BIOMOLECULAR MARKERS FOR THE ASSESSMENT OF GENETIC POTENTIAL IN BIOREMEDIATION PROJECTS

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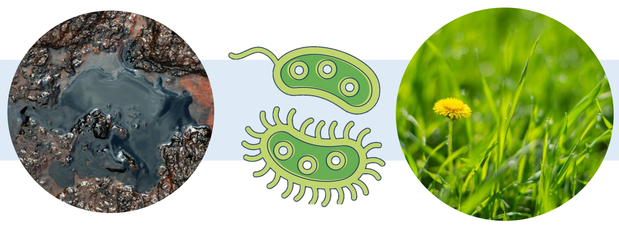
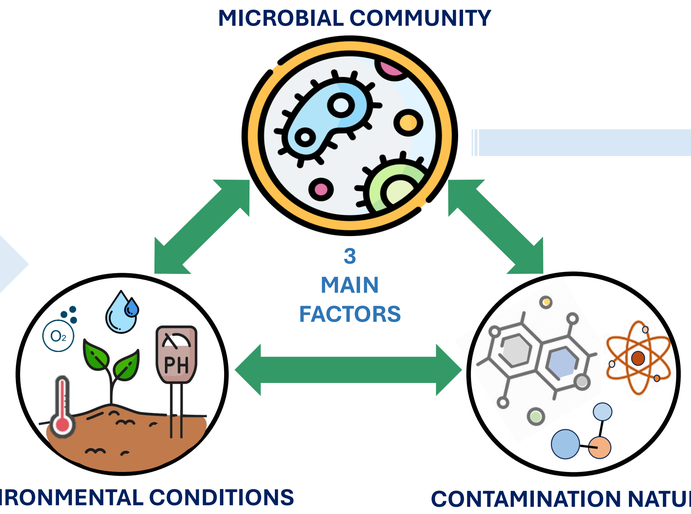
BIOREMEDIATION is based on the natural hability of microorganism to transform contaminants using them as growth substrate and energy source.



Determination of the **biodegradative potential** of the contaminated site

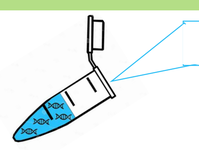


GENETIC POTENTIAL:
The presence, in the microbial community genomes, of the genes that encodes for the enzimes involved in the degradation pathways.

MAIN GENES = BIOMOLECULAR MARKERS

BIOMOLECULAR MARKERS: DNA sequences involved in a reaction of interest: selected on the contamination and the biological processes under analysis.

HOW CAN WE ANALYSE THEM?

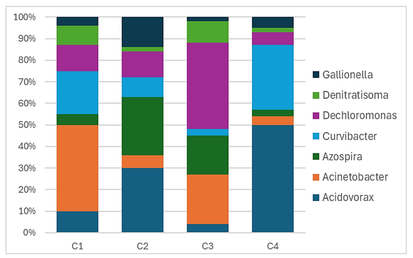


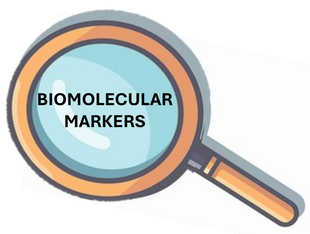
BIOMOLECULAR ANALYSES: genomic DNA extraction + complementary analyses (qualitative and quantitative)

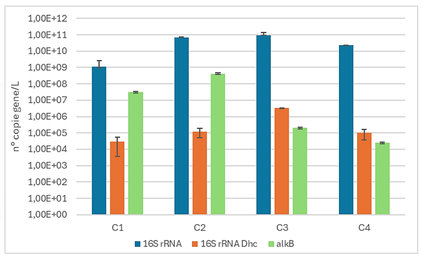
QUALITATIVE ANALISIS

Next-Generation Sequencing (NGS)

Taxonomic classification of the microbial community with relative abundances of specific groups.







QUANTITATIVE ANALYSIS

Quantitative PCR (qPCR)

Absolut quantitication of specific taxonimic or metabolic genes.

HOW CAN WE USE THEM IN BIOREMEDIATION PROJECTS?

Case study 1: SITE CHARACTERISATION

Matrix: soil; **Contamination:** aliphatic hydrocarbons and BTEXs;

SITE BACKGROUND

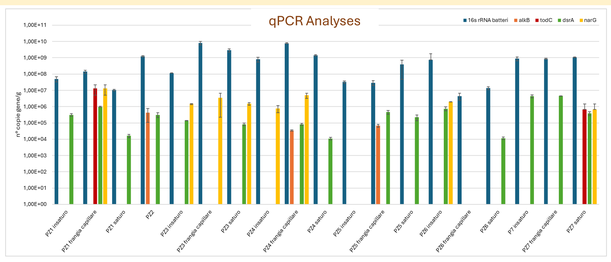
Petroleum products deposit

WHAT WE DO: **Microbial characterisation**

WHY: to investigate the biodegradative potential of the native microbial community and define the best site-specific biological strategy for the site remediation.

HOW: on the basis of chemical results we selected biomarkers and n. 19 samples for microbiological analysis, trying to investigate all the possible conditions:
- Samples from unsaturated, capillary fringe and saturated soil
- Samples from contaminated and not contaminated zones.

ANALYSES: **Next Generation Sequencing – NGS** of bacterial16S rRNA gene
Quantitative PCR (qPCR)
- bacterial**16S rRNA** gene (total bacteria)
- ***alkB*** biomarker gene for aerobic degradation of aliphatic HC
- ***todC*** biomarker gene for aerobic degradation of BTEXs
- ***dsrA*** biomarker gene for sulfate reducers
- ***narG*** biomarker gene for nitrate reducers



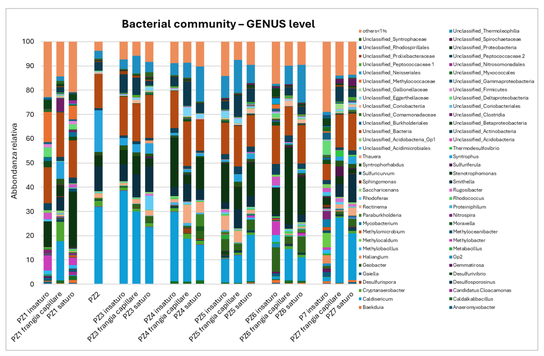
qPCR ANALYSES:

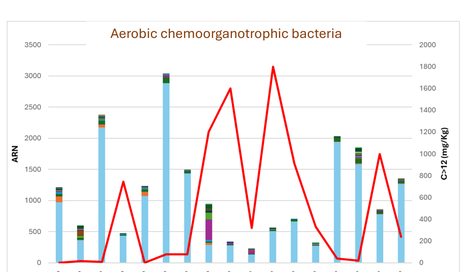
Aerobic	>	anaerobic
alkB		*dsrA*
todC		*narG*

NGS ANALYSES:

Samples from superficial and not-contaminated soils are characterised by a mostly **AEROBIC community**:
- *Xanthomonadales & Bacillales*

Samples from deep and contaminated soil are characterised by an **ANAEROBIC community**:
- *Caldisericales & Syntrophobacterales*





NGS DATA ELABORATION:

Functional analyses is a tool through which it is possible to attribute the membership of specific bacterial groups (orders and/or genera) identified through NGS sequencing to functional groups, also defining their normalized relative abundance (ARN).
It is useful to compare functional data with chemical ones, to investigate possible relations between them.
→ in this case it is evident the correlation between contamination and anaerobic community.

Case study 2: MONITORING

Matrix: groundwater; **Contamination:** chlorinated aliphatic hydrocarbons (CAHs)

SITE BACKGROUND

11.500 m² site with previous petroleum products deposit, waste disposal and paraffines production;

2009: chemical characterisation

2010-2017: risk analysis → groundwater contaminated by CAHs

2018: integrative characterisation → **microbial characterisation** to investigate the presence of biodegradative processes and their nature

2019: on the basis of characterisation results, **definition of a biological remediation strategy** → MNA

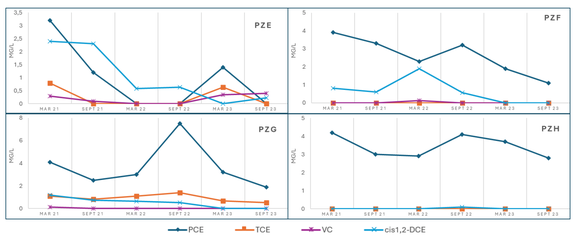
2021: **start of Monitoring of Natural Attenuation (MNA)**

MONITORING OF NATURAL ATTENUATION (MNA)

Duration: 3 years
Times: every 6 months

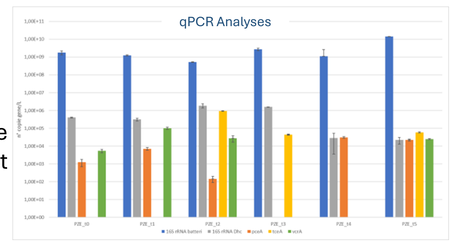
BIOMOLECULAR ANALYSES:
Next Generation Sequencing – NGS of bacterial16S rRNA gene
Quantitative PCR (qPCR)
- bacterial**16S rRNA** gene (total bacteria)
- **16S rRNA *Dehalococcoides spp.***, model bacteria for reductive dechlorination of PCE to ethene.
- ***pceA*** biomarker gene for anaerobic degradation of PCE and TCE.
- ***tceA*** biomarker gene for anaerobic degradation of TCE and DCE.
- ***vcrA*** biomarker gene for anaerobic degradation of DCE and VCM.

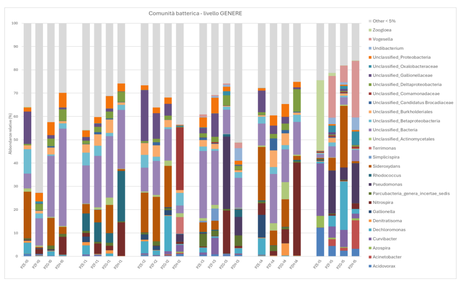
RESULTS



CHEMICAL & ISOTOPIC ANALYSES:
reduction of contaminant concentrations in all monitored piezometers
→ it can be attributed to biological processes of reductive dechlorination (anaerobic) and co-metabolism (aerobic).

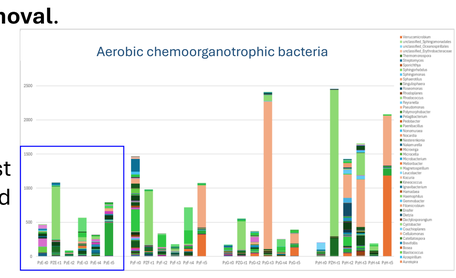
MICROBIOLOGICAL ANALYSES - qPCR:
increase in gene degradation potential during monitoring, with the greatest potential at the final time in the PZE, the one with best chemical results.





MICROBIOLOGICAL ANALYSES - NGS:
Mostly anaerobic populations, but non-strictly reducing conditions. Presence of bacteria for both anaerobic (*Aquabacterium, Dehalococcoides, Dechloromonas, Desulfosporosinus)* and co-metabolic (*Pseudomonas, Curvibacter, Nistrospira Acinetobacter)* **CAHs removal**.

NGS DATA ELABORATION:
Functional analysis and PCA → PZE is the piezometer that evolves least over time, maintaining anaerobic conditions the most. This could explain the best results for reductive dechlorination in this piezometer.



CONCLUSIONS

Results show the microbial community composition and functions, representing a precious instrument for the understanding of on-going processes and potential ones present on site.

Definition of the best site-specific remediation strategy.

Results show the effectiveness of the chosen remediation strategy, based on natural attenuation processes, allowing possible interventions to improve contaminant degradation and the site remediation.

Achievement of bioremediation objectives.

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