SYNERGIC ACTION OF ORGANIC MATTER-MICROORGANISM-PLANT IN SOIL BIOREMEDIATION

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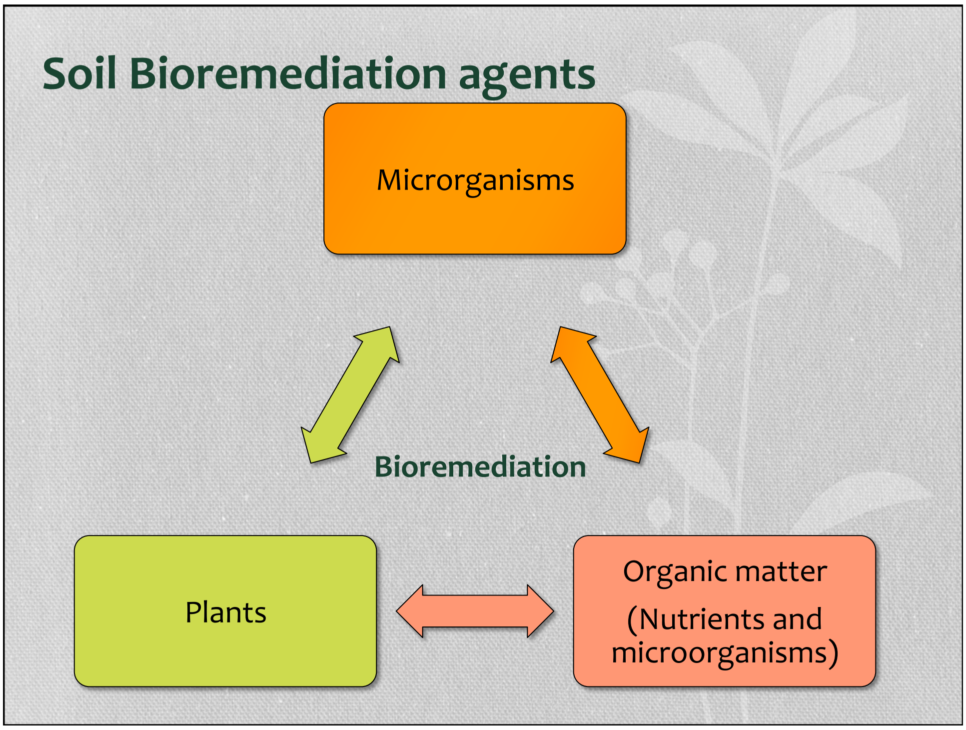
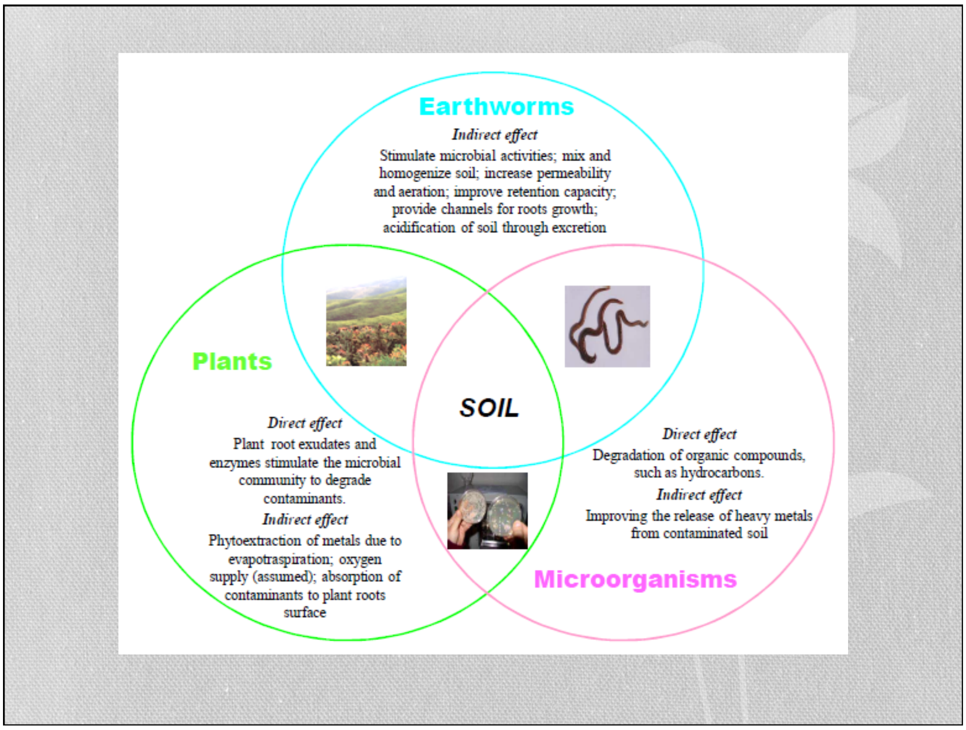
What is **Bioremediation**?

- Range of **clean up methodologies** by using **natural organisms** (**Bacteria, Plant, Fungi**) to **degrade organic contaminants** or to **convert inorganic contaminants** to environmentally less toxic or non-toxic compounds

- Bioremediation utilizes the natural role of microorganisms in **transformation, mineralization**, or **complexation** capabilities addressed to organic and inorganic environmental pollutants (Bollag et al., 1994)


Soil Bioremediation agents
Microrganisms
Bioremediation
Plants
Organic matter
(Nutrients and microorganisms)


Earthworms
Indirect effect
Stimulate microbial activities; mix and homogenize soil; increase permeability and aeration; improve retention capacity; provide channels for roots growth; acidification of soil through excretion
Plants
Direct effect
Plant root exudates and enzymes stimulate the microbial community to degrade contaminants.
Indirect effect
Phytoextraction of metals due to evapotraspiration; oxygen supply (assumed); absorption of contaminants to plant roots surface
SOIL
Direct effect
Degradation of organic compounds, such as hydrocarbons.
Indirect effect
Improving the release of heavy metals from contaminated soil
Microorganisms

Earthworm action

- Mix and give air to soil and substrate under decomposition
- Release microrganisms and enzymatic proteins for organic compound degradation
- Increase the nutrient availability
- Increase soil permeability
- Incorporate organic matter into the soil
- Digest organic matter and produce a humus rich and biologically active casting
- Produce hormon-like substances

Earthworms ensure the continuous monitoring of material toxicity (bioindicators)

Eisenia fetida
High adaptability to different environmental condition and residues
High sexual reproduction

Eisenia fetida
- Temperature: 20-25 °C
- Moisture content: 60% - 90%
- Oxygen requirement: aerobicity
- Ammonia content of waste: <0,5 mg/g
- Salt content of waste: <0,5%
- pH: >5 and <9

BIOREMEDIATION

MICROORGANISM ACTION

Main agents of Bioremediation
- **Bacteria**
- **Fungi**
- **Actinomycetes**

- **Biostimulation** or **bioenhancement:** addition of nutrients and air to stimulate authoctonous microorganisms
- **Bioaugmentation:** addition of selected microorganisms
- The microorganisms break down contaminants by using them as a **food source** or **cometabolizing** them with a food source.

Decontamination processes are mainly aerobics
- **Aerobic processes** require an oxygen source, and the end products typically are CO_2 and H_2O.
- **Anaerobic processes** are conducted in the absence of O_2, and the end products can include CH_4, H_2, H_2S, N_2.

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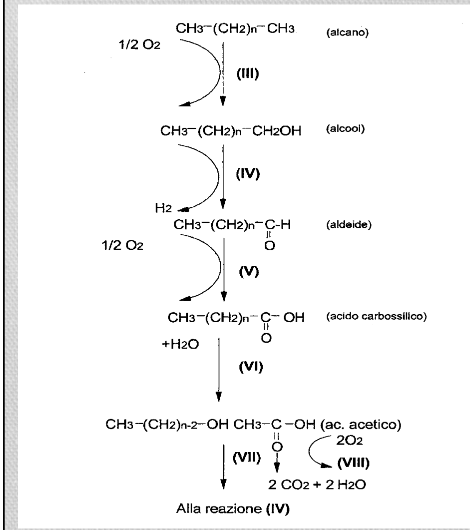
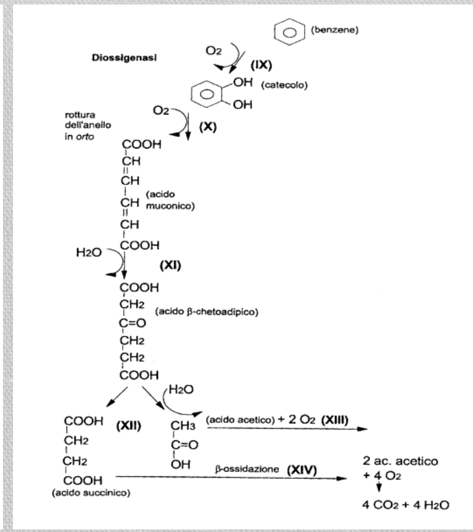


Scheme of microbial degradation of n-alcane



Scheme of microbial degradation of benzene

ORGANIC MATTER ACTION

Key factor for soil quality and fertility at three levels:

Chemical (sink of nutrients)

Biological (stimulation of microbial activity, addition of microorganisms)

Physical (improvement of structure and water retention capacity)

Types of organic matter

- fresh (e.g. sewage sludge, organic fraction of urban residues)
- stabilised (e.g. compost)

Adding organic matter to soil

Stabilized organic matter

- (e.g. **compost**)
- Low availability of nutrients
- Relatively homogeneous, reduced volume
- Biologically stable
- Non-offensive odor
- Moist-dry
- Weed seeds, pathogens absent

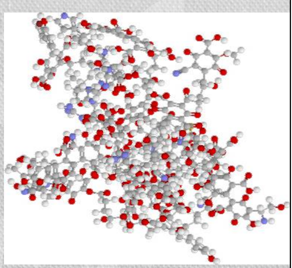
Fresh organic matter

- (e.g. **sewage sludge, organic fraction of urban residues**)
- High availability of nutrients: N forms, P, K, etc.
- Heterogeneous, high volume
- Biologically active
- Strong odor
- Wet
- Contains weed seeds, pathogens

Application of Fresh organic matter

Advantages

1. Short-term action: rich in available nutrients and substrates; Rich in microorganisms
2. Long-term action: promotion of formation *in situ* of humic substances specific for that soil



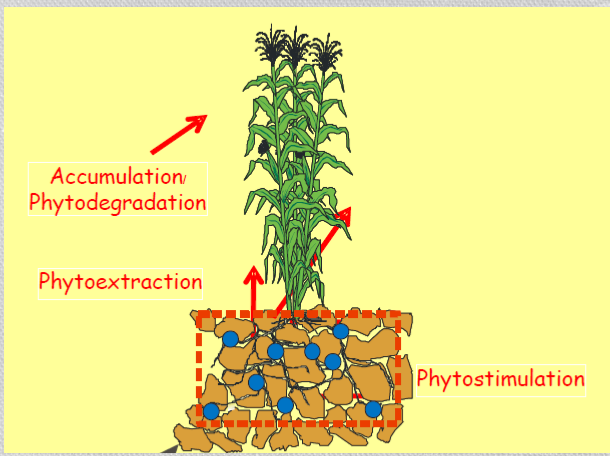
Humic substances

Stable organic polyphenol-polycarboxilic polymers resistant to microbial attack

Main Roles:

- Sink of nutrients and bio-energy
- Attenuation of toxicity in soil-water environment (heavy metals, organic, micro- and macropollutants)
- Protection of soil from erosion (organo-mineral complexes)
- Expression of molecular biodiversity and bio-functionality in soil (humo-enzyme complexes, proteomics, soil resilience)

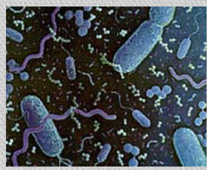
PLANT ACTION



- Plants can take up toxic substances, in particular, heavy metals.
- Specific plants accumulate different quantities of inorganic contaminats (iperaccumulators, tolerants).

Plants are not passive media in the pollutant absorption

Roots exudates…



…generate an environment that promote bacterial activity and degrade bioavailable pollutants

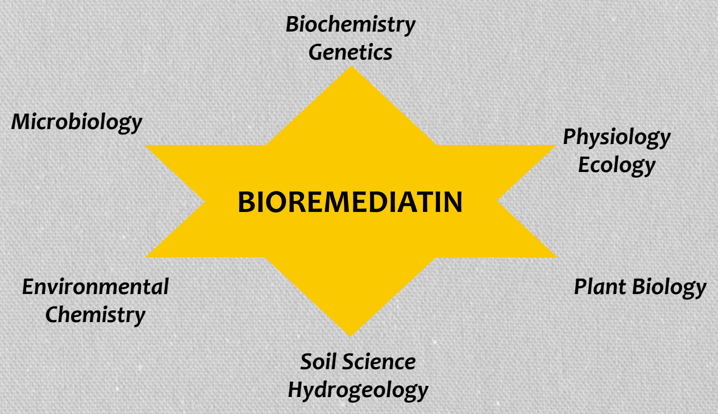
… contain several organic substances influencing microbial diversity

… contain enzymes and bind compounds able to modify pollutant bioavailability and absorption

Phytoremediation

- **Phytoextraction** involves contaminant uptake by plant roots, with subsequent accumulation in plant tissue. Phytoextraction typically is used to address inorganic contaminants, such as metals, metalloids, and radionuclides.
- **Phytosequestration**, also referred to as **phytostabilization**, is a mechanism that immobilizes contaminants—mainly metals—within the root zone, limiting their migration. Immobilization of contaminants can result from adsorption of metals to plant roots, formation of metal complexes, precipitation of metal ions (e.g., due to a change in pH), or a change to a less toxic redox state. Phytosequestration can occur when plants alter the chemical and microbial makeup of the soil (e.g., through the production of exudates or carbon dioxide), which impacts the fate and transport of the soil metals. (US EPA 2006)

A multidisciplinary approach



Indicators for monitoring soil decontamination

✓Physical
✓Chemical

1. Reduction of contaminants
2. Agronomical recovery (improvement of chemical-nutritional characteristics)

✓Biological and biochemical (Bioindicators)

1. Functional recovery
2. Potential detoxification activity

Bioindicators **are frequently used to:**

1. **detect changes in the natural or anthropized environments**
2. **monitor the presence of pollution and its effect on the ecosystem**
3. **monitor the progress of environmental clean up and restoration**

BIOINDICATORS AT ECOSYSTEM LEVEL

Enzyme activities (hydrolases, oxidoreductases, etc.) may be used as biomarker of functional evolution of natural or anthropized soil ecosystems

They have an effect on:

- decomposition of the organic substances
- mineralization and release of nutrients
- fertility and productivity of soil
- response of the soil to ecological and functional recover practices after natural or human stresses

However, the measurement of the enzyme activities gives only an indication on soil **potential activity**

There are, in fact, considerable difficulties in relating enzyme activities determined *in vitro* (usually involving buffers, constant temperatures, excess (and sometimes artificial) substrates, etc.) to those occurring *in situ*

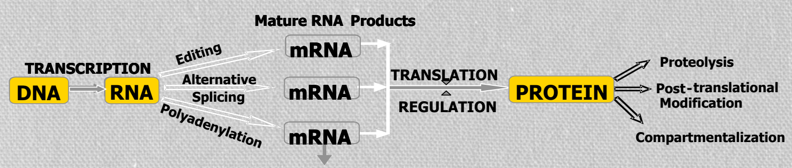
BIOINDICATORS AT ECOSYSTEM LEVEL

- Microbial biomass **quantity** and **biodiversity**

Molecular biology has revolutionized the study of microorganisms in the soil environment giving the possibility to have information of microbial biodiversity and function directly *in situ*

The current molecular toolbox involves "**omics**" approaches which encompasses a range of DNA-based technologies and new methods for the study of RNA and proteins extracted from soil samples.

Genomics
Transcriptomics
Proteomics



CASE STUDIES (LABORATORY)

1. SOIL BIOREMEDIATION: COMBINATION OF EARTHWORMS AND COMPOST FOR THE ECOLOGICAL REMEDIATION OF A HYDROCARBON POLLUTED SOIL

Brunello Ceccanti, Grazia Masciandaro, Carlos Garcia, Cristina Macci and Serena Doni
Water, Air and Soil Pollution (2006) 177:383-397

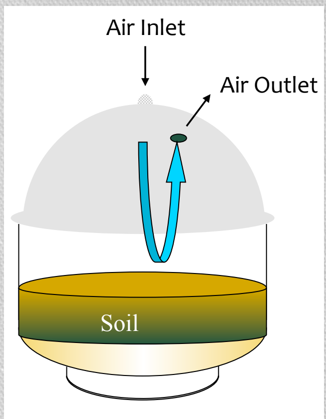
Main characteristics of soil and compost

	Soil	Compost
E.C. (1/10) (dS m^{-1})	4.0 ± 0.2	3.5 ± 0.17
pH (1/10)	7.3 ± 0.44	7.9 ± 0.31
SO_4^{2-} (mg kg^{-1})	15891 ± 476	—
Cl^- (mg kg^{-1})	351 ± 14	—
NO_3^- (mg kg^{-1})	153 ± 4.6	—
TOC (mg g^{-1})	42.3 ± 1.3	180 ± 10.8
WSC (mg g^{-1})	0.85 ± 0.03	2.23 ± 0.07
N-tot. (mg g^{-1})	2.0 ± 0.08	8.33 ± 0.52
NH_3 (mg kg^{-1})	19.2 ± 0.95	40 ± 0.8
P-tot. (mg kg^{-1})	394 ± 19.1	1999 ± 60
P-ass. (mg kg^{-1})	3.32 ± 0.13	50.3 ± 4.03
Sand (%)	14.2 ± 0.85	—
Silt (%)	82.7 ± 5.7	—
Clay (%)	3.15 ± 0.12	—
ATP (ng ATP g^{-1})	764 ± 43.2	—
TPH (mg g^{-1})	11.27 ± 0.56	—

E.C., Electrical Conductivity; Pass, available phosphorus; Ptot, total phosphorus; TOC, Total Organic Carbon; TPH, Total Petroleum hydrocarbons; WSC, Water-Soluble Carbon.

Italian legislation 152/06
Column B:
0.25+0.75 (C<12+C>12)

Experimental Layout



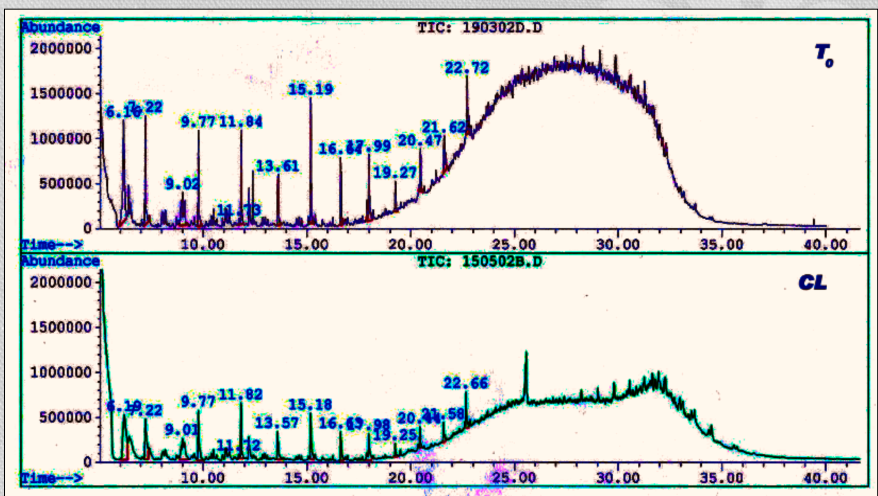
The monitoring of the bioremediation system consisted of samplings carried out at 30, 60 and 90 days from the beginning of experimentation (3 months)

SOIL TREATMENTS:
BIOSTIMULATION+BIOAUGMENTATION

1. Mixture of microorganisms-enzymes-nutrients (MEN):
   $(NH_4)H_2PO_4$, NH_4NO_3
   (10:1:0,2 of carbon, nitrogen and phosphorus)
   - Bacillus subtilis
   - Bacillus megaterim
   - Bacillus thuringiensis
   - Yeast Saccaromices
   - Enzymes Oxidase

   (1 kg of soil : 4 g of mixture)
2. Compost (1 kg of soil : 100 g of compost)
3. Compost + earthworms (Eisenia fetida); 10 adult earthworms
4. SOIL WITHOUT TREATMENT

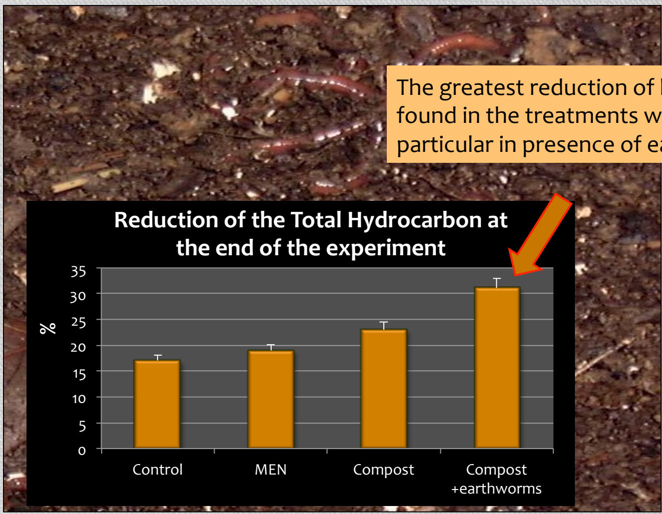
SOIL DECONTAMINATION



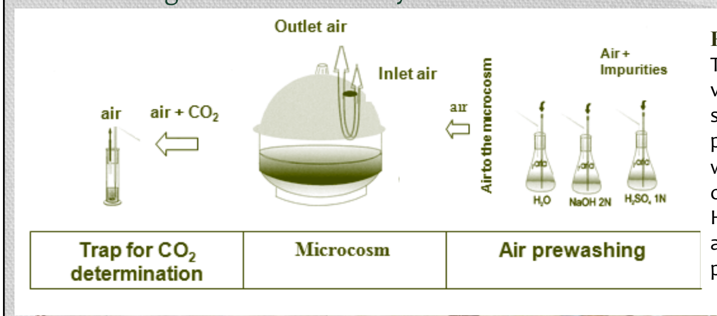
GC-profile of hydrocarbons extracted at T_o (initial time) and after three monts from the beginning of experimentation in the compost+earthworms treatment

SOIL DECONTAMINATION

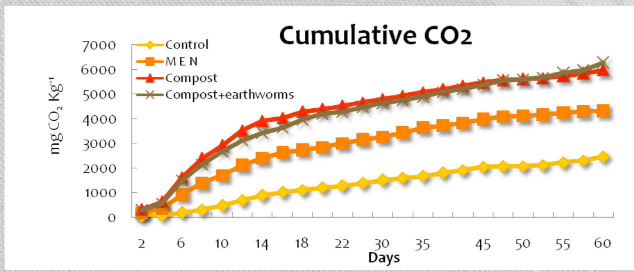
The greatest reduction of hydrocarbons was found in the treatments with compost, in particular in presence of earthworms



The respirometric activity was determined by a titrimetric method measuring the CO2 released by microbial biomass



Principle:
The CO_2 was collected in a vial containing NaOH solution. The carbonate produced was precipitated with $BaCl_2$ and the excess of NaOH was titrated with HCl, using phenolphthalein as indicator of the end point of reaction

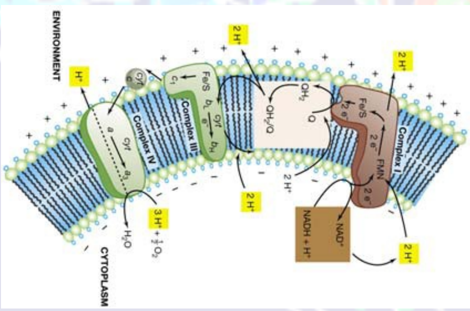


Intense microbiological activity expressed as carbon dioxide evolution during the time, in particular in the compost treatments with a tendency to stabilize at the end of the experiment

Dehydrogenase activity

Indicator of global microbial metabolism

$$R\text{-}H_2 + NAD^+ \rightarrow NADH + R + H^+$$

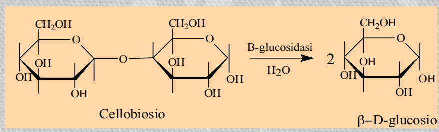


catalyze the oxidation of organic compounds with the removal of two hydrogen atoms that are transferred to the molecule of NAD^+

Hydrolitic enzyme activities

β-Glucosidase enzymes catalyze the final limiting step of cellulose degradation

Carbon cycle



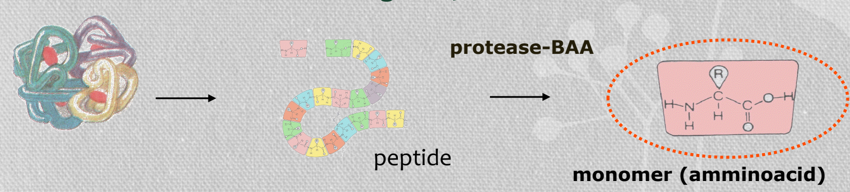
Phosphatase enzymes catalyze the hydrolysis of various organic and inorganic phosphate esters

Phosphorus cycle

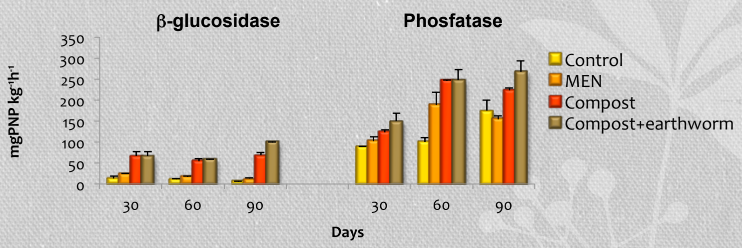
$$R\text{-}O\text{-}PO_3^{2-} + H_2O \longrightarrow R\text{-}O\text{-}H + HO\text{-}PO_3^{2-}$$

Protease and Urease enzymes are involved in breaking down proteins and releasing ammonia

Nitrogen cycle

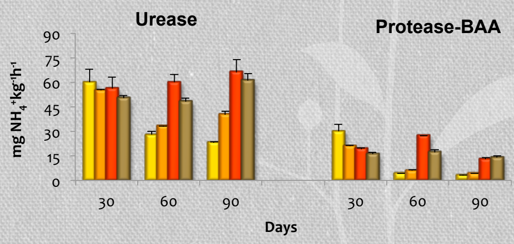


$$NH_2C(=O)NH_2 \xrightarrow[H_2O]{Urease} CO_2 + 2NH_3$$



•Stimulation in the hydrolitic enzyme activities representative of C, N and P cycles, in particular in the compost treatments

•The treatment with MEN increased during the time phosphatase activity due to the microbial turn over releasing organic P compounds



Conclusions

- ➢The highest biochemical activity in the **treatments with compost** suggested the highest stimulation of microbiological processes linked to the organic compounds/contaminants degradation

- ➢The decrease in TPH, in particular in the compost+earthworm treatment (>30%) confirms the effectiveness of the bioremediation system.

Compost+earthworm treatment (organic matter-microorganism-earthworm interaction) creates a biological microenvironment better affecting the soil decontamination and functional reclamation

CASE STUDIES (PILOT SCALE)

2. BIOREMEDIATION OF POLLUTED SOIL THROUGH THE COMBINED APPLICATION OF PLANTS, EARTHWORMS AND ORGANIC MATTER

Soil characterization

mg/kg	Zn	Pb	Ni	Cu	Cr	Cd	Total hydrocarbons
pollution	617	440	145	308	70	4	2990

lower than law limit for urban use (green)

higher than law limit for industrial use (red)

D.Lgs.152/2006	Zn mg/kg	Pb mg/kg	Ni mg/kg	Cu mg/kg	Cr mg/kg	Cd mg/kg	C<12 mg/kg	C>12 mg/kg
Urban use	150	100	120	120	150	2	10	50
Industrial use	1500	1000	500	600	800	15	250	750

Bioremediation approach

The use of biological treatments to clean up hazardous materials in the environment

Plants

- Improve soil structure
- Microbial increase at root zone
- Remove, stabilize degrade contaminants

\+

Earthworms

- Improve aerobic conditions
- Increase microbial and biochemical soil activity
- Contaminant degradation

Organic matter

Bioenhancement

Bioaugmentation

The use of earthworms, organic matter and plants could be an alternative and valid natural technology to improve the soil remediation

Plant selection

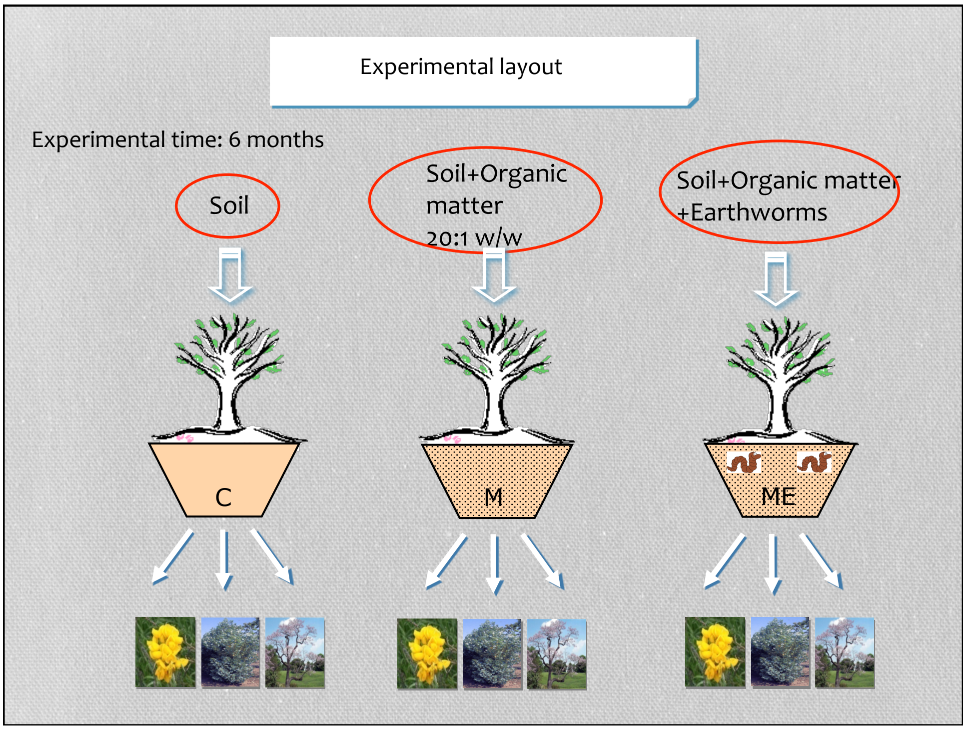
Plant characteristics:

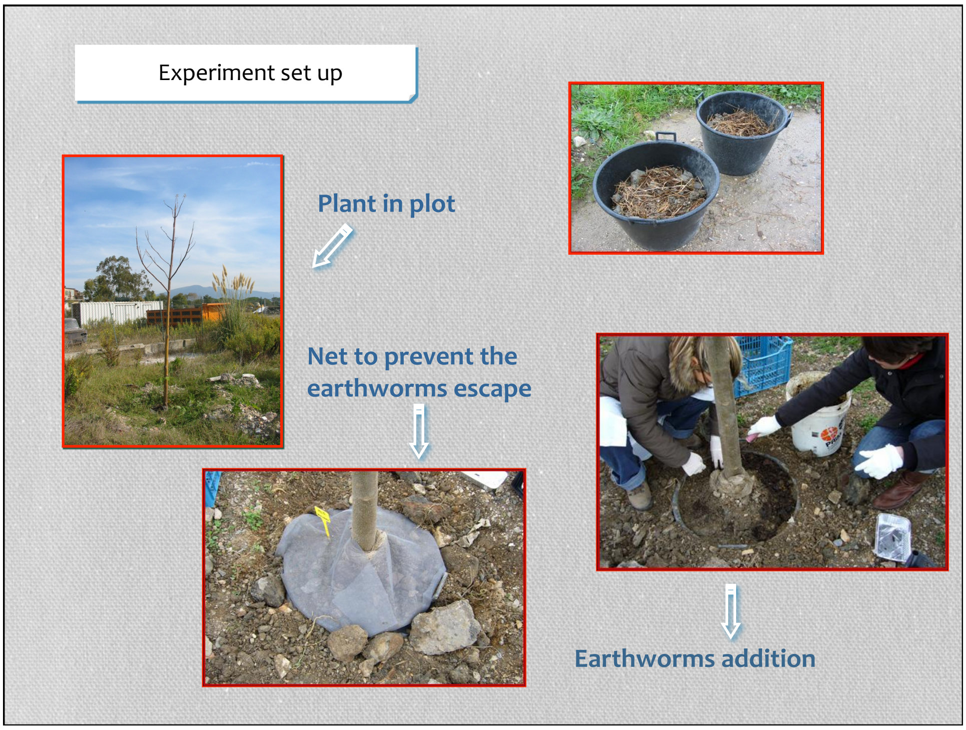
✓Fast growth.
✓Suitability to develop in the substrate.
✓Adaptability to the local climatic conditions.
✓Capability to extract heavy metals and/or improve soil properties for the hydrocarbon degradation.

Populus nigra var. Italica
(Lombardy poplar)

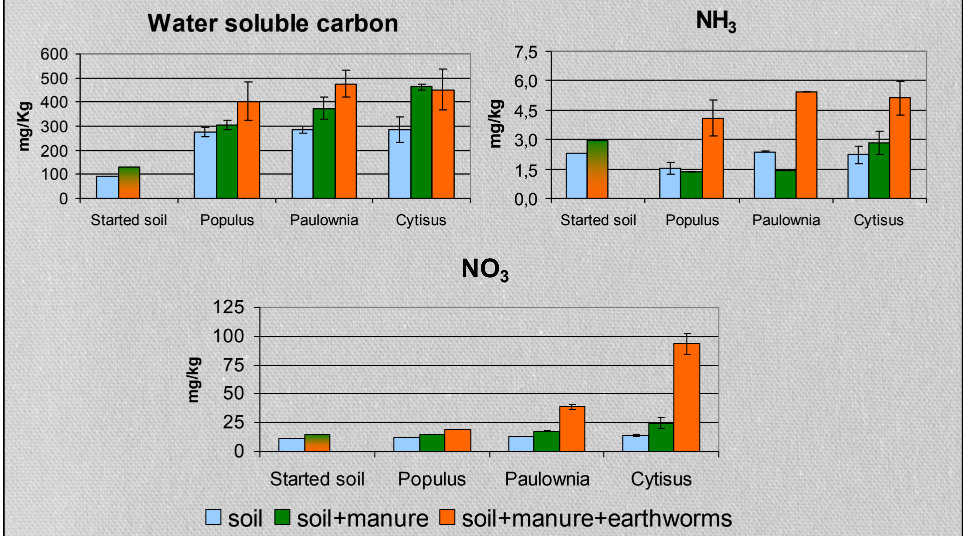
Cytisus scoparius
(Scotch broom)

Paulownia tomentosa
(Princesstree)


Experimental layout
Experimental time: 6 months
Soil
Soil+Organic matter 20:1 w/w
Soil+Organic matter +Earthworms
C
M
ME

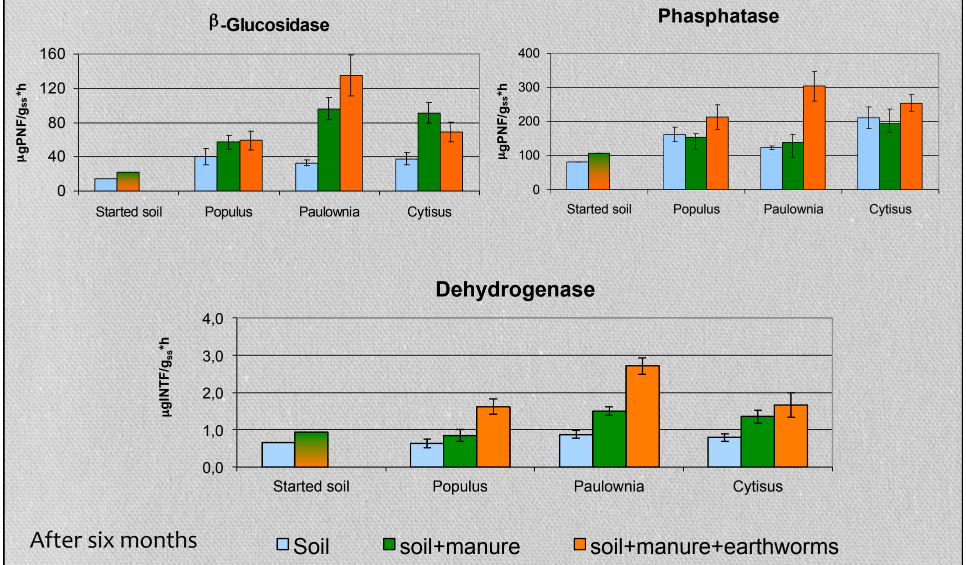

Experiment set up
Plant in plot
Net to prevent the earthworms escape
Earthworms addition

Chemico-nutritional improvement



After six months

Biochemical improvement

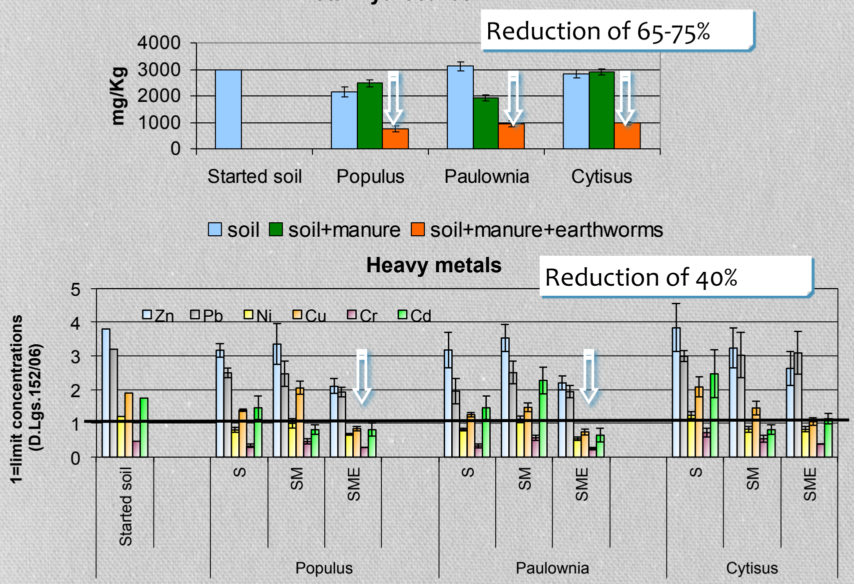


After six months

Remediation efficiency

After six months

Total Hydrocarbon



S=Soil **SM**=soil+manure **SME**=soil+manure+earthworms

Conclusions

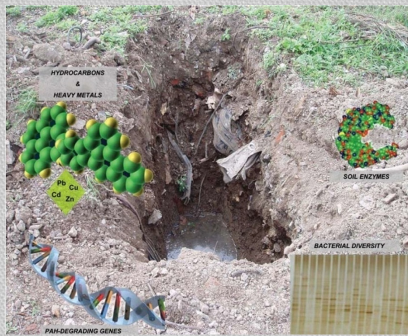
✓The treatments with organic matter and earthworms, stimulating soil metabolic processes, achieved the best result in the hydrocarbon degradation even preserving the biochemical quality of the soil.

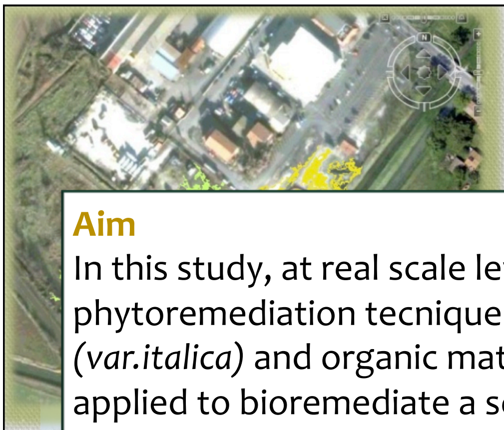
✓Populus nigra and Paulownia tomentosa were more efficient than Cytisus scoparius in the heavy metal extraction.

CASE STUDIES (REAL SCALE)

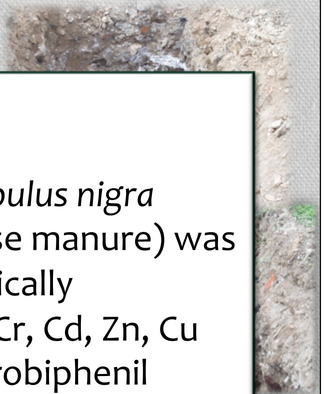
3. IN SITU PHYTOREMEDIATION OF A SOIL HISTORICALLY CONTAMINATED BY METALS, HYDROCARBONS AND POLYCHLOROBIPHENYLS

Serena Doni, Cristina Macci, Eleonora Peruzzi, Mariarita Arenella, Brunello Ceccanti and Grazia Masciandaro
J. Environ. Monit. (2012) 14(5): 1383-1390





Soil contaminated by organic and inorganic pollutants.
Municipality of San Giuliano Terme - Pisa (Italy).



Aim

In this study, at real scale level, the phytoremediation tecnique with *Populus nigra (var.italica)* and organic matter (horse manure) was applied to bioremediate a soil historically contaminated by heavy metals (Pb, Cr, Cd, Zn, Cu and Ni), hydrocarbons and polychlorobiphenil (PCB).

1. Soil removal until clay basement

2. Soil storage

3. Removal of waste with sieve

4. Control of clay basement pollution

5. Replacing of mixed soil

6. Soil sample collection

Bioremediation at real scale

Treated surface: 1.5 ha

Tree plantation 2 x 2 m
Approximately 3 years old
and
Organic matter application (20 t ha^{-1})

1. Plant selection (*Popolus nigra*)

- Common in the Mediterranean area
- Tolerance and fast growth
- Suitability to develop in the substrate
- Adaptability to the local climatic conditions
- Capability to extract heavy metals and/or improve soil properties for the hydrocarbon degradation.

Popolus nigra

2. Organic matter

The organic matter addition, improving the chemical physical and biological characteristics of soil, enhances the soil health and provides a medium satisfactory for plant growth.

Horse manure

Monitoring

Treatments:
1. Horse manure (control)
2. Horese manure+*Populus nigra* (plant)

Experimental time: 2 years

The monitoring of the bioremediation system consisted of samplings carried out at 0-30 cm twice a years.

Bio-indicators		Chemical indicators
General parametres	Specific parameters	Contaminats
Dehydrogenase activity Microbial diversity Metaproteomic analysis	β-glucosidase, Phosphatase activities Catechol 2,3-dioxygenase activity PHA-degrading genes	Heavy metals Hydrocarbons Polychlorobiphenyl

Soil characteristics

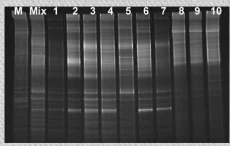
	Initial soil	Italian legislation 152/06 Column A	Italian legislation 152/06 Column B
Total Organic C (%)	1.29		
Total N (%)	0.118		
NH_4^+ (mg kg^{-1})	4.97		
Total P (mg kg^{-1})	823		
Ni (mg kg^{-1})	131	120	500
Pb (mg kg^{-1})	554	100	1000
Cu (mg kg^{-1})	137	120	600
Cr (mg kg^{-1})	40.3	150	800
Cd (mg kg^{-1})	2.99	2	15
Zn (mg kg^{-1})	628	150	1500
Total Petroleum Hydrocarbon (mg kg^{-1})	1140	10 + 50 (C<12+ C>12)	250+ 750 (C<12+ C>12)
PCB (mg kg^{-1})	15.4	0.06	5

DGGE

(denaturing gradient gel electrophoresis)

Purified PCR product (primer 16S rDNA)

↓ DGGE



Urea-formamide-polyacrilamide gel
(run 17h at 58°C to separate the fragments)
The gel was silver stained

↓

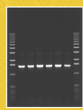
Band excision from DGGE gel

↓

DNA isolation and amplification by PCR →

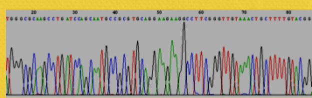
Fragments were:

Purified (Agarose gel) →



Cloned → Vector + 16S rDNA

Sequenced (automatic DNA sequencer)



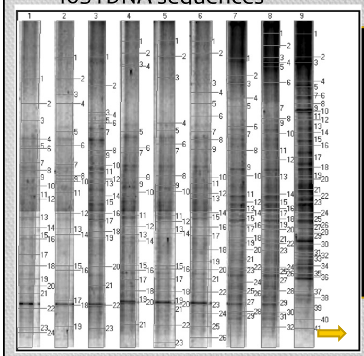
Sequence identifications (GenBank databases)

Microbial diversity of the contaminated soil (initial soil characterization)

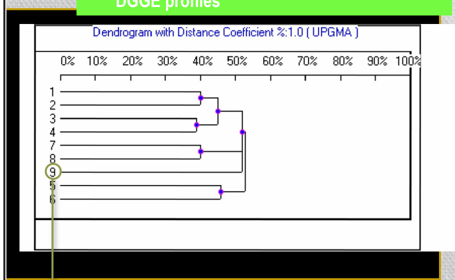
DGGE

Based on amplification of a GC hyper-variable zone, and separation of DNA fragments in polyacrylamide gels with a linear gradient of denaturants

16S rDNA sequences



Cluster analysis and diversity indexes from the DGGE profiles



Uncontaminated soil

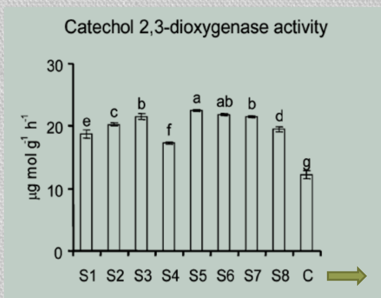
Community complexity (DGGE analysis) was inversely related to the contamination level.

Sequence Analysis of DGGE bands

Query	Closes relative	Accession number	Identity %	Phylogenetic class	Phylogenetic taxa
B2	Unidentified eubacterium	AJ232846	89	Firmicutes;	Clostridia; Clostridiales; Eubacteriaceae
B3	Uncultured eubacterium clone F10.48s	AF495406	89	Firmicutes;	Clostridia;Clostridiales; Eubacteriaceae
B4	Uncultured bacterium clonesJC2701R_55	AY349521	93	Bacteria;	environmental samples
B5	Proteobacterium UMB 38	DQ147584	100	Proteobacteria;	unclassified
B6	Uncultured soil bacterium clonesM25_Pitesti	DQ378245	99	Bacteria;	environmental samples
B7	Thermoactinomyces sacchari	AJ251779	95	Firmicutes;	Bacilli; Bacillales; Thermoactinomycetaceae; Laceyella
B8	Cinetobacter .calcoaceticus	X81668	99	Proteobacteria;	Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter
B9	Uncultured soil bacterium clone C129s	AF507687	98	Bacteria;	environmental samples
B10	Uncultured Firmicutes bacteriums clone GASP-WC2W3_D03	EF075358	98	Firmicutes;	environmental samples
B11	Monochloroacetic-acid-degrading bacterium 'Band C'	AF532191	96	Bacteria;	unclassified Bacteria
B12	Cellvibrio sp. R-20952	AJ786793	98	Proteobacteria	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae
B13	Uncultured actinobacterium clone LW7s	DQ973232	100	Actinobacteria	environmental sample
B15	Roseomonas sp. 1 LOT M4	AY624051	100	Proteobacteria;	Alphaproteobacteria; Rhizobiales; Methylobacteriaceae; Roseomonas
B18	Bacterium Ellin 5273	AY234624	96	Actinobacteria	Acidimicrobidae; unclassified Acidimicrobidae
B21	Bacteroidetes bacterium MH2-1	EF187349	97	Bacteroidetes/Chlorobi group;	Bacteroidetes; unclassified
B22	Bacterium TSA-5.4	AF240150	96	Bacteria;	unclassified Bacteria
B23	Uncultured earthworm cast bacteriums clone c251	AY154605	96	Bacteria;	unclassified Bacteria
B25	Uncultured Firmicutes bacteriums clone GASP-WB2S1_H07	EF073821	99	Firmicutes;	environmental samples
B26	Caulobacter subvibrioides (ATCC 15264)	X94470	96	Proteobacteria;	Alphaproteobacteria; Sphingomonadales
B28	Sphingobacteriaceae str. Ellin 160	AF409002	90	Bacteroidetes/Chlorobi group;	Bacteroidetes; Sphingobacteria;
B29	Spirosoma sp. RODSPM9	EF451725	92	Bacteroidetes/Chlorobi group;	Bacteroidetes; Sphingobacteria; Sphingobacteriales; Flexibacteraceae; Spirosoma
B31	Mycobacterium doricum strain DSM	AF547917	98	Actinobacteria	Actinobacteridae; Actinomycetales; Corynebacterineae; Mycobacteriaceae; Mycobacterium

BIOCHEMICAL ACTIVITY of the contaminated soil (initial soil characterization)

- Catechol 2,3-dioxygenase activity in PAH-contaminated soils, which has not so far been analysed, could provide accurate information on the capacity of the soil to degrade these organic components and on potential soil recovery



Uncontaminated soil

PCR-SSCP (SINGLE-STRAND CONFORMATION POLYMORPHISM) analysis diversity of the PAH degrading genes

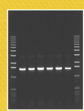
DNA amplification by PCR (primers P1.1f and P2.2r)

The highest number of PAH-degrading genes and catechol 2,3-dioxygenase activity were found in contaminated soils

PAH-degrading genes, and catechol 2,3-dioxygenase activity analysed in this study may be appropriate tools for monitoring contamination and bioremediation in soils.

Fragments were:

Purified (Agarose gel)

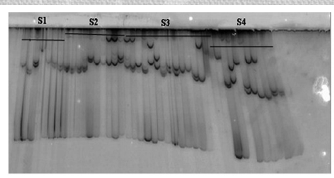


Cloned → Vector + PAH degrading DNA

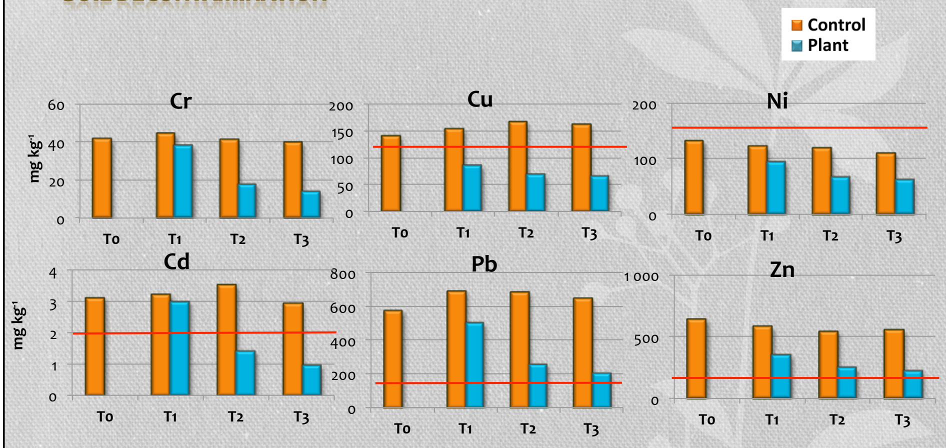
PCR (primers P1.1f and P2.2r)

SINGLE-STRAND CONFORMATION POLYMORPHISM (SSCP) screening

gel was silver stained



SOIL DECONTAMINATION

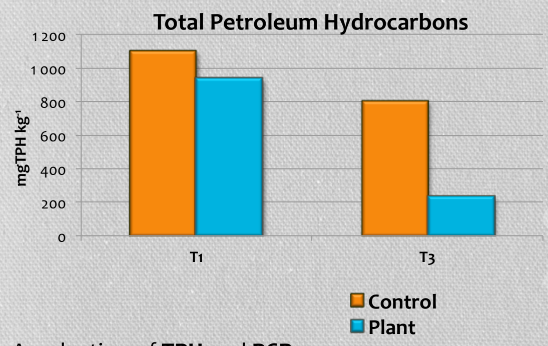


A reduction of **heavy metals (50%)** was observed in the plant treatment with respect to untreated soil

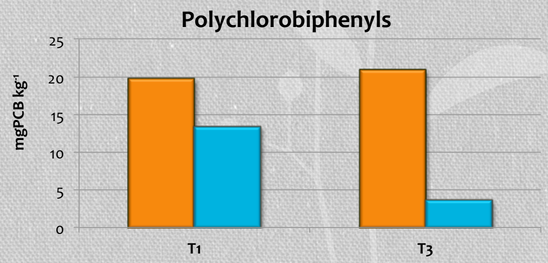
— Law limit for urban use (Column A)

Industrial use (Comun B) in mg Kg-1: Cr 800; Ni 500; Pb 1000; Cu 600; Cd 15; Zn 1500

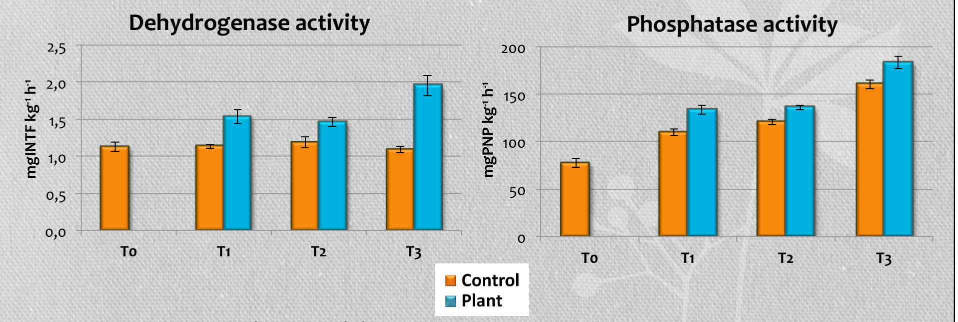
SOIL DECONTAMINATION

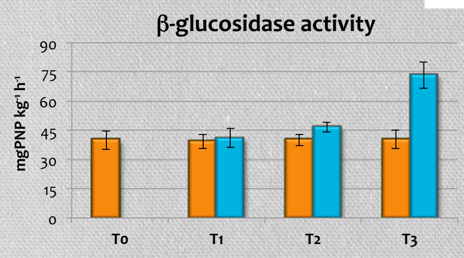


A reduction of **TPH** and **PCB (70%)** was observed in the plant treatment with respect to untreated soil



FUNCTIONAL RECOVERY

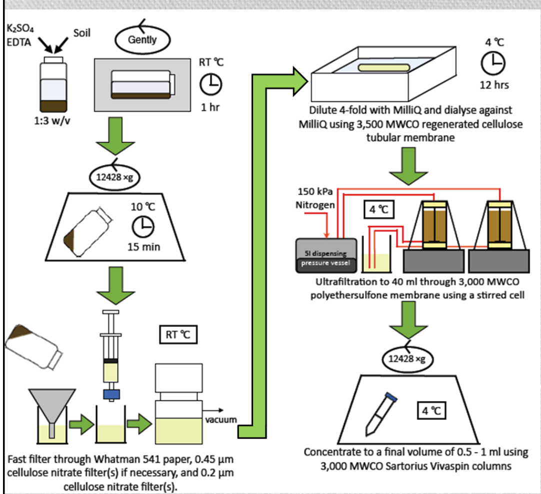


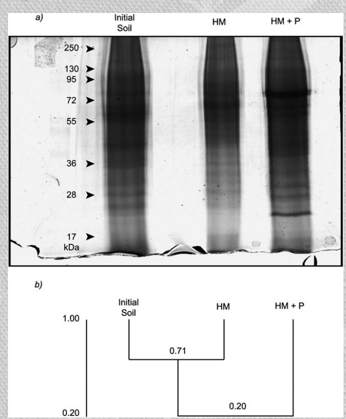


A stimulation of the **metabolic soil processes** (increase in β-glucosidase, phosphatase and dehydrogenase activities) was showed during the time in the planted compared to untreated soil

Metaproteomic analysis of the soil secretome*

*the total proteins present in the soil but without any cell lysis





At the end of experimentation, preliminary protein SDS-page results have permitted the purification of a greater number of proteins (SDS-PAGE bands) in planted (HM+P) with respect to unplanted (HM) and initial soil, thus indicating the improvement of the HM+P soil functional status

Conclusions

- The decrease during the time of both inorganic (Heavy metals, 50%) and organic (PCB and TPH 70%) contaminants indicated the effectiveness of the Phytoremediation system
- The increase in biological parameters (Dehydrogenase, β-glucosidase and phosphatase activities) in planted compared to untreated soil indicated the activation of microbial metabolism favoured by the **plant roots-microorganisms interaction**
- Metaproteomic analysis has permitted the purification of a greater number of proteins (SDS-page bands) in planted with respect to untreated soil

CASE STUDY (REAL SCALE)

4.LANDFARMING: AN EFFICIENT TECHNOLOGY FOR SOIL DECONTAMINATION

European Project "Indicators and thresholds for desertification, soil quality, and remediation" INDEX (STREP contr n 505450 2004/2006)

M. Ros, I. Rodriguez, C. Garcia, T. Hernandez. (2010). Microbial communities involved in the bioremediation of an aged recalcitrant hydrocarbon polluted soil by using organic amendments. Bioresource Technology 101 6916–6923

Landafarming

- Landfarming is an *In situ* bioremediation technology based on the spreading and incorporation of organic amendments in soil to increase organic contaminants degradation.

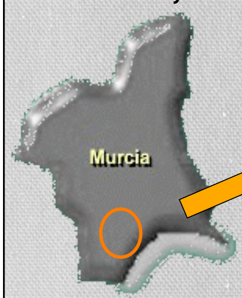
The organic amendments are a source of **microbial biomass** and enzymes and provide **nutrients** and easily biodegradable substrates to stimulate organic contaminants degrader microorganisms activity

Exprimental layout

	Refinery sludge[a]	Fresh sewage sludge	Sewage sludge compost
pH (H_2O)	–	7.46	7.53
EC (1:10) (μS cm^{-1})	–	2250	2800
TOC (g kg^{-1})	162.3	350	360
Total N (g kg^{-1})	–	45	27
Total P (g kg^{-1})	–	24.8	15.9
Total K (g kg^{-1})	–	6.1	4.4
Hydrocarbon content (g kg^{-1})	220.7	–	–
Pb (mg kg^{-1})	10	173	109
Cd (mg kg^{-1})	<1.2	1.8	0.7
Ni (mg kg^{-1})	24	51	14
Zn (mg kg-1)	57	1385	859
Cu (mg kg^{-1})	22	337	270
Cr (mg kg^{-1})	21	132	22

The clayey subsoil confers a degree of impermeability, which makes the soil suitable for recycling refinery sludge through landfarming

Oil refinery sludge had been added to soil for more than **10 years**



*Piezometer at 10 and 20 m of depth to control the leachate
* Semi-arid area

Treatments:
✓Soil without organic amendment (US);
✓Soil amended with 180 t ha^{-1} of fresh sewage sludge (FS);
✓Soil amended with 180 t ha^{-1} of sewage sludge compost (CS).

Exprimental layout

Sewage sludge and compost were spread on the soil surface and mixed with soil by plowing

Establishment of a spontaneous vegetation cover

MONITORING

Soil samples (0–20 cm) were collected at the beginning of the experiment and after 4 and 8 months of bioremediation

Main spontaneous vegetation cover

PINO

LENTISCO

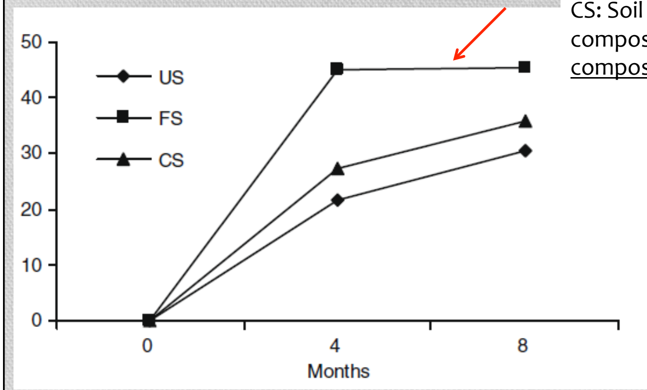
SOIL DECONTAMINATION

Hydrocarbon degradation (%)

US: Soil without organic amendment

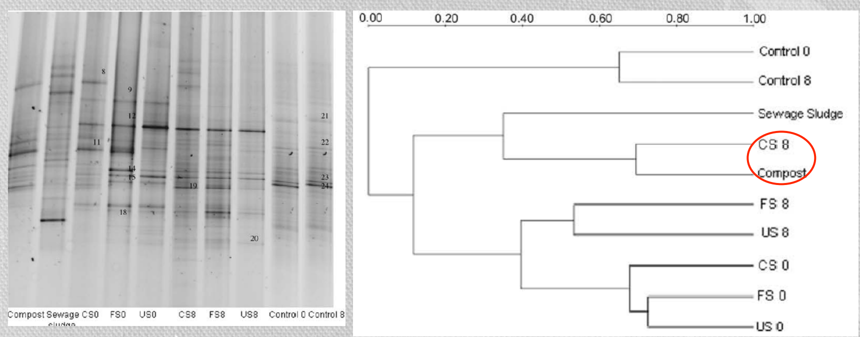
FS: Soil amended with <u>fresh sewage sludge</u>

CS: Soil amended with composted <u>sewage sludge compost</u>



The higher rate of hydrocarbon degradation observed in the soil amended with <u>fresh sewage sludge</u> (FS) could be due to its higher content of nutrients such as N and P

Denaturing gradient gel electrophoresis profiling-sequence analysis of PCR-amplified 16S soil rRNA fragments (**PCR-DGGE**) of the different treatments, 0 and 8 months after treatments (US, FS and CS).



❖In general, the bacterial communities of the different bioremediation treatments changed during the experiment

❖At the end of the experiment, compost amended soil showed a similar bacterial community of compost, suggesting a major influence of compost microorganisms in the decontamination processes

Conclusions

- The application of organic matter both fresh and stabilized, permitted the establishment of a spontaneous vegetation cover
- The higher rate of hydrocarbon degradation was observed in the soil amended with fresh sewage sludge
- In general, the bacterial communities of the different bioremediation treatments changed during the experiment
- At the end of the experiment, compost showed a similar bacterial community to compost amended soil

Main Conclusions

Organic matter application (fresh sewage sludge, compost, ect..) and the presence of plants positively affected the decontamination and functional recovery of soil:

✓ Increase of available nutrients and activation of the metabolic processes

✓ Stimulation of microbial activity and Increase in biodiversity

✓ Reduction in organic and inorganic contaminats

All the above may constitute a valid alternative to the current decontamination systems