DnD biotech
Discover New Directions

MIBIREM

Life surfing

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INTEGRATED SUSTAINABLE APPROACH TO LINDANE BIODEGRADATION

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AGENDA

- DND BIOTECH: WHO WE ARE
  - SERVICES & PRODUCTS
- LINDANE & HCH ISOMERS
- LINDANE CASE STUDY
  - ACTIVITIES
  - RESULTS
  - NEXT STEPS
- ROBONOVA®
- MIBIREM PROJECT

DND BIOTECH: WHO WE ARE

- Headquarter in Pisa, Italy
  Working in Europe, South America and Middle East
- Multidisciplinary team
- Environmental engineering and biotechnology
- Design & operation of pilot plants
- Scale-up of bioremediation systems
- Zeolites experts

Cosimo Masini CEO

Federica Brogioli Biotechnologist

Burak Anadolu Environmental engineer

Aurora Bellandi Agronomist

Francesco Panattoni Chemist

Marianna Tardani Chemist

SERVICES &PRODUCTS

- Environmental remediation consultancy
- Bioremediation treatment design
- Lab and pilot scale testing of on site and in-situ biopiles
- Microbial community analysis by metagenomics and bioinformatics
- Production and supply of biomass for bioaugmentation
- Prototype scale testing:
  - Biostimulation
  - Bioaugmentation
  - Bio-soil-washing
  - Permeable Reactive Barriers
- Zeolites for water filtration and soil remediation

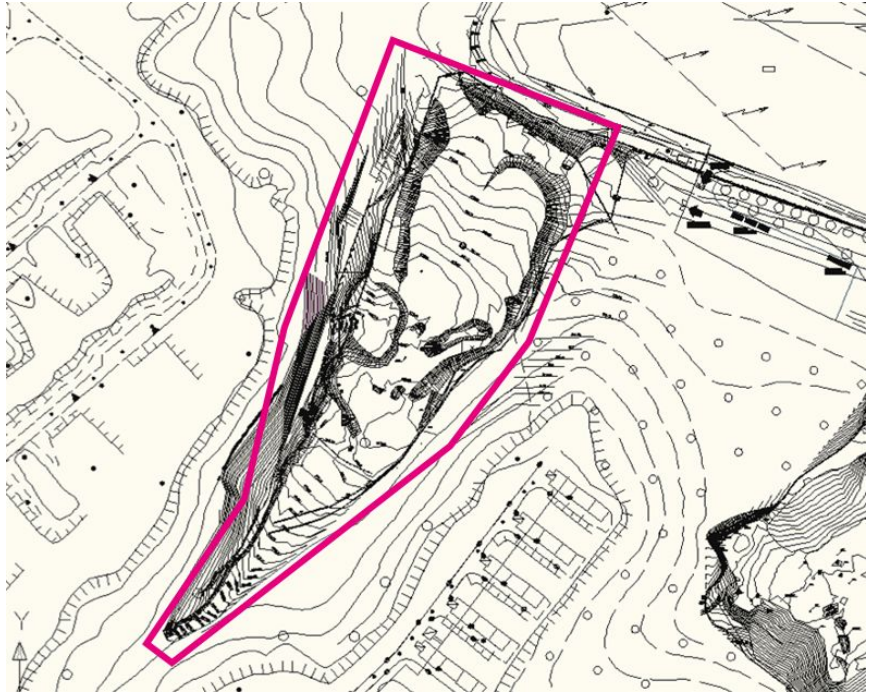
LINDANE & HCH ISOMERS

- 1945 – 2000: lindane (γ-HCH) used as broad-spectrum insecticide and treatment against ectoparasites
- Inefficient production process: 1 ton of lindane = 8 to 12 tons of waste isomers (technical lindane, t-HCH)
- Waste isomers dumped at production facilities → huge uncontrolled landfills
- > 4.8 million tons of HCH waste present worldwide
- HCH isomers barely degrade in the environment, bio-accumulate through the food chain and present a risk to human health and the environment
- In 2000: lindane and HCH isomers banned in EU
- In 2009: α, β, γ-HCH added in Stockholm Convention on POPs
- Bacterial degradation: anaerobic and aerobic dehalogenation (mineralization)
- Fungal degradation: extracellular ligninolytic enzymes

CASE STUDY

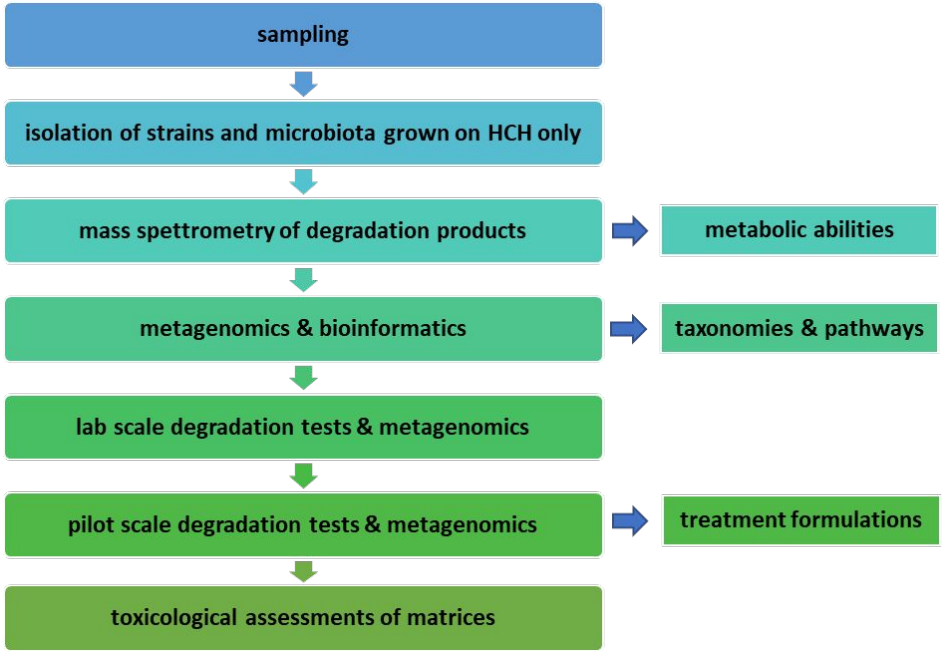
- Industrial area of Colleferro and the surrounding Valle del Sacco
- Production site from mid ‘40s to late ‘70s
- Two disposal waste areas in disuse reported high concentrations of HCH isomers (α, β, γ)
- 2005: high concentrations of β isomer detected in cow milk/dairy products from farms nearby → epidemiological surveillance
- Disposal areas characterized and contaminated soil permanently separated
- River sediments have adsorbed HCH, release during floods
- Study for the remediation of lindane

CASE STUDY



ACTIVITIES

- Soil, water and sediments sampling
- Microbial analyses: strains isolation, mass spectrometry and metagenomics
- Lab scale: degradation tests under different conditions, treatment protocols and microbial inocula
- Pilot scale: field testing of selected treatment protocols with Robonova®



SAMPLE SELECTION

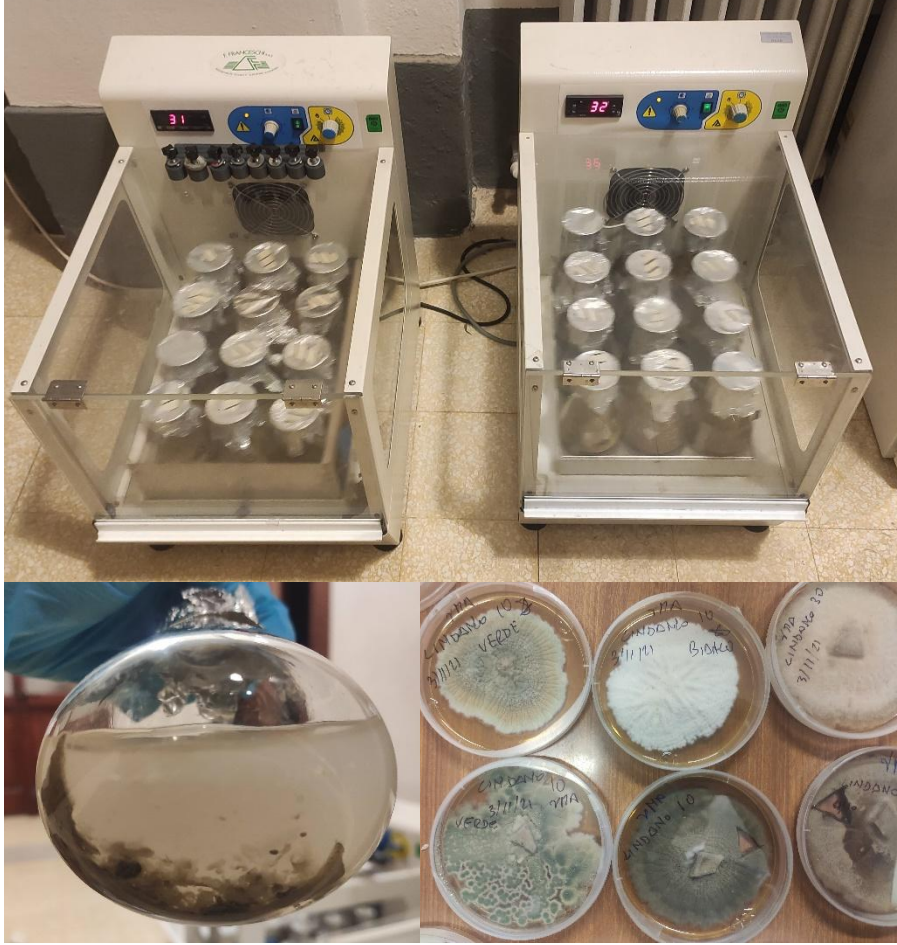
- AG10-13m7A, AN10-11m7AB and AS11-12m4ABG → compare the microbiome at varying HCH isomers but similar soil type/sampling depth
- AT03-04m4D → identify the microbiota possibly associated with DDT only
- AG09-10m7ABGDL and AS10-11m4ABGDO → assess the effect on the microbial community of chlordane or aldrin
- AU01-02m5GDO and AS09-10m4ABDO → assess the effect on the microbial community of a common background contamination and different HCH isomers
- O09-10m5ADEC → assess the effect on the microbial community of light hydrocarbons and HCH
- O11-12m4CTR and AS/13-14m7CTR → uncontaminated soil as reference samples

Parameters	Values range
Sampling depth	1 - 14 m
Soil type	anthropic, volcanic, loamy sand, loamy clay
Benzene	0.005 - 114 ppm
DDT	0.004 - 4.433 ppm
Aldrin	0.002 - 0.433 ppm
Chlordane (cis + trans)	0.002 - 0.537 ppm
α-HCH	0.026 - 58.7 ppm
β-HCH	0.009 - 305 ppm
γ-HCH (Lindane)	0.061 - 8.5 ppm
Light hydrocarbons (≤ C12)	0.004 - 315 ppm
Heavy hydrocarbons (C12-C40)	5.6 - 78 ppm

RESULTS

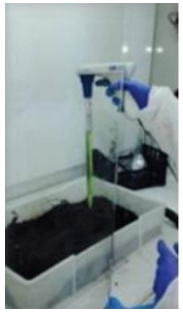
- Selected sample: AN7-8m5ABDLO = 7-8m depth, loamy sand, α-HCH (45 ppm) + β-HCH (305 ppm) + DDT (0.125 ppm) + aldrin (0.175 ppm) + cis+trans chlordane (0.537 ppm)
- 1g of soil incubated in BSM e HCH, 125rpm at 30°C for 1 month → 4x transfers of 1ml → plating
- ✔ Isolated three fungal strains and five bacterial strains, grown only on HCH as carbon source
- ✔ Established one mixed microbial community, currently under test at lab scale for HCH degradation

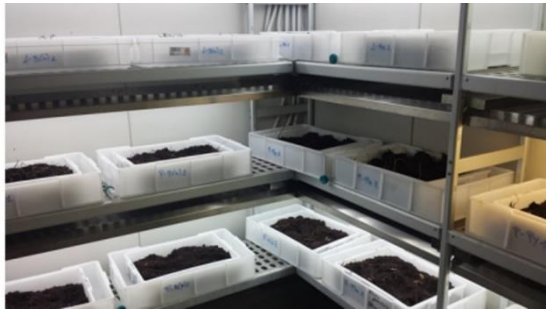
- GCXGC MS-TOF analytical method:
  - sensitivity per isomer of 15.25 femtograms (ppb in heptane solvent)
  - R^2 on a 5-point calibration curve is 0.086 - 0.999 for α/β/γ/δ-HCH
  - detection range is 15.25 - 122 ppb



NEXT STEPS

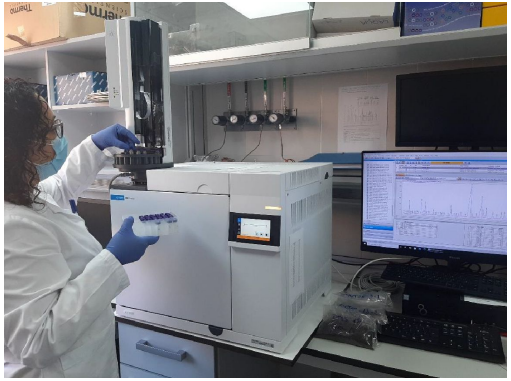
- Mass spectrometry (GCXGC MS-TOF) study of the metabolic intermediates and final degradation products → identification of metabolic abilities
- Metagenomics on isolates → taxonomical identification & degradation pathways
- qPCR assays for quantitative microbial monitoring
- Lab scale tests (mesocosms)
- Robonova® pilot tests & metagenomics for data correlation



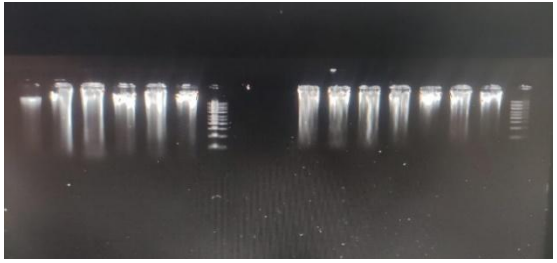


Soil samples from test site

Soil samples physico-chemical characterisation



DNA isolates for metagenomics analysis



Mesocosm test preparation

Soil Testing Facility

ROBONOVA

The use of the RoboNova plant allows to define important key-parameters to proceed with the design of a full-scale plant:

- Machinery
- Treatment formulation
- Degradation kinetics and expected duration
- Energy, environmental impact and emissions
- Monitoring plan





Air filter
Injection port
Soil turner
Ferment er

MIBIREM PROJECT

- Awarded in Horizon Europe frame 2021-2027
- Consortium of 11 partners: SMEs, universities & research centres, industries
- Aim: exploitation of microbiomes able to degrade certain contaminants of interest (petroleum hydrocarbons, pesticide HCH and cyanides)
- Creation of a toolbox to identify, analyse, cultivate and upscale the microbiomes for bioremediation applications, while ensuring safety and policy alignment

We have not inherited the Earth from our parents, we have borrowed it from our children.

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