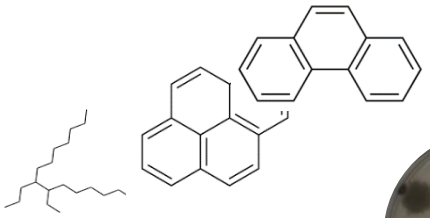


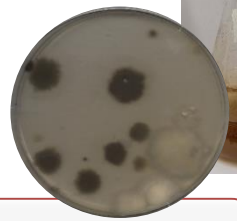


Total isolates	309 isolates	256 isolates
De-replicated strains	**219 strains**	**133 strains**

352 strains

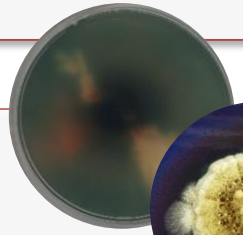
LIFE-BIOREST Outline





219 fungi, 133 bacteria

- **Which are the microorganisms adapted to this ecological niche?**

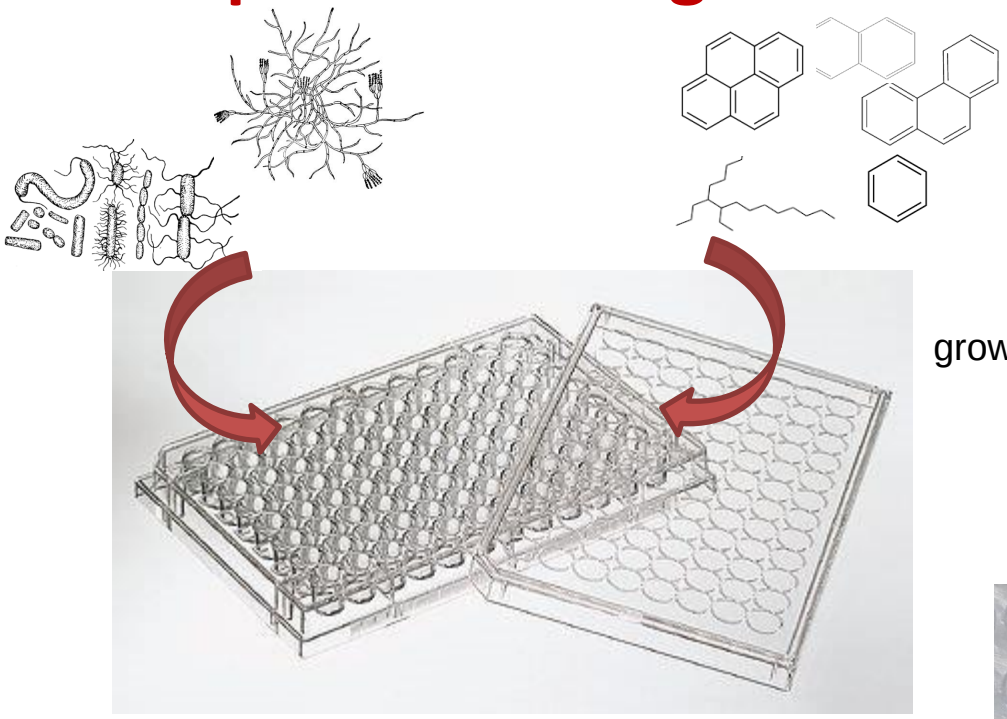


- **Which are the best performing strains?**
- Degradation skills, production of enzymes, biosurfactants, etc.

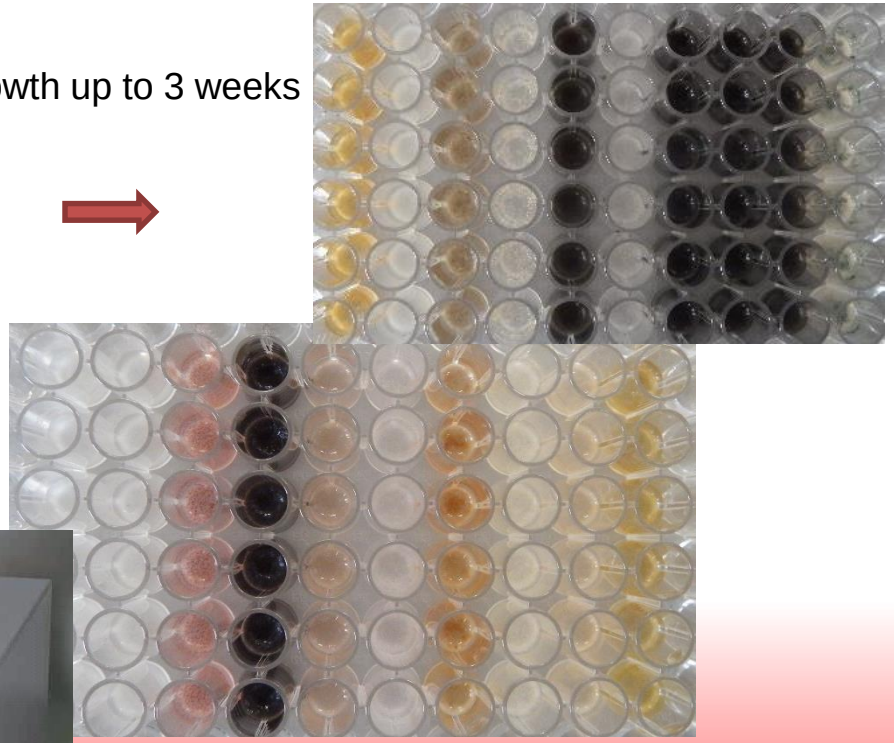
- **Will be the microorganisms effective in real conditions?**
- Degradation assessment: microcosm, mesocosm and *in situ* biopile

Microplate Screening

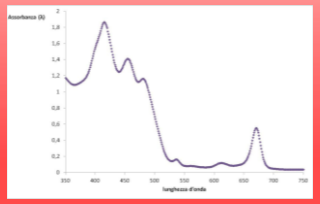
Set up of a innovative microplate screening for the quantitative evaluation of the pollutants biotransformation.

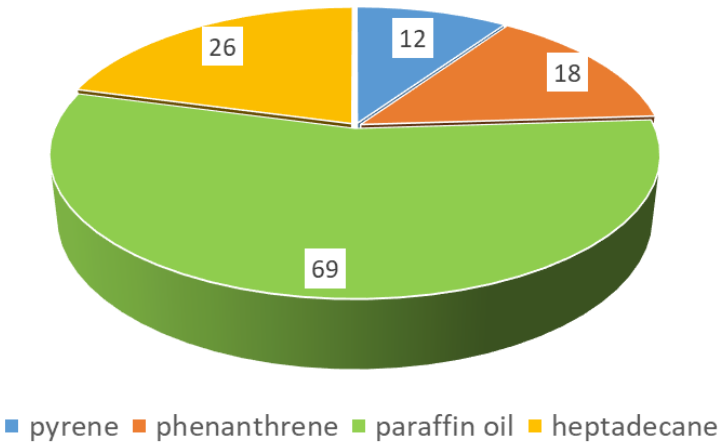


growth up to 3 weeks



spectrophotometric analysis of the fungal and bacterial growth

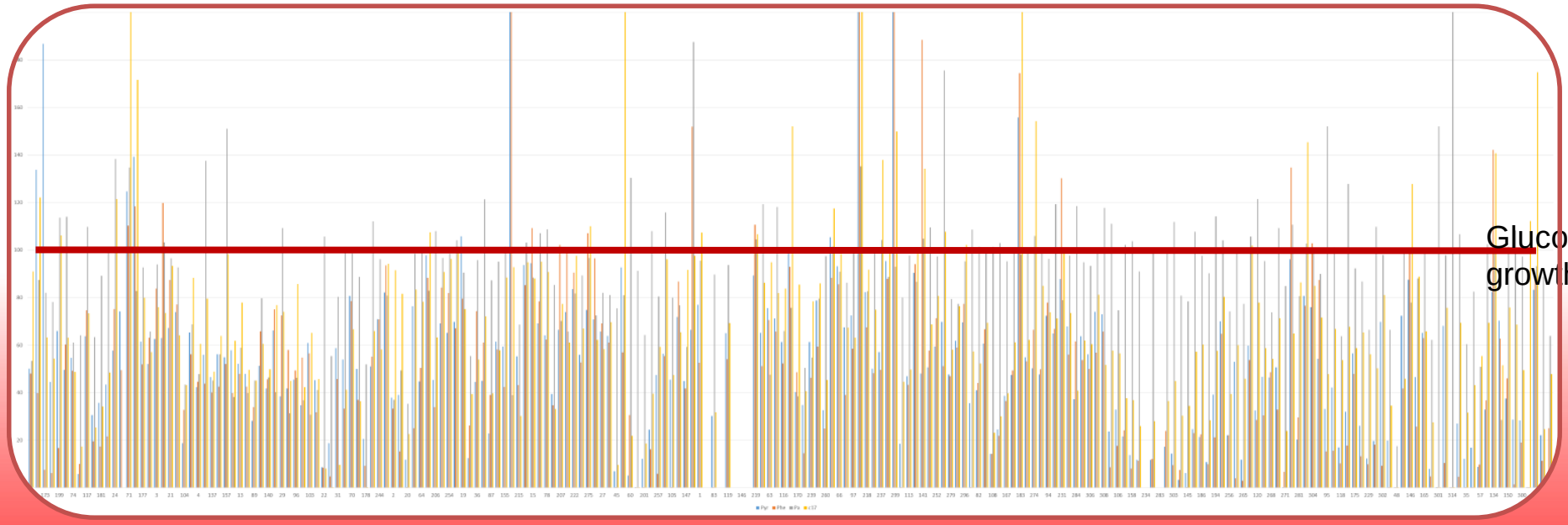


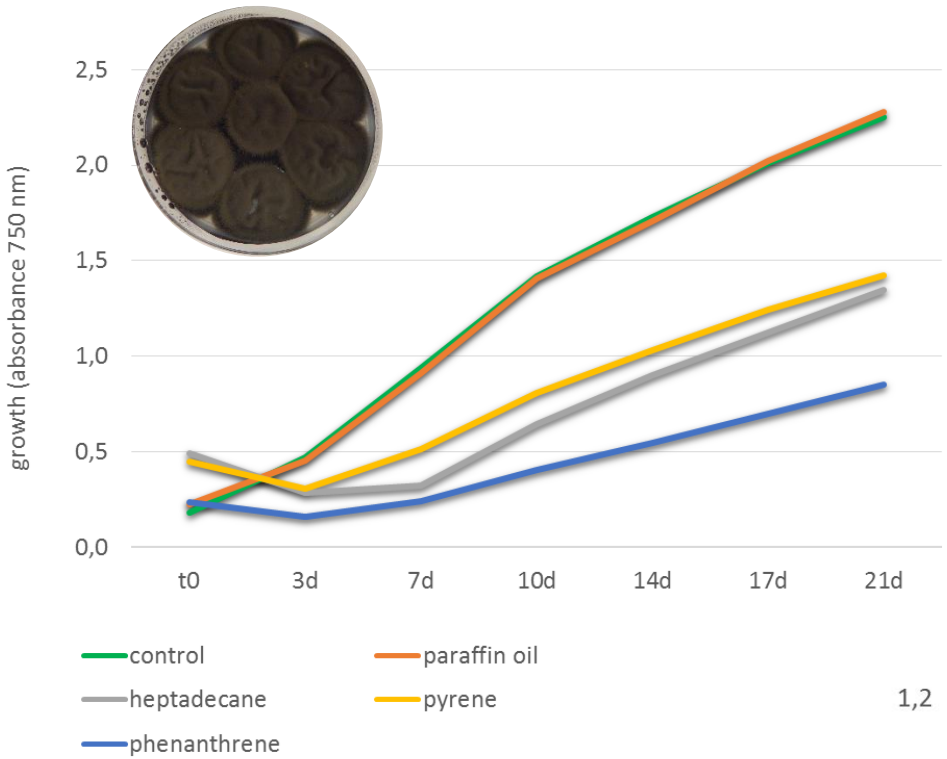


Number of strains grown more than in the presence of a pollutants than glucose

Many fungi (53 %) grew more than the control (glucose) in the presence of at least one substrate.

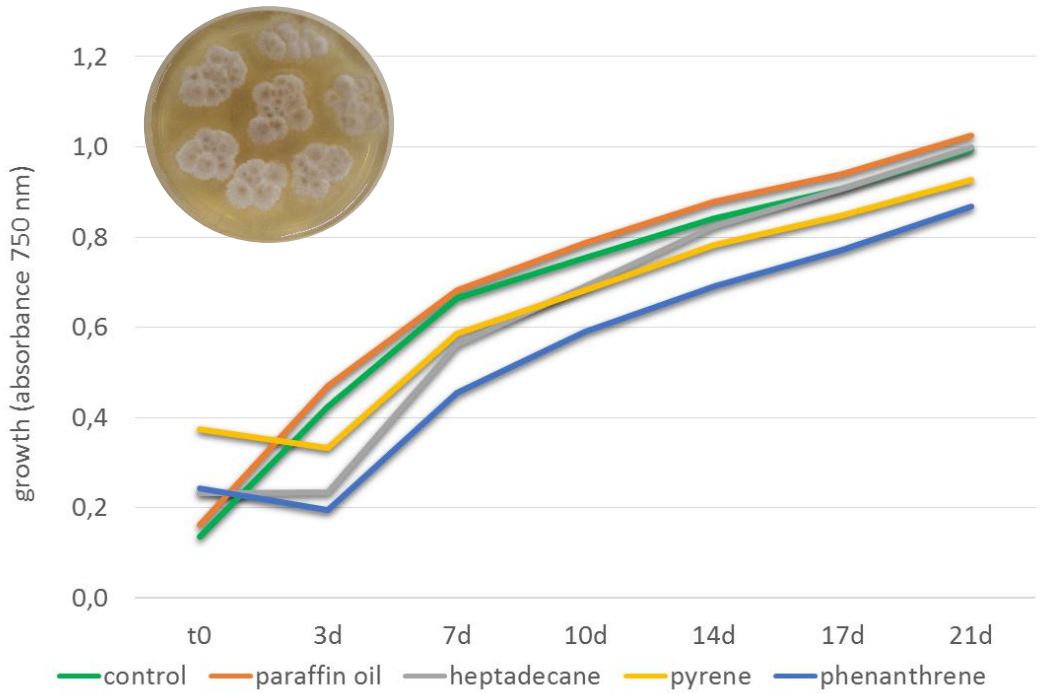
Alkanes are easily used as source of nourishment (often better than the control with **glucose**).



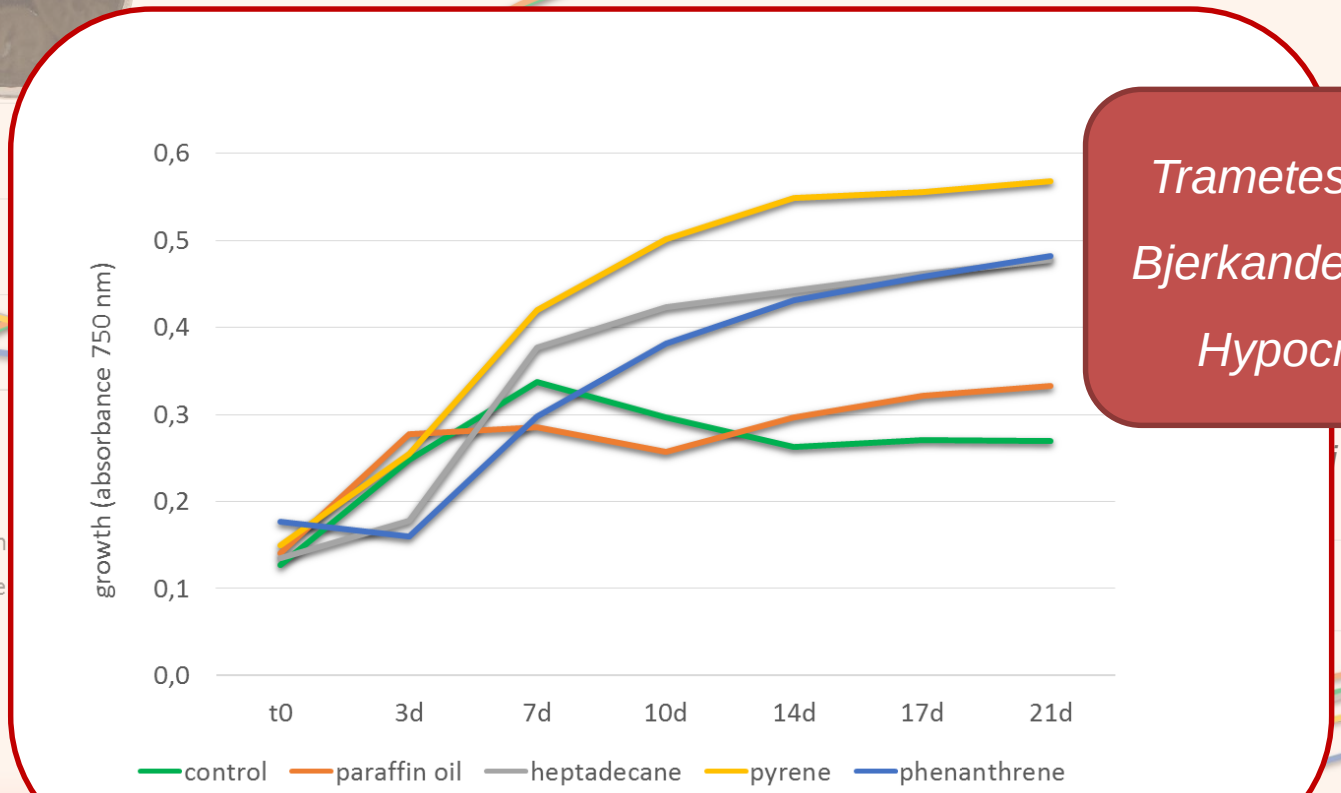


Cladosporium cladosporioides

Fusarium solani



Cladosporium cladosporioides



Trametes gibbosa

Bjerkandera adusta

Hypocrea lixii

Biosurfactants Screening

70% of strains showed a positive response in at least one test.

Blue agar test

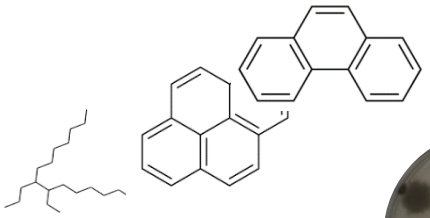
Drop collapsing assay

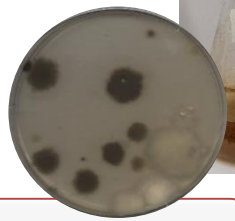
Oil dispersion test

Emulsification Activity

WORK IN PROGRESS

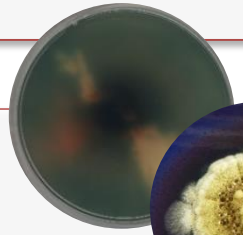
LIFE-BIOREST Outline





219 fungi, 133 bacteria

- **Which are the microorganisms adapted to this ecological niche?**



24 fungi, 21 bacteria

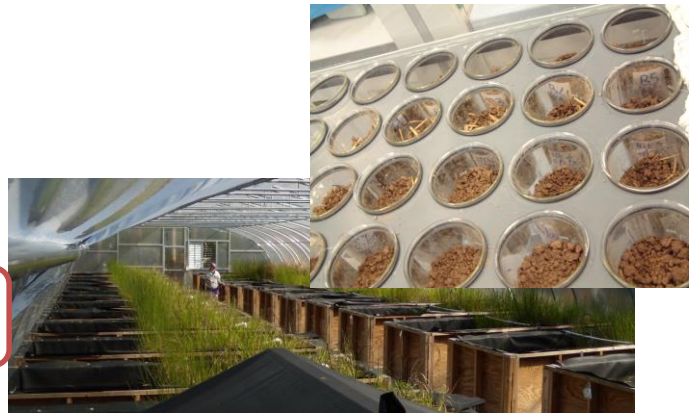
- **Which are the best performing strains?**
- Degradation skills, production of enzymes, biosurfactants, etc.

- **Will be the microorganisms effective in real conditions?**
- Degradation assessment: microcosm, mesocosm and *in situ* biopile

Microcosms

Mesocosms

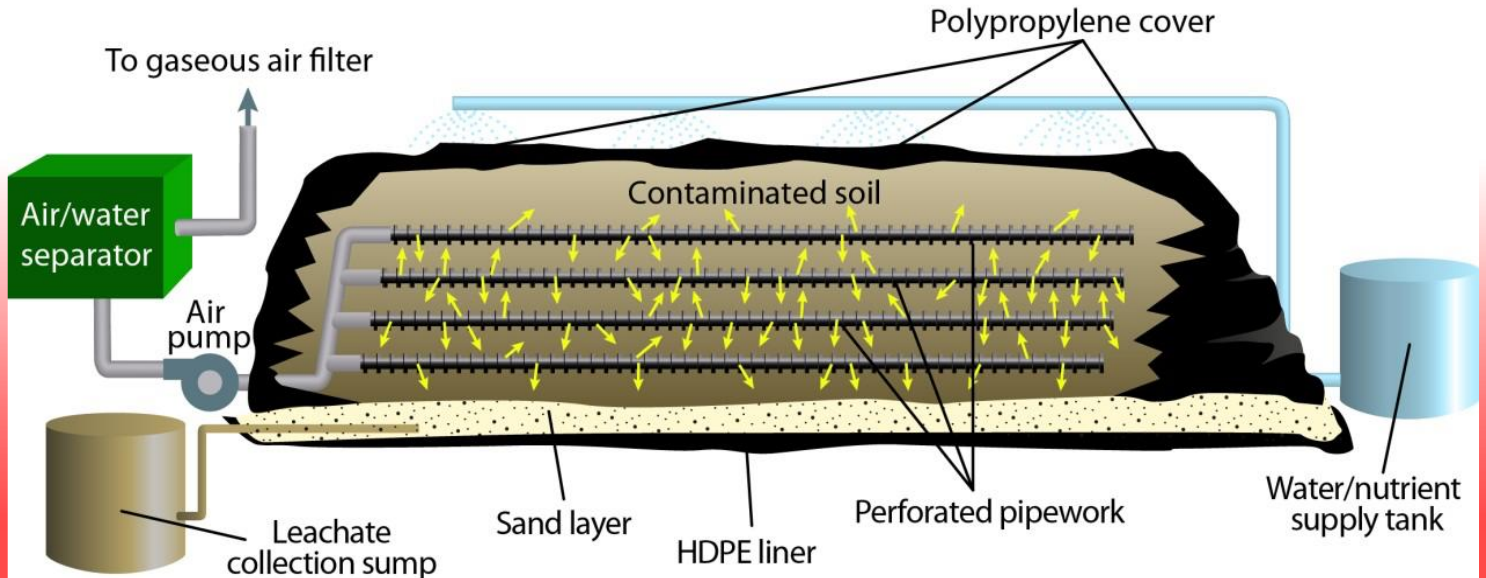
***in situ* biopile**



500 g

10 kg

500 ton

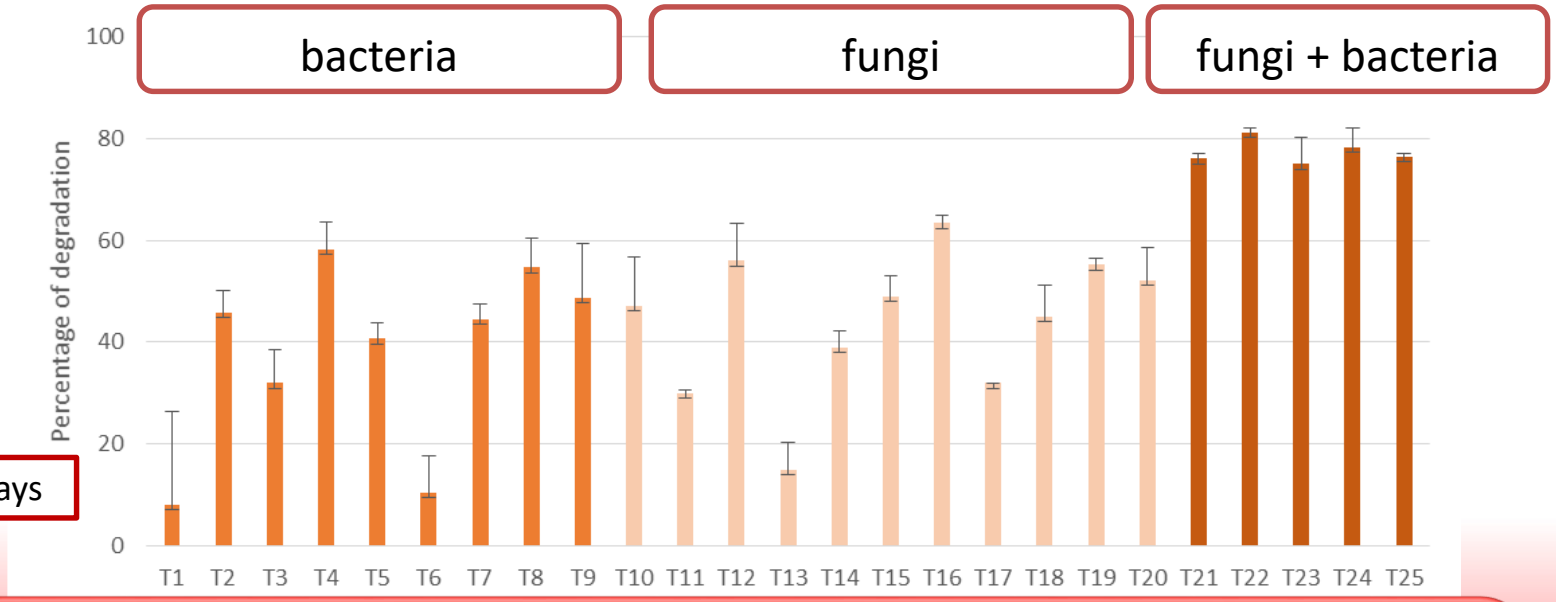


Microcosms trials

Colonized carriers were inoculated in 500 g soil (3 months): thesis with only bacteria or fungi (as single or mixed consortia) and thesis with both bacteria and fungi.

pyrene

20 days



Fungi and bacteria synergically transformed the target pollutants. Combined consortia resulted faster and more efficient against the most recalcitrant pollutants.

Mesocosms trials

Six microbial consortia were selected and inoculated in 10 kg soil.

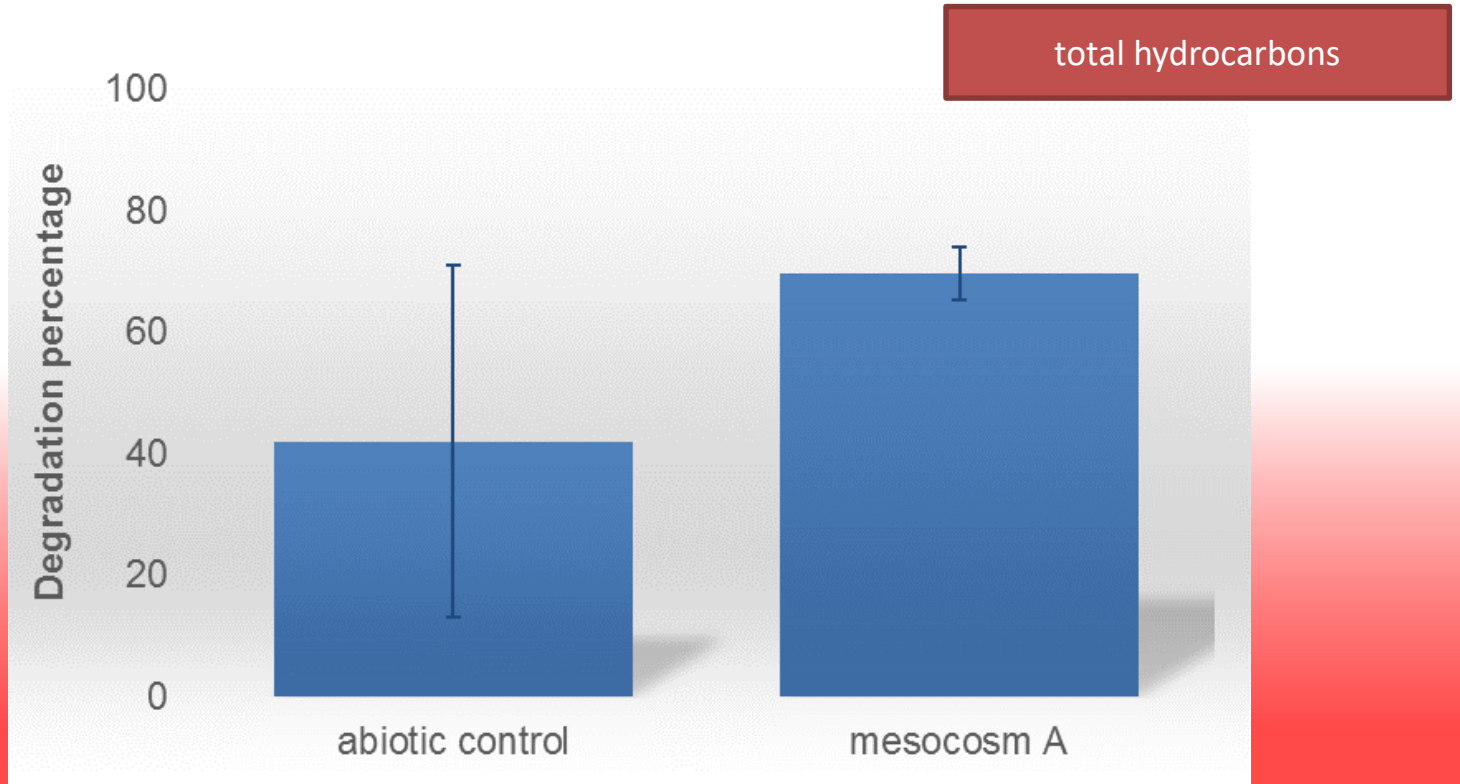
Experiments last for 3 months. Three replicates were set up.

The total hydrocarbons content was reduced up to 70 %, but the **choice of the microbial consortia** came to be a critical issue.

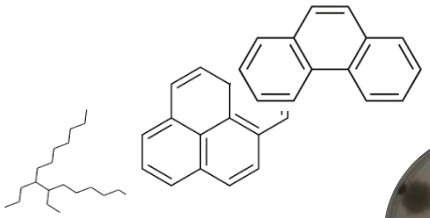
Among the six selected consortia, the **yields varied from 20 to 70 %**.

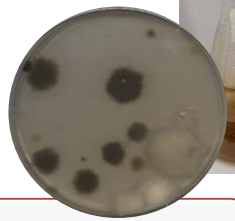
The most concentrated pollutants (e.g. phenanthrene, fluoranthene and pyrene) were almost completely transformed.

- Bioaugmentation with the selected **microbial consortium** guarantees a **high and stable degradation**.
- The control gave variable results due to the heterogeneous quali- and quantitative distribution of the autochthonous microbiota.



LIFE-BIOREST Outline





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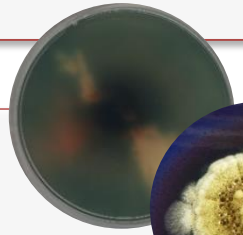

24 fungi, 21 bacteria

- **Which are the best performing strains?**
- Degradation skills, production of enzymes, biosurfactants, etc.

selected consortium

- **Will be the microorganisms effective in real conditions?**
- Degradation assessment: microcosm, mesocosm and *in situ* biopile

400 m^3 of soil has been inoculated with the best performing consortia (fungi and bacteria). Chemical and ecotoxicological analysis are carried out to follow the microbial degradation.

21 m x 15 m x 4 m (h)

WORK IN PROGRESS

GRAZIE PER L'ATTENZIONE,

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LIFE15 ENV/IT/000396