IN SITU REMEDIATION OF CHLORINATED SOLVENTS-CONTAMINATED GROUNDWATER: FROM SITE CHARACTERISATION TO THE MONITORING OF NATURAL ATTENUATION (MNA) BY BIOMOLECULAR AND ISOTOPIC TOOLS.

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Abstract: Inappropriate storage of chemicals and/or waste, improper disposal techniques and accidental spills are among the most common causes of groundwater pollution. This work dealt with the study and the treatment of a **chlorinated solvent-contaminated site**. An Emergency Safety System (MISE), consisting of a *pump&stock* hydraulic barrier, has been applied. However, the high costs associated to this system led to the evaluation of the feasibility of applying **bioremediation** as an alternative.

At first, the temporal trend of the chlorinated ethylenes and BTEX concentrations was analysed, processing chemical data regarding groundwater contamination. Moreover, a chemical and **microbiological-biomolecular characterisation** was performed to define the biodegradative potential of the site, in relation to chlorinated ethylenes and BTEX, the concentrations of which resulted over the law limits. In particular, Next Generation Sequencing (NGS) of the bacterial 16S rRNA gene and quantitative PCR (qPCR) analyses of target genes (*tceA*, *vcrA*, *bvcA*, *etnC*, *bssA*, *todC*) were carried out for the taxonomical and functional characterisation of the microbial community.

Results highlighted the presence of a strong anaerobic potential for the reductive dechlorination of chlorinated ethylenes, suggested by the presence of *tceA* gene in the piezometers contaminated by trichloroethylene (TCE) and of *vcrA* gene in both the piezometers contaminated by vinyl chloride monomer (VCM). Moreover, the *etnC* gene, involved in the aerobic oxidation of VCM, was not found in any piezometer, suggesting the absence of aerobic degradation potential towards low chlorinated ethylenes. However, the presence of the *bssA* and *todC* genes (biomarkers for the anaerobic and aerobic BTEX degradation, respectively), in the BTEX-contaminated piezometers, showed the co-presence of both aerobic and anaerobic degradation potential. From NGS results, microbial populations with specific metabolic capabilities were also identified.

Considering the characterisation results, a biological-based treatment strategy was proposed and a full-scale remediation project was designed and approved. In detail, for the central area of the site, **Monitored Natural Attenuation** (MNA) was selected, considering the presence of specific microorganisms and metabolisms potentially involved in the anaerobic reductive dechlorination process of chlorinated ethylenes. For the monitoring of this process, chemical, biomolecular and isotopic analyses were proposed and performed (4 selected piezometers) every six-month for three years. Biomolecular analyses comprised the quantification through qPCR of total bacteria, of *Dehalococcoides spp*., of *pceA*, *tceA* and *vcrA* genes and the NGS sequencing of bacterial 16S rRNA. The **isotopic analyses** (Compound Specific Isotope Analysis-CSIA) were carried out to evaluate the ratio $^{13}C/^{12}C$ of the target contaminants. Chemical analyses were integrated with isotopic and biomolecular ones for data interpretation and results showed the abatement of contaminant concentrations through biological processes, suggesting the effectiveness of the selected strategy. Moreover, an Air/Bio-Sparging (A/BS) technique was applied instead of the hydraulic barrier to aerobically remove the residual lower chlorinated ethylenes and BTEX.

This case study is an excellent demonstration of how bioremediation can be applied, from the site characterisation to the implementation of the selected strategy, showing that the use of innovative tools for the study and the monitoring of the site, such as isotopic and biomolecular analyses, is a significant and useful support for the choice of the best strategy and the calibration of the intervention.

Key words: *bioremediation*, *chlorinated solvent-contaminated site, isotopic analyses, microbiological-biomolecular characterisation, Monitored Natural Attenuation.*

Commentato [CM1]: Spiegare acronimo per esteso alla prima apparizione nel testo (sigla tra parentesi)

Commentato [CM2]: A nostro avviso, va riformulata la frase in modo più chiaro