Compound-Specific Isotope Analysis and Microbial Molecular Data for Effective Monitoring of a Bioremediation Pilot Trial at a heavily contaminated 1,2-DCA area: laboratory and field results.

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Objectives and case study in Northern Italy

- **Objective of the whole project:** ongoing work with the Politecnico di Milano and the University of Milano_Bicocca is focused on the application of the integrated molecular and isotopic approach for site characterization and monitoring in diverse sites in Italy and with different contaminants. This work implies the development of new molecular tools and CSIA protocols where needed.

- **Objective case study:** verify the possibility to accelerate in-situ natural biodegradation process of 1,2-dichloroethane (1,2-DCA) through **Enhanced In Situ Bioremediation (EISB)**.

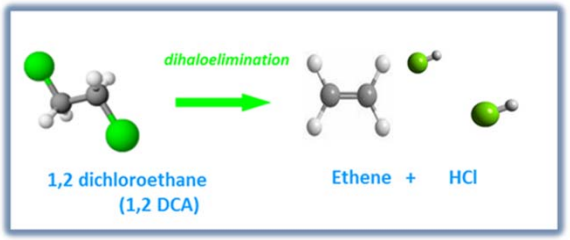
1,2 - DCA

Level of contamination:

Up to ~5-6 g/L

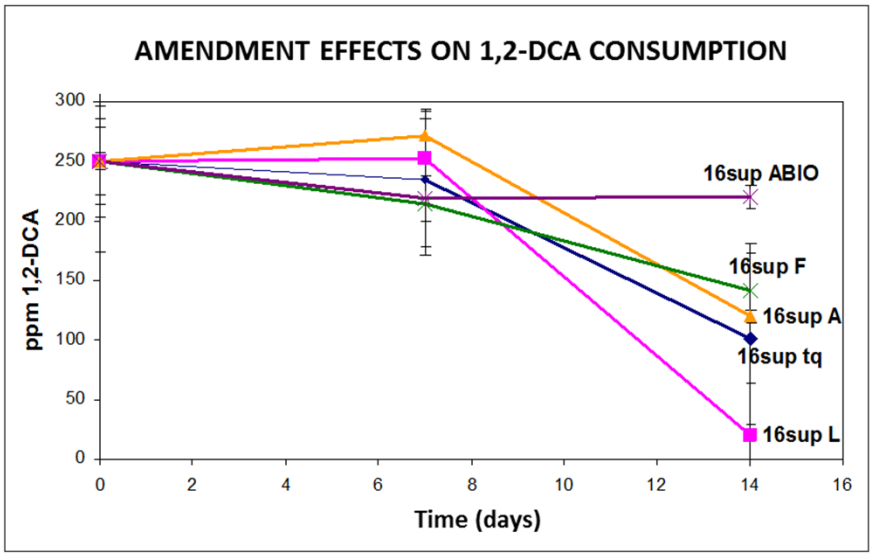
No DNAPL in the monitoring wells

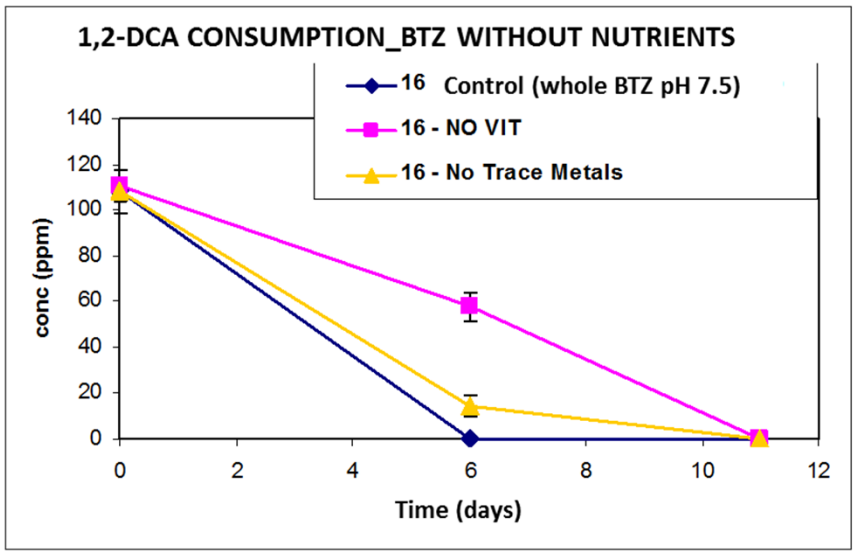
Laboratory results on groundwater samples (1/4)



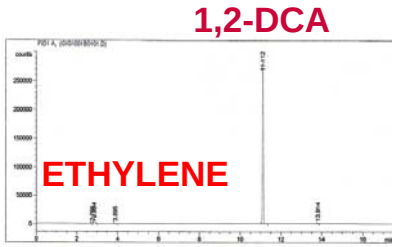
Laboratory activities identified:

- DI-HALOELIMINATION as the biodegradative reaction of 1,2- DCA;
- sodium lactate as the best amendment;
- the importance of nutrients (salts, trace elements and vitamins).

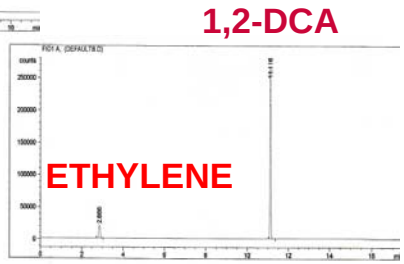




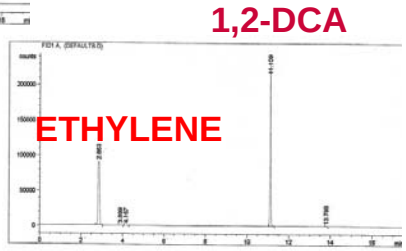
Laboratory results on groundwater samples (2/4)



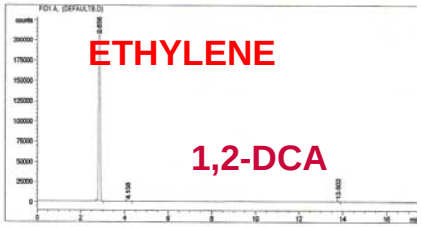
day 0



day 2



day 4



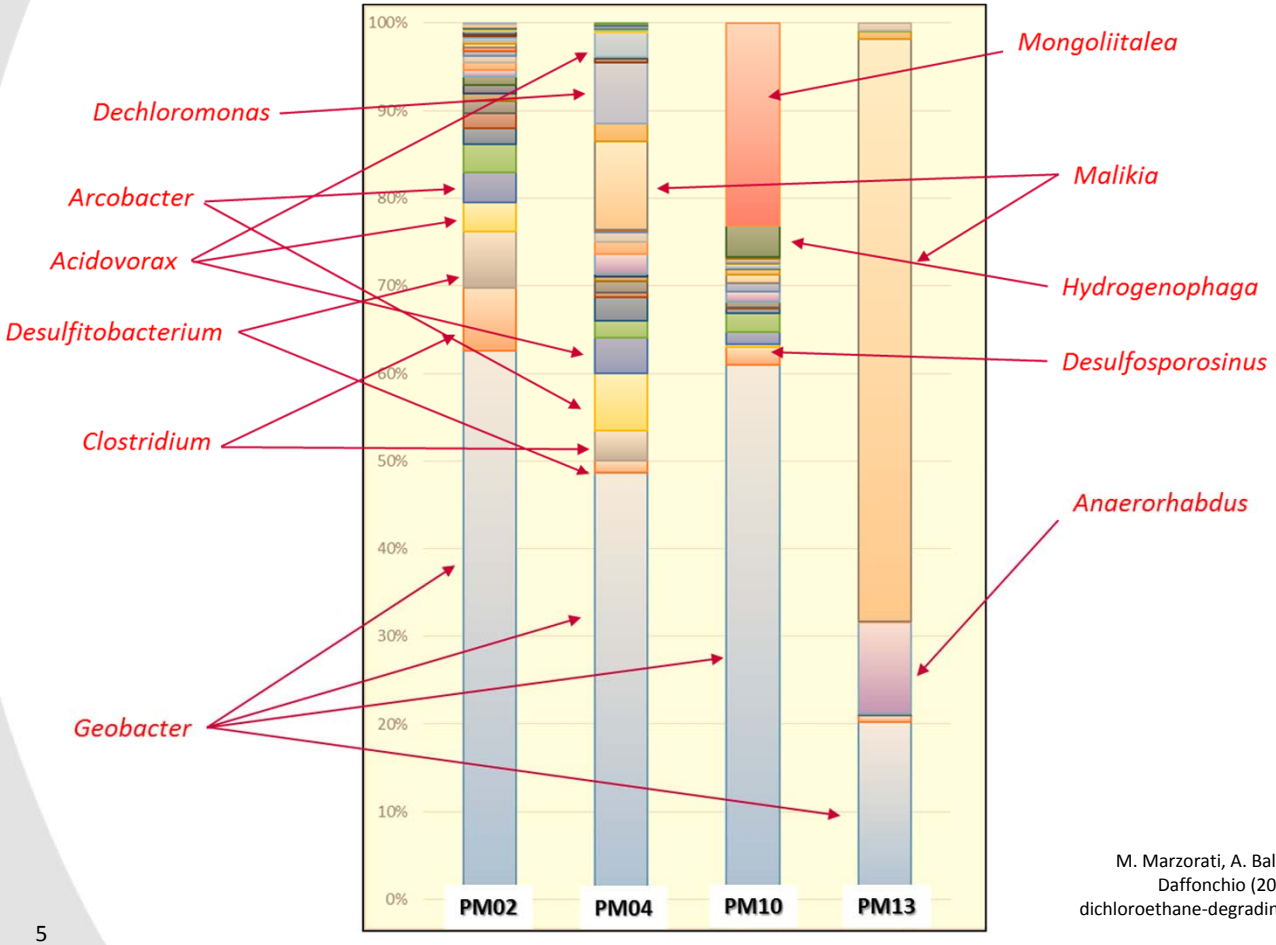
day 6

No CVM increase

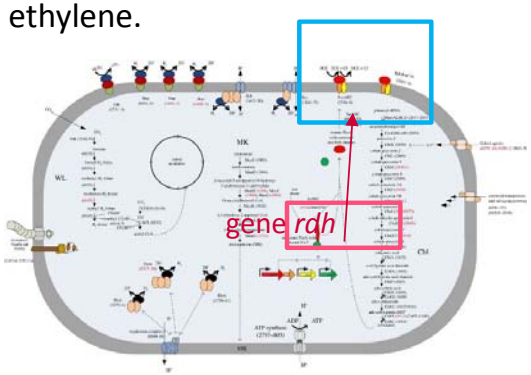
Kinetic of 1,2-DCA consumption:

- the dehalogenating pathway is confirmed;
- a dehalogenating microflora is present.

Laboratory results on groundwater samples (3/4)



- Anaerobic microbial community.
- *Geobacter* highly present.
- The protein **dehalogenase** catalyses the dehalogenating reaction from 1,2-DCA to ethylene.

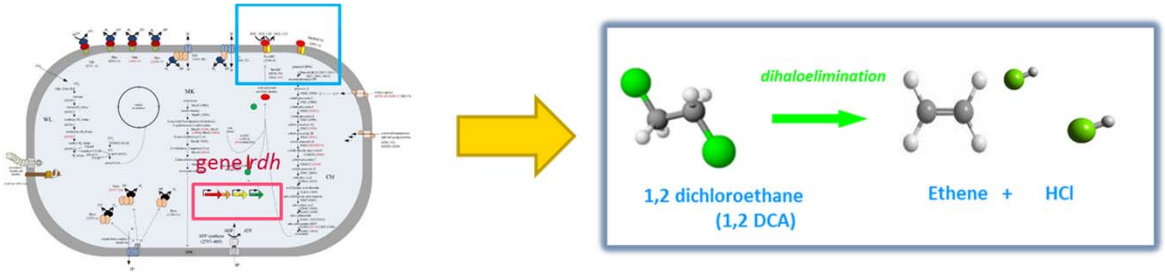


- The dehalogenating bacteria are specifically recognized by probes for the gene of the dehalogenase (**rdh group VI** molecular marker).
- Thus, the levels of **dealogenase rdh** can be used as index of the concentration of the dehalogenating bacteria in the aquifer.

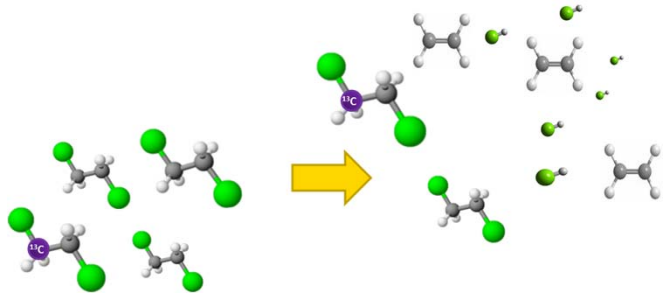
M. Marzorati, A. Balloj, F. de Ferra, L. Corallo, G. Carpani, L. Wittebolle, W. Verstraete and D. Daffonchio (2010). Bacterial diversity and reductive dehalogenase redundancy in a 1,2-dichloroethane-degrading bacterial consortium enriched from a contaminated aquifer. Microbial Cell Factories, 9. 12. doi: 10.1186/1475-2859-9-12

Laboratory results on groundwater samples (4/4)_^{13}C CSIA

- The dehalogenase protein catalyses the dehalogenating reaction from 1,2-DCA to ethene.

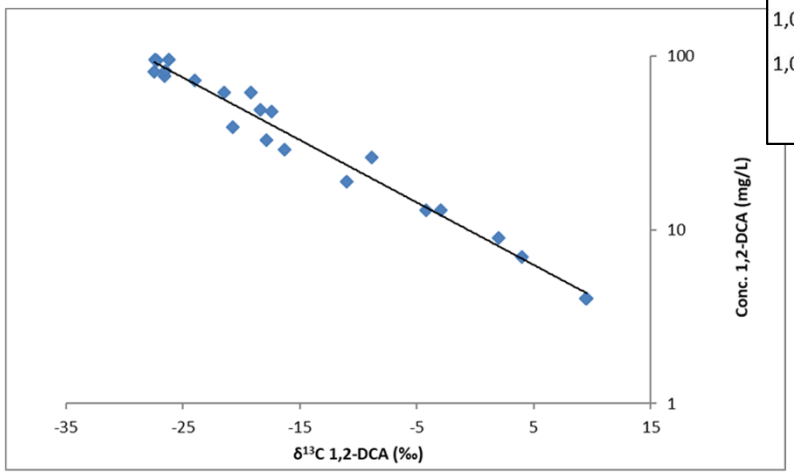


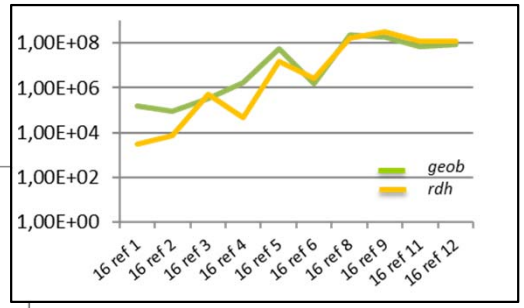
- The dehalogenating reaction from 1,2-DCA to ethene is slightly faster on 1,2-DCA molecules containing the lightest isotope of carbon atoms.



- Therefore, in the case of biodegradation, the isotopic $^{12}C/^{13}C$ ratio of the remaining 1,2-DCA shifts to more enriched values in the heavy isotope (^{13}C).

Estimation of the dehalogenation rate of 1,2-DCA to ethene from the comparison between *in vitro* kinetics and isotopic values of the residual contaminant





ε (enrichment factor) calculated by the Rayleigh equation is comprised between -7 ‰ and -8 ‰ (as it results from the equation corresponding to the line in figure). Form the literature enrichment values ranged from -4 ‰ to -30 ‰.

This shows the isotopic data from samples taken during an *in vitro* biodegradation experiment of 1,2-DCA in the presence of lactate.

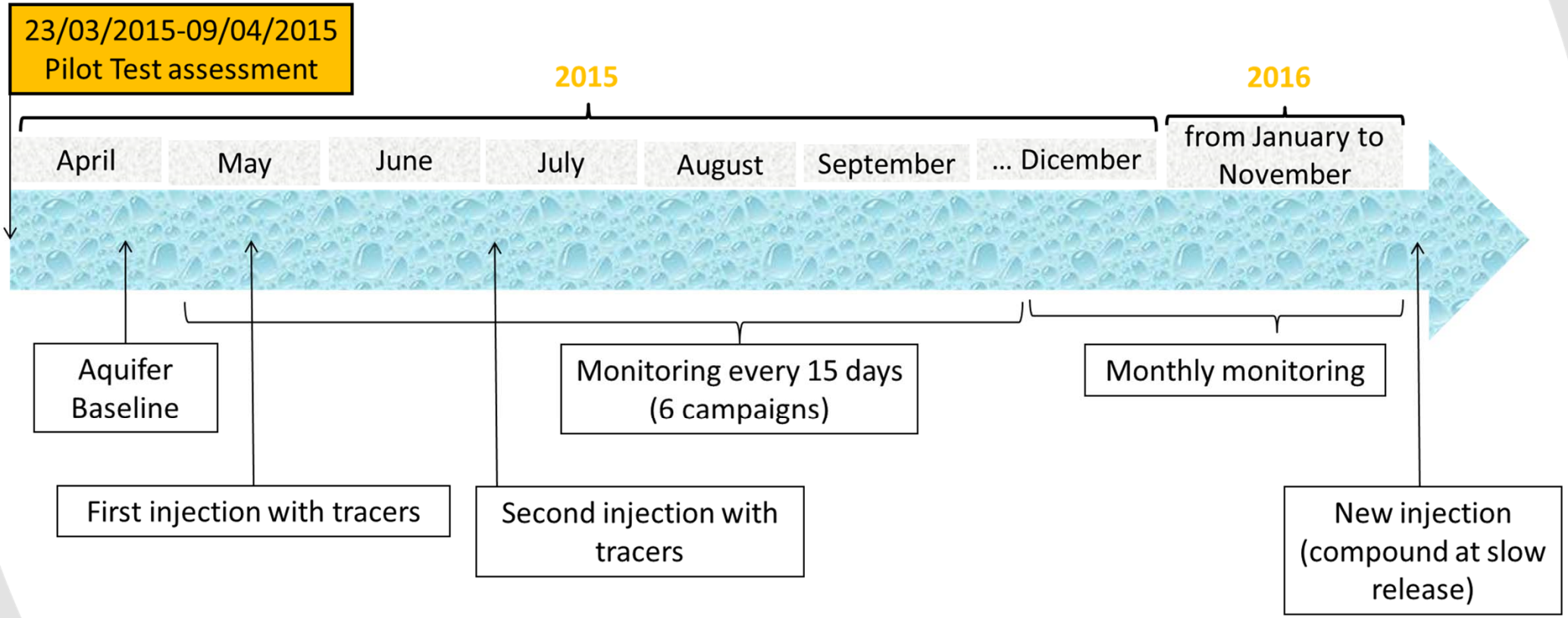
Pilot test (1/4)

A **field pilot test**, based on the injection of lactate + salts (in the formula and concentration to reproduce the medium BTZ used in laboratory), started in **May 2015.**

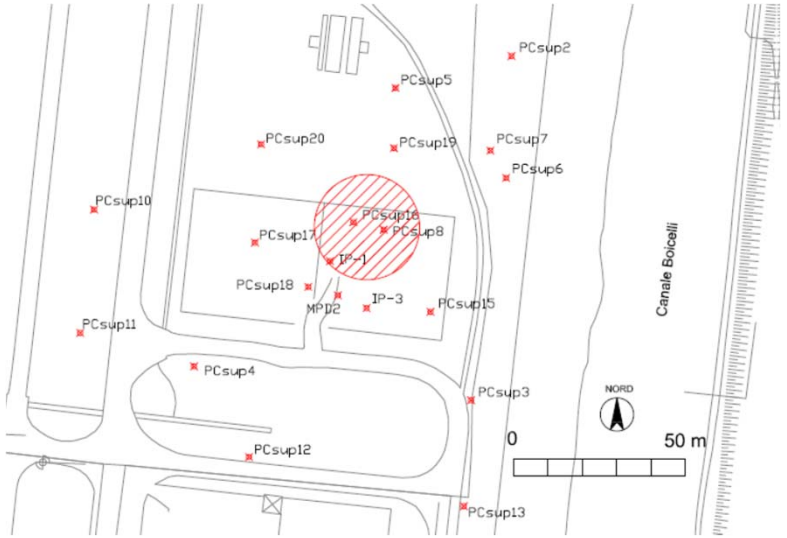
Objectives

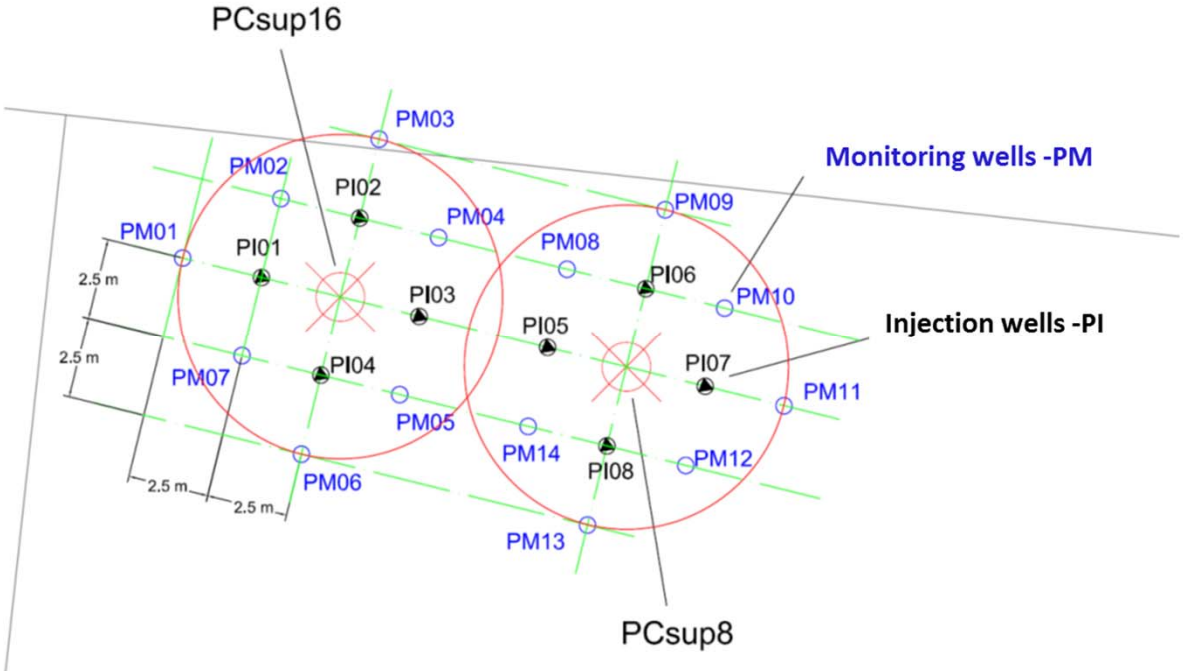
- Field evaluation of the mechanism of natural attenuation (di-haloelimination) highlighted in the laboratory;
- field evaluation of the efficacy of the amendment (lactate) selected in laboratory;
- test and engineering of the injection mode.

Pilot test (2/4)_Time line activities



Pilot test (3/4) _ Area PZPEC023

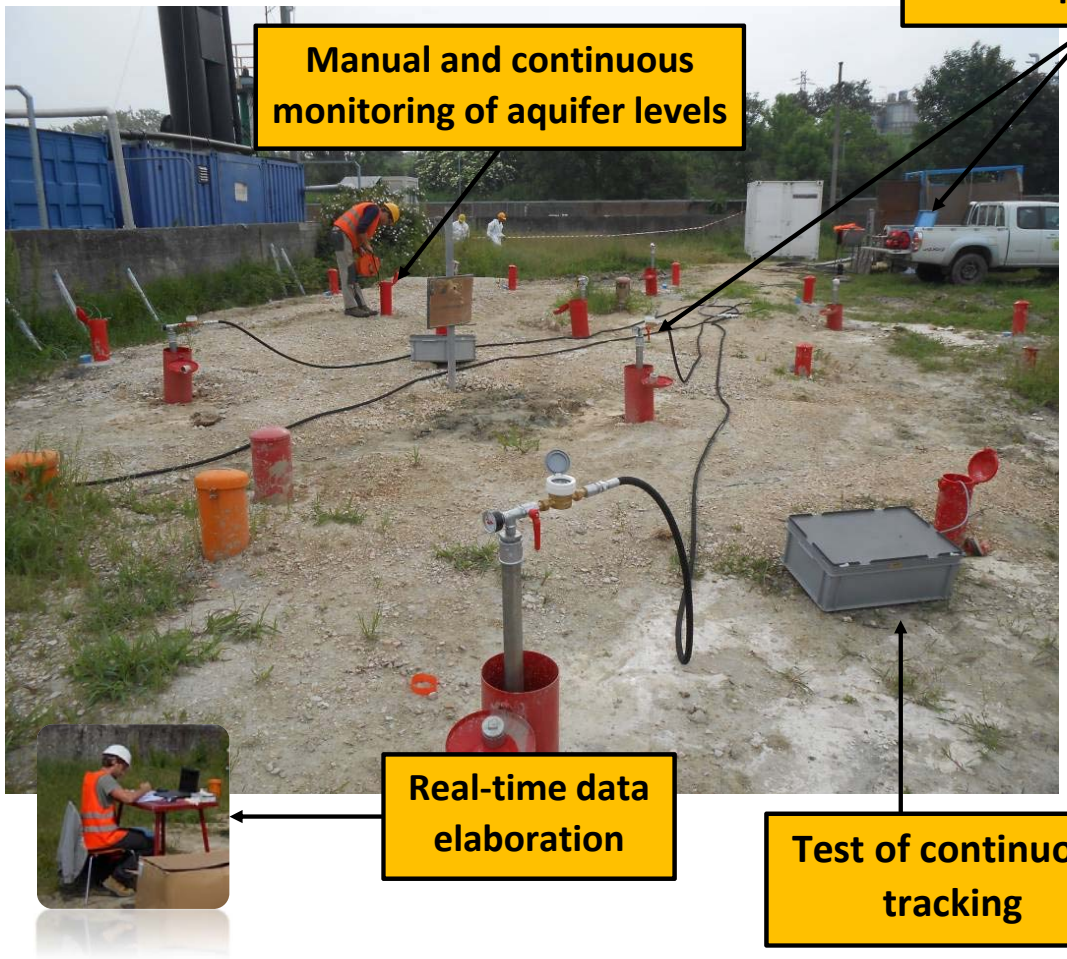




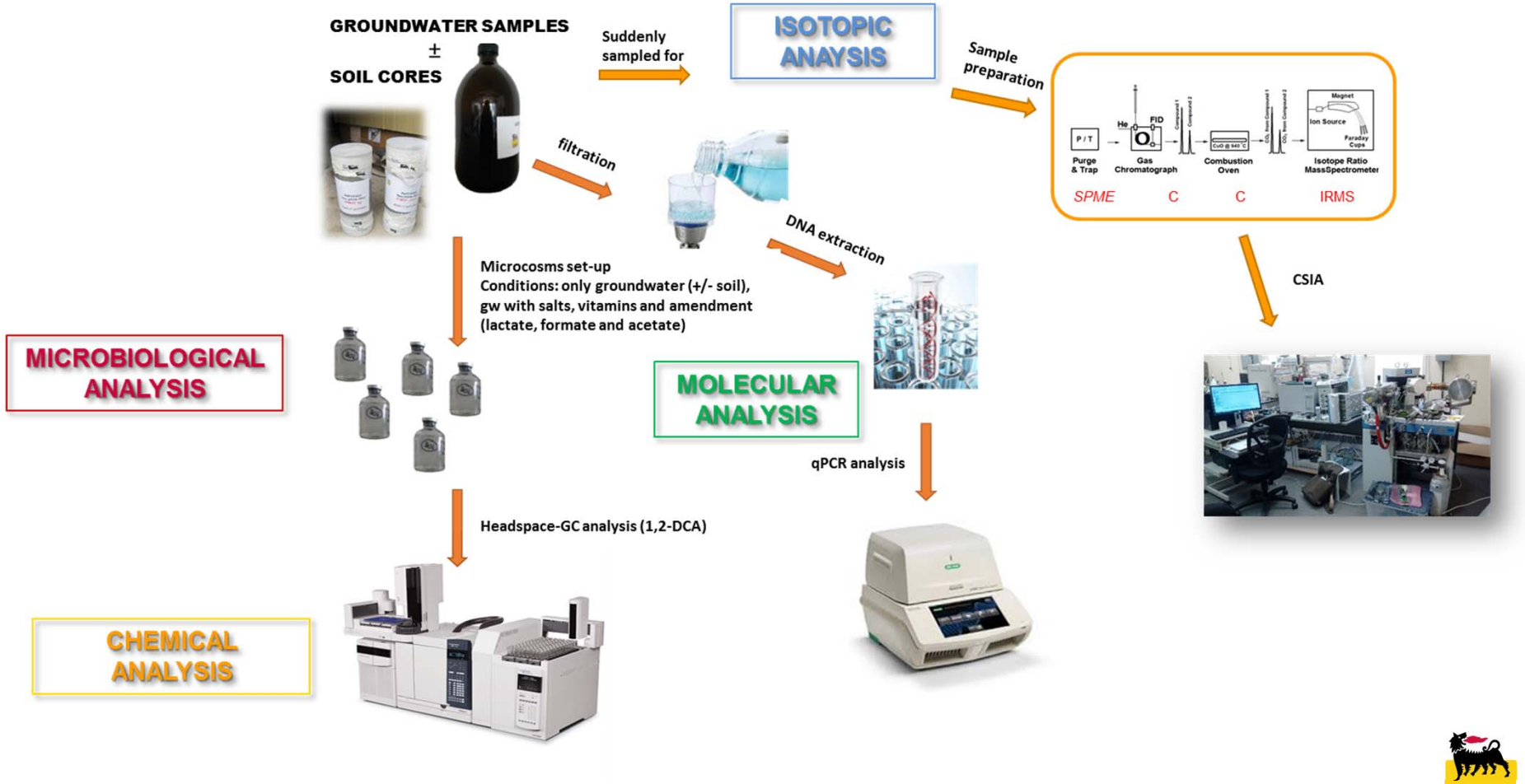
- 8 injection wells (PI)
- 14 monitoring wells (PM)

The depth affected by the field test correspond to the upper confined aquifer (about 8-11 m from ground level).

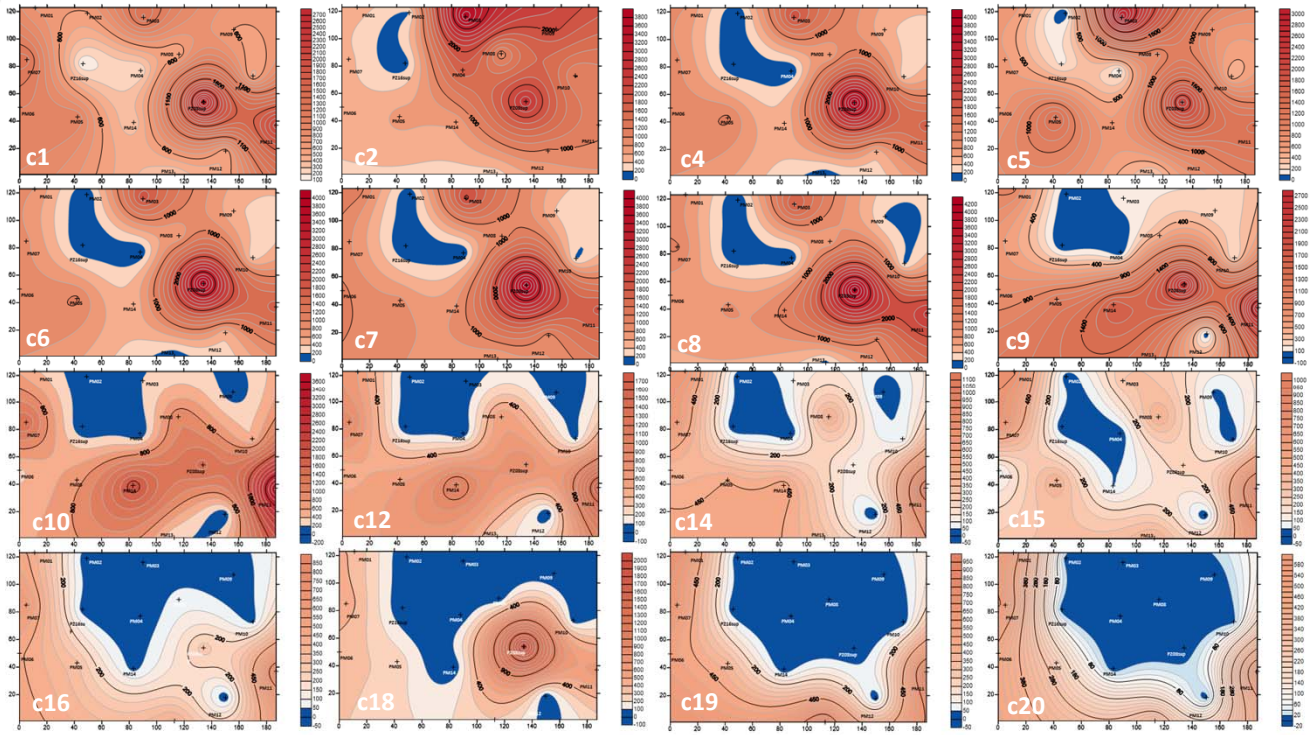
Pilot test (4/4)_Injection: field operations



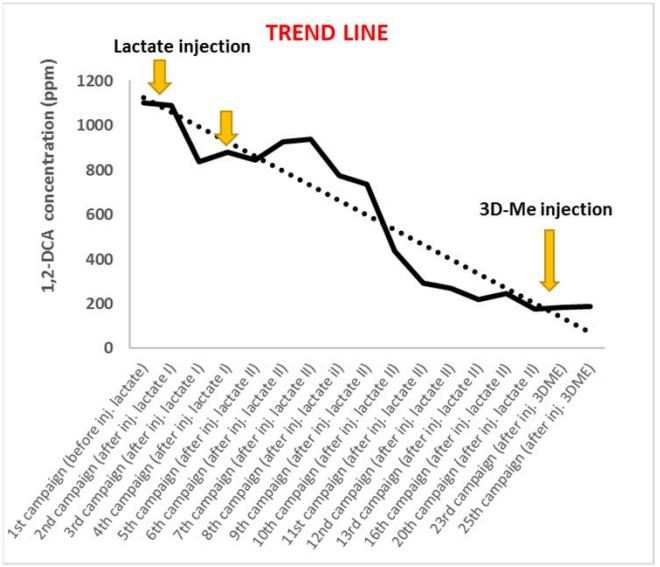
Monitoring of pilot test _ Experimental plan



Field results (1/4)_Chemical analysis: 1,2-dichloroethane monitoring



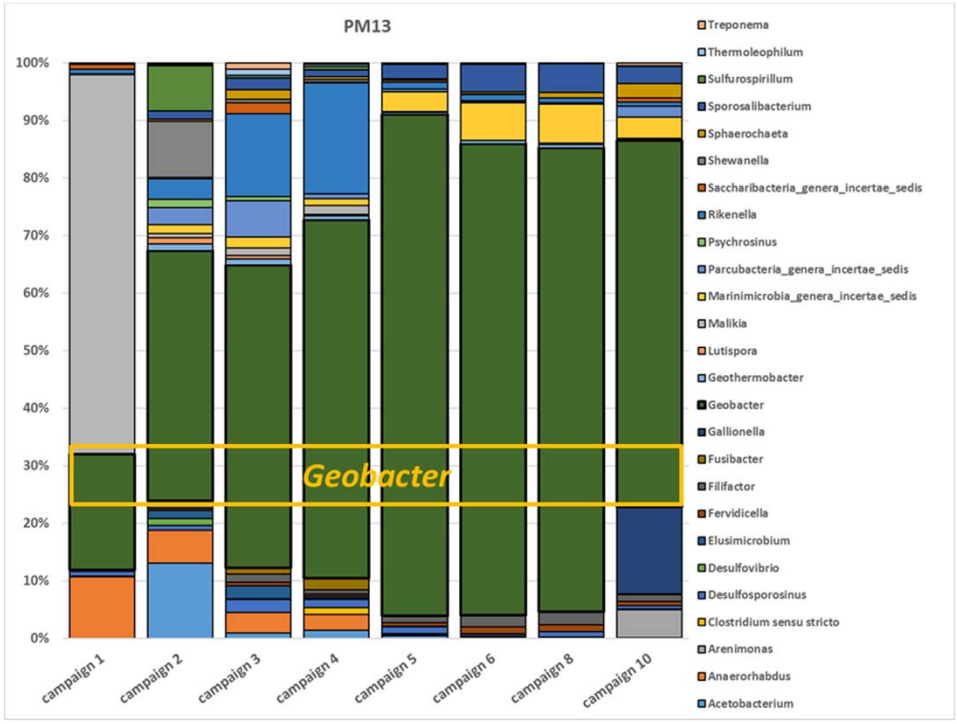
No CVM increase



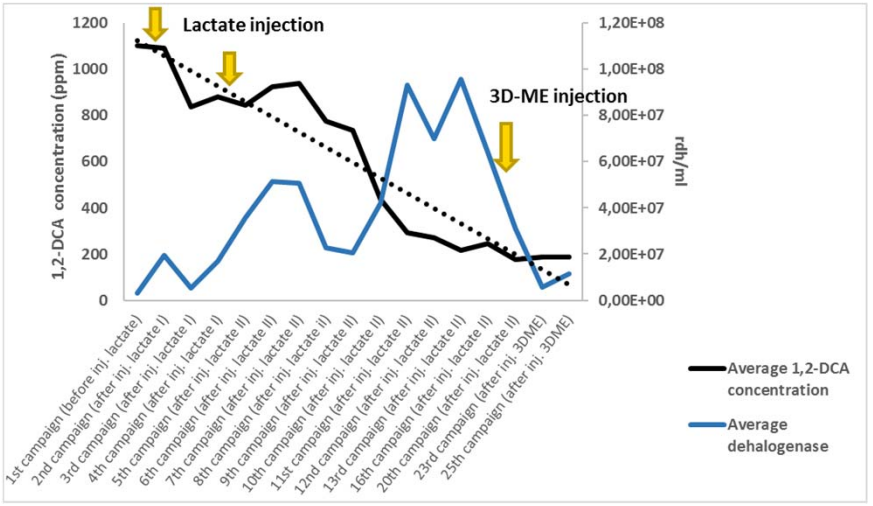
Levels of 1,2-DCA (ppm) in the area of the pilot test during the monitoring campaigns c1,2,4,5,6,7,8,9,10,12,14,15,16,18,19,20 The indicated points corresponds to the 16 monitored piezometers (14 PM + PZsup8 and PZsup16).
Legend: lower values of DCA in blue (<100 ppm), higher values in red (>2000 ppm). The values are normalized among each other.

Field results (2/4)_Microbiological and molecular monitoring during the pilot test

Changes in microbial communities during the pilot test



Trends in 1,2-DCA concentration and quantity of dehalogenation during the pilot test



Degradation and rdhA levels show a specular trend during the pilot test. The increase of rdhA levels **(black line)** are related to the decrease in 1,2-DCA concentration **(blu line)**.

The peak of the dehalogenase concentration **(black line)** is observed around 9-11 months after the 2nd injection (XV - XVII campaign).

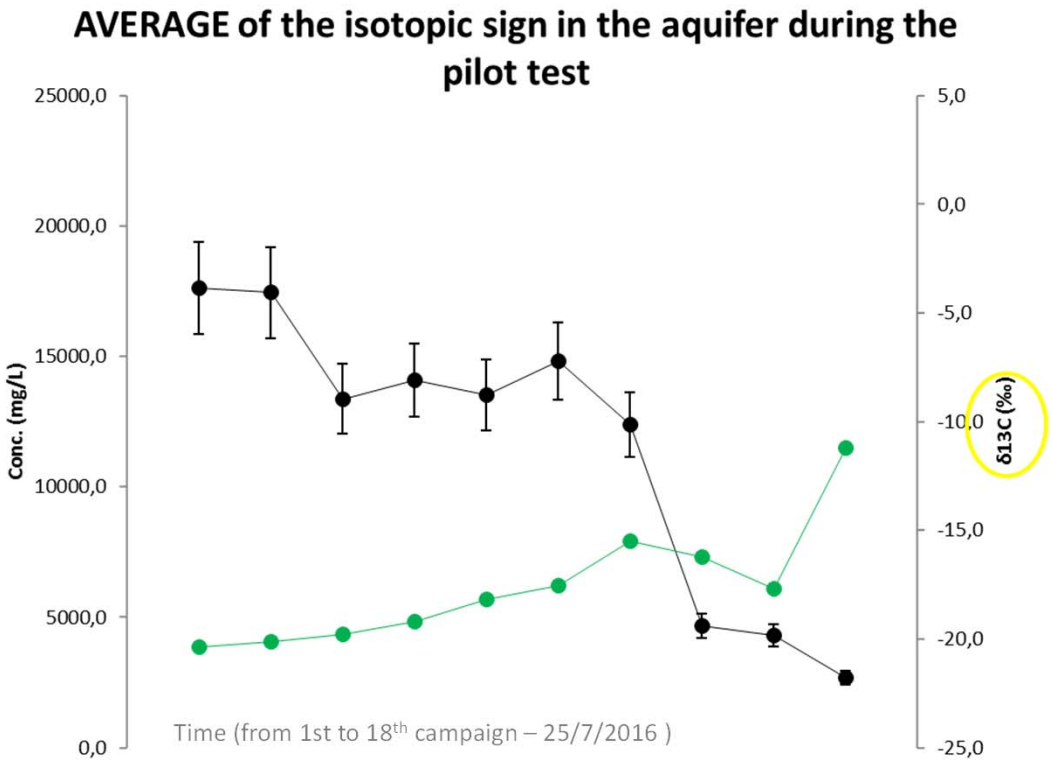
Field results (3/4)_ Changes in the isotopic sign during the pilot test

$$\delta^{13}C = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{Campione} - \left(\frac{^{13}C}{^{12}C}\right)_{std}}{\left(\frac{^{13}C}{^{12}C}\right)_{std}} \times 1000$$

Rayleigh equation

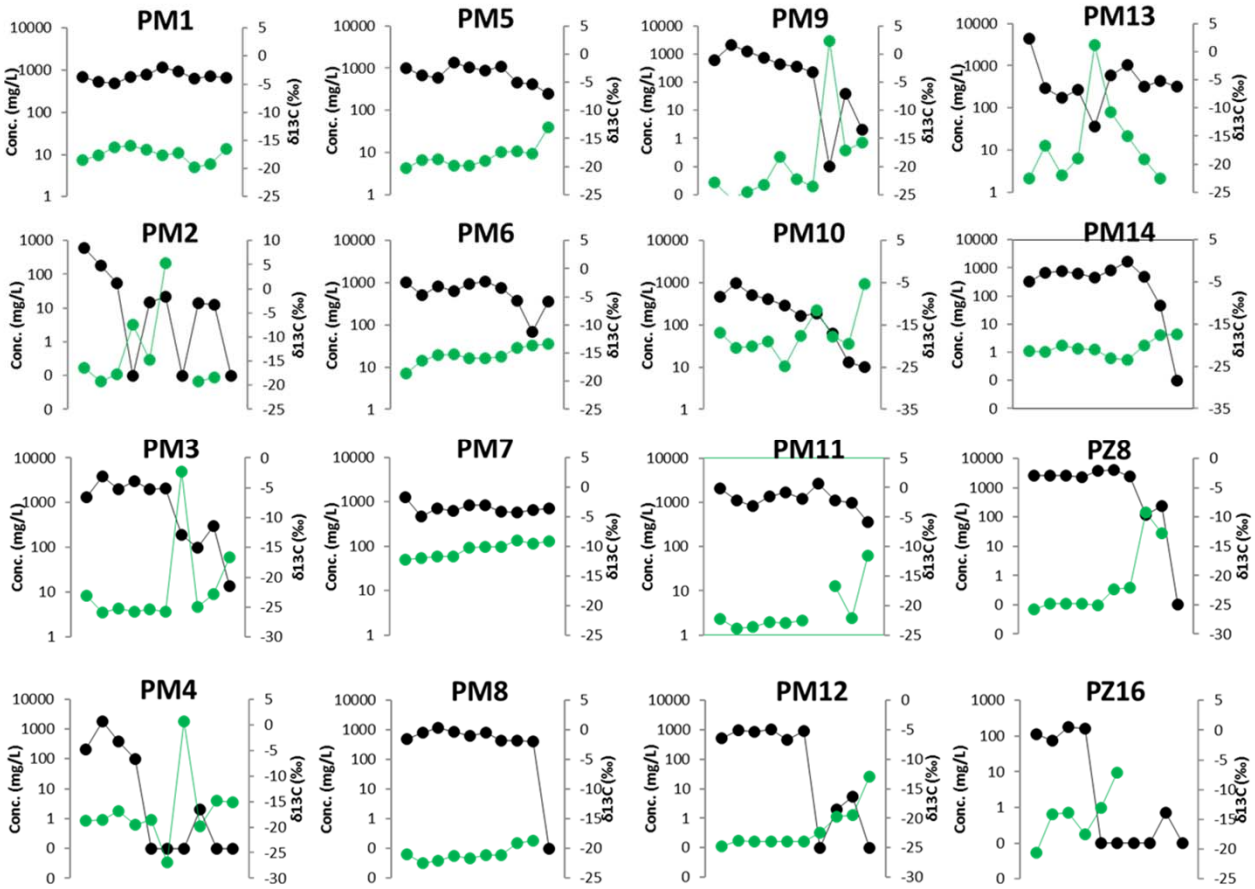
1,2-DCA concentration

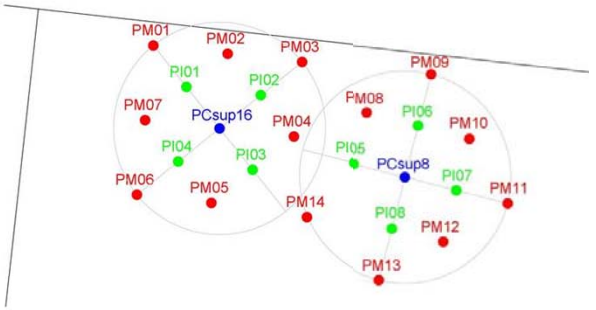
Isotopic sign

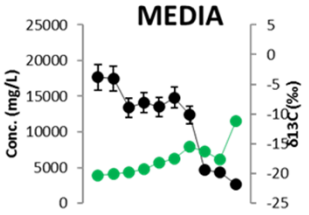


The isotopic $^{12}C/^{13}C$ ratio of the remaining 1,2-DCA shifts to more enriched values during biodegradation.

Field results (4/4)_ Changes in the isotopic sign during the pilot test







Estimated removal of contaminant: calculation from isotopic analysis and chemical data

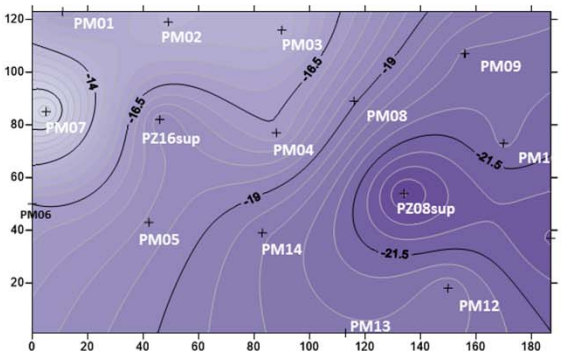
- The 1,2-DCA removal was calculated for each well and each CSIA time point (Rayleigh equation). It was estimated a removal of 92.7 kg contaminant at the 18th campaign (after 14 months from the lactate injection) and of 103.8 kg of contaminant at the 21st campaign.

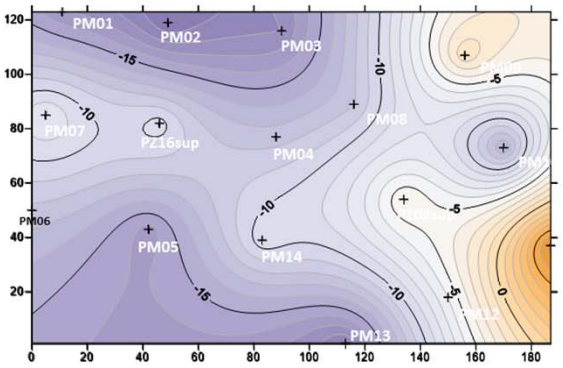
- The 1,2-DCA removal estimated from chemical data (chlorine and ethene accumulation up to the onset of ethene consumption in situ) was found to be slightly higher, but in the same order of magnitude, with a value of 178 kg.

- Enzyme activity was also estimated as about one third of the activity observed in active stable cultures in microcosms.

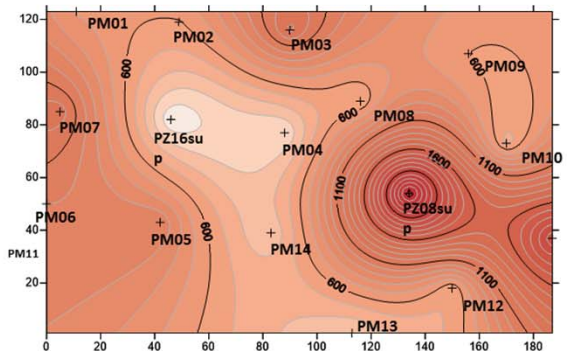
Whole monitoring of the pilot test

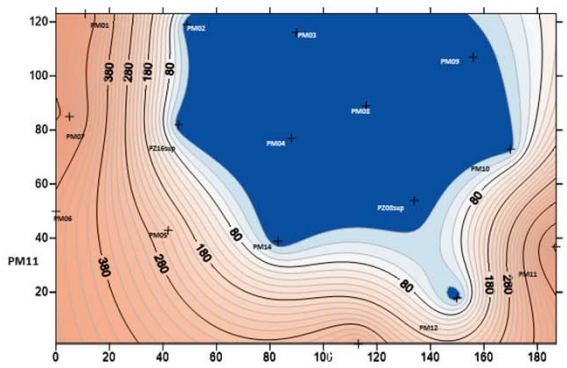
Isotopic analysis

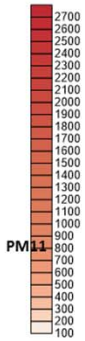




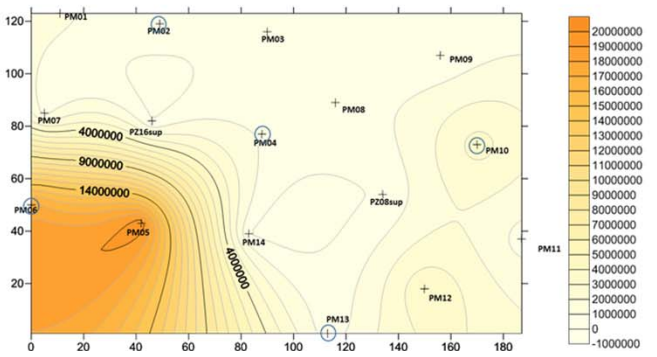
Chemical analysis

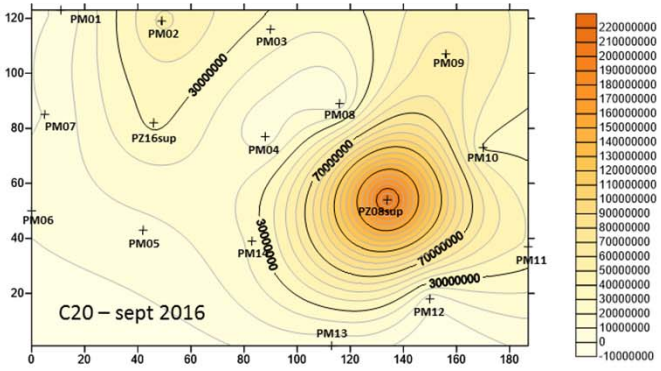






qPCR analysis





Conclusions

- Robust natural attenuation was demonstrated by the presence and activity of biological markers such as dehalogenases (new genes and RNA expression), species-specific markers (qPCR and metagenomics) and 1,2-DCA isotopic analysis in cultures and *in situ*. Data from laboratory cultures, from well water and from stable consortia defined the occurring reactions and optimal amendments to accelerate the reaction.

- The molecular and isotopic data recorded during the monitoring campaigns supported the hypothesis of a strong acceleration of the natural biodegradation phenomena of 1,2-DCA in the upper aquifer during the pilot test.

- The application of the isotopic tool in the environmental field strengthens microbiological analyses by providing an independent data on the biodegradation of chemical species in the environment and reducing uncertainty and risk in bioremediation treatments.

Thanks for your attention

Acknowledgements:
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