A MOLECULAR APPROACH TO LINDANE BIODEGRADATION

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Discover New Directions

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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

PROJECT

MIBIREM

- Awarded in Horizon Europe frame 2021-2027
- Consortium of 11 partners: SMEs, universities & research centres, industries

➢ **Aim:** creation of a toolbox to identify, analyse, cultivate and upscale the microbiomes for bioremediation applications & development of a predictive modelling tool

➢ **Strategy:** exploitation of microbiomes to implement a sustainable and cost-effective approach for widespread diffused contamination (petroleum hydrocarbons, pesticide HCH and cyanides)

➢ **Method:** identifying and studying microbial population and species that can degrade effectively Lindane and its isomers & testing their efficiency in laboratory and pilot scale trials

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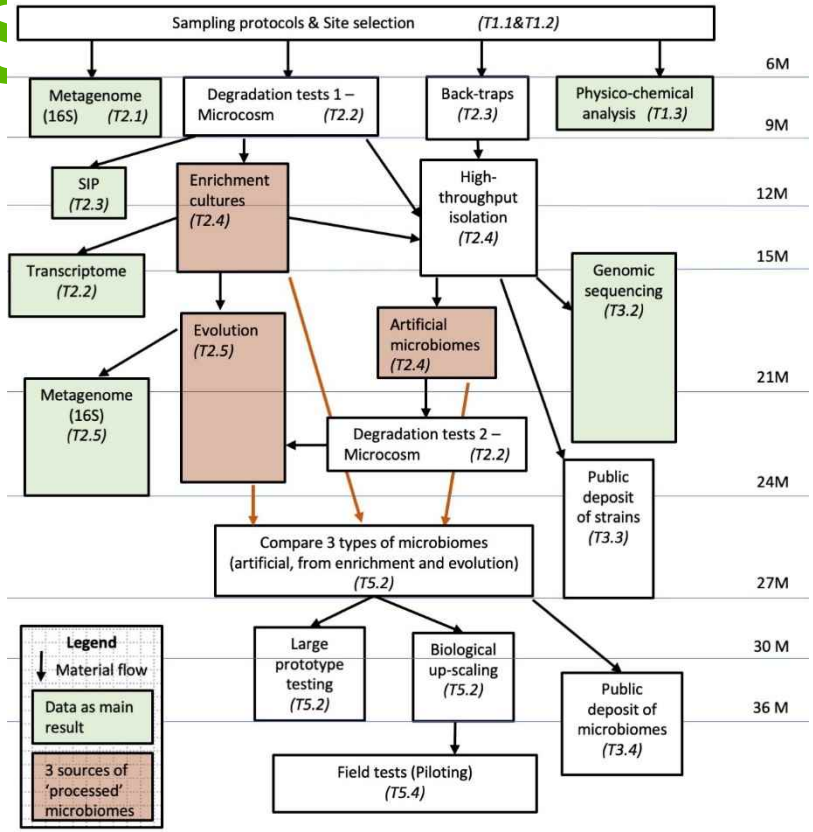
ACTIVITIES

- Soil/groundwater sampling & physical-chemical characterization
- Microbiota enrichment, selective evolution & metagenomics
- Strains isolation & genome sequencing
- Degradation tests (microcosms)
- Field testing with Robonova®
- Design of predictive tool for bioremediation feasibility



PREDICTIVE TOOL

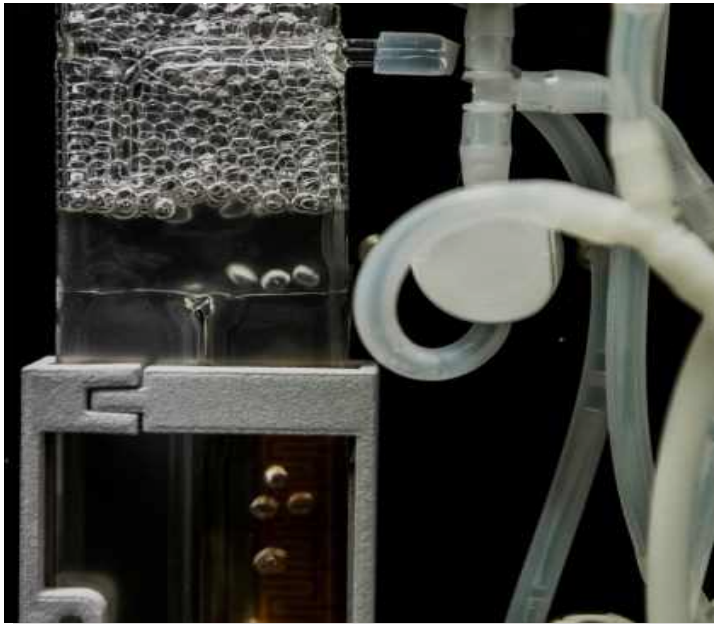
- For a successful application of bioremediation:
  - Understand and control microbial networks responsible for the degradation of the contaminants
  - Recognise links between the degrading microbiome and chemical and physical site characteristics
  - Run numerical simulation: groundwater flow, transport of dissolved substances, geo-chemical processes (adsorption, oxidation,..), occurrence of natural biodegradation

- Combine modelling of microbiological, chemical, hydrological, and physical processes with statistical data analysis
- Link and analyse interplay of degrading microbiome, contaminants and physical-chemical site characteristics

  → predictive tool for decision-making on bioremediation feasibility

SELECTIVE EVOLUTION

- High environmental selective pressure process to improve bacterial abilities and efficiency to degrade the contaminants of interest
- Artificial selection of bacteria in continuous culture:
  - Population dilution creates a Darwinian competition for remaining in the system. Only bacteria growing at the given conditions can be selected over time
  - Exploration of random genome mutations to perform desired biological reactions without losing bacterial fitness

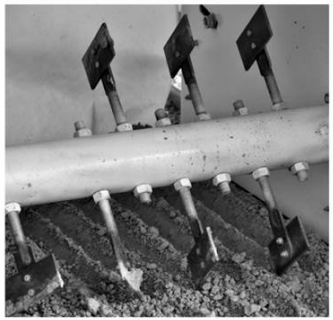


ROBONOVA®

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

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ISOMERS - PRODUCTION HISTORY

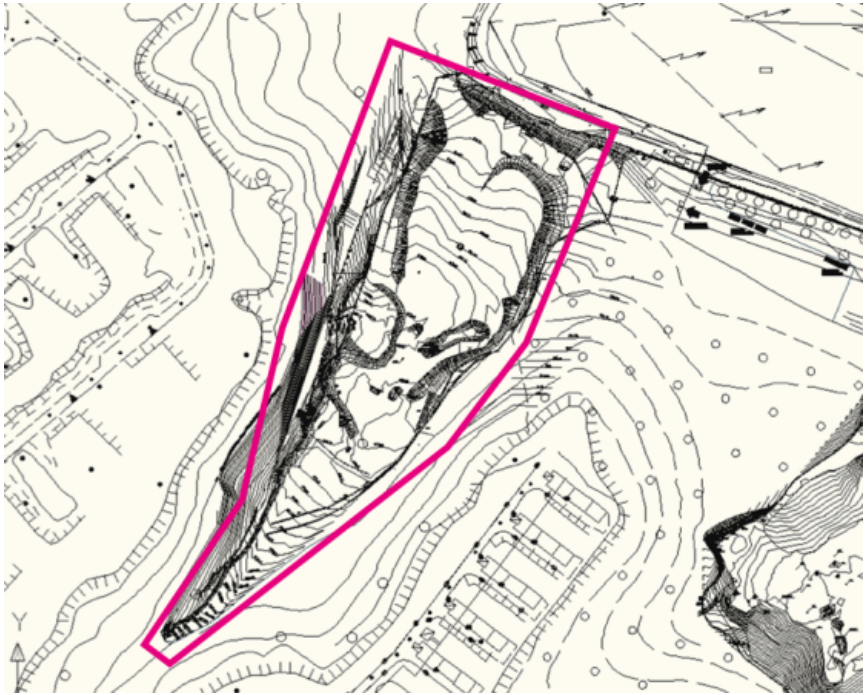
- 1945 – 2000: lindane (γ-HCH) used as broad-spectrum insecticide
- Inefficient production process: 1 ton of lindane = 8 to 12 tons of waste isomers (technical lindane, t-HCH)
- Waste isomers dumped at production facilities → huge uncontrolled landfills
- > 4.8 million tons of HCH waste present worldwide
- 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: added α, β, γ-HCH in Stockholm Convention on POPs

ISOMERS - REMEDIATION TECHNOLOGIES

- SOURCE ZONE (high concentrations):
  - Excavation
  - Pump & treat
  - Chemical oxidation
  - Incineration

- DIFFUSED CONTAMINATION (low concentrations):
  - Bacterial degradation: dehalogenation anaerobic and aerobic (mineralization)
  - Fungal degradation: extracellular ligninolytic enzymes

CASE STUDY

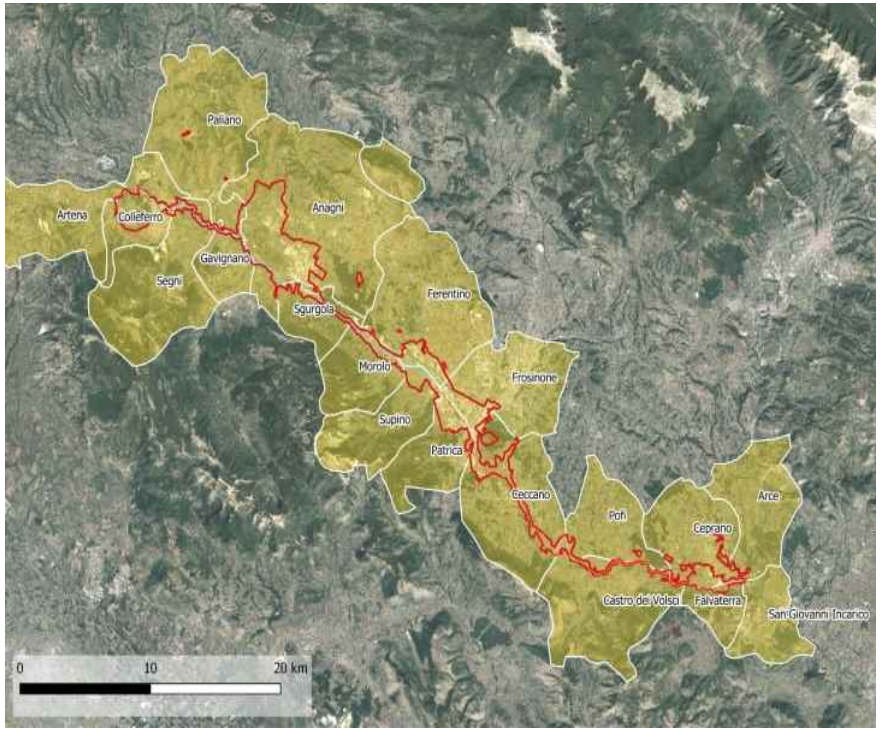


CASE STUDY

- The industrial area of Colleferro is included in the Remediation Site of National Interest “River Sacco basin”: 6.000 ha
- The site includes a former chemical industry active since 1913 in the production of pesticides, ammunition and railway parts
- Lindane production site from mid 40s up to late 70s
- 2 uncontrolled industrial waste disposal areas with high concentration of HCH isomers (α, β, γ), total potential volume of approx. 10.000 m3, of which:
  - About 2.500 m3 of so-called "white soil“: a solid matrix in which HCH and isomers represent ≈ 50% w/w
  - 1.000 m3 of contaminated soils in which HCH and isomers represent ≈ 10% w/w.

CASE STUDY

- 1990: Two illegal landfills of chemical waste were discovered close to the industrial plant
- 2005: Milk samples (34/244) at dairy farms in the area contained β-HCH 30 times above the limit by law (0,003mg/kg) → correlation with β-HCH contamination in soil and forage in the Sacco Valley
- Farming for human/animal consumption and pasture in terrains in the flooding areas and soil within 100 meters from the river embankments were prohibited (still ongoing)
- Emergency characterization of soils, waters and river sediments performed in the Sacco River basin
- Phase I, North-western part: Diffused contamination of DDT, DD, DDE, alfa, beta, gamma-HCH found in 9 municipalities
- Phase II, South-eastern part: Similar diffused contamination found in 7 municipalities
- Constant biomonitoring of milk and forage performed by the Authorities. A progressive reduction of Beta-HCH was found in milk and



SAMPLES 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

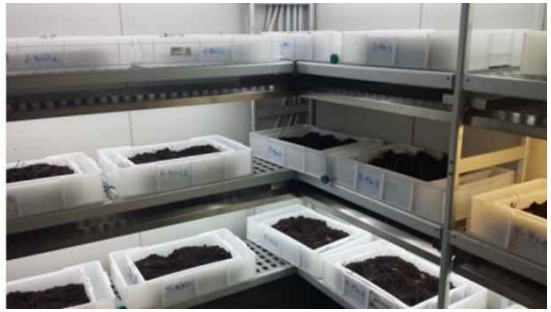
Parameters	Values range
Sampling depth	1 - 14 m
Soil type	anthropic, volcanic, loamy sand, loamy clay
Benzene	0.005 - 114 ppm
DDT	0.004 - 4.433 ppm
Aldrin	0.002 - 0.433 ppm
Chlordane (cis + trans)	0.002 - 0.537 ppm
α-HCH	0.026 - 58.7 ppm
β-HCH	0.009 - 305 ppm
γ-HCH (Lindane)	0.061 - 8.5 ppm
Light hydrocarbons (≤ 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 and HCH, 125 rpm at 30°C for 1 month → 4x transfers of 1 ml → 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)
  - ✓ R2 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



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

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