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*Characterization by the use of ^{37}Cl , ^{13}C and ^2H -
Compound-Specific Isotope Analysis (CSIA), Biological
Molecular Techniques (BMTs) and Numerical Modeling
of a Site Contaminated by Monochlorobenzene.*

M. Marchesi (Politecnico di Milano, Isotope Tracer Technologies Europe Srl Milano, Italy), I. Pietrini (ENI Group, Milano, Italy), M. Antelmi, L. Alberti (Politecnico di Milano, Milano, Italy), T. Stella, A. Franzetti (Università Statale di Milano-Bicocca, Milano, Italy), D. Antonelli, F. de Ferra, G. Carpani (ENI Group, Milano, Italy), R. Aravena, (University of Waterloo, Waterloo, Canada) and O. Shouakar-Stash (Isotope Tracer Technology Inc, Canada).



OBJECTIVES

1999 ¹³C-CSIA

2006 ³⁷Cl-CSIA

2013 ²H-CSIA

2014

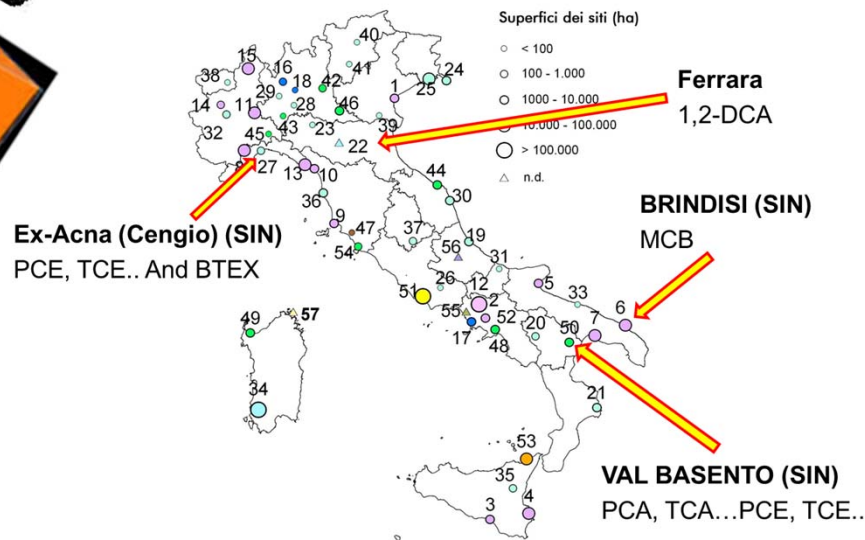
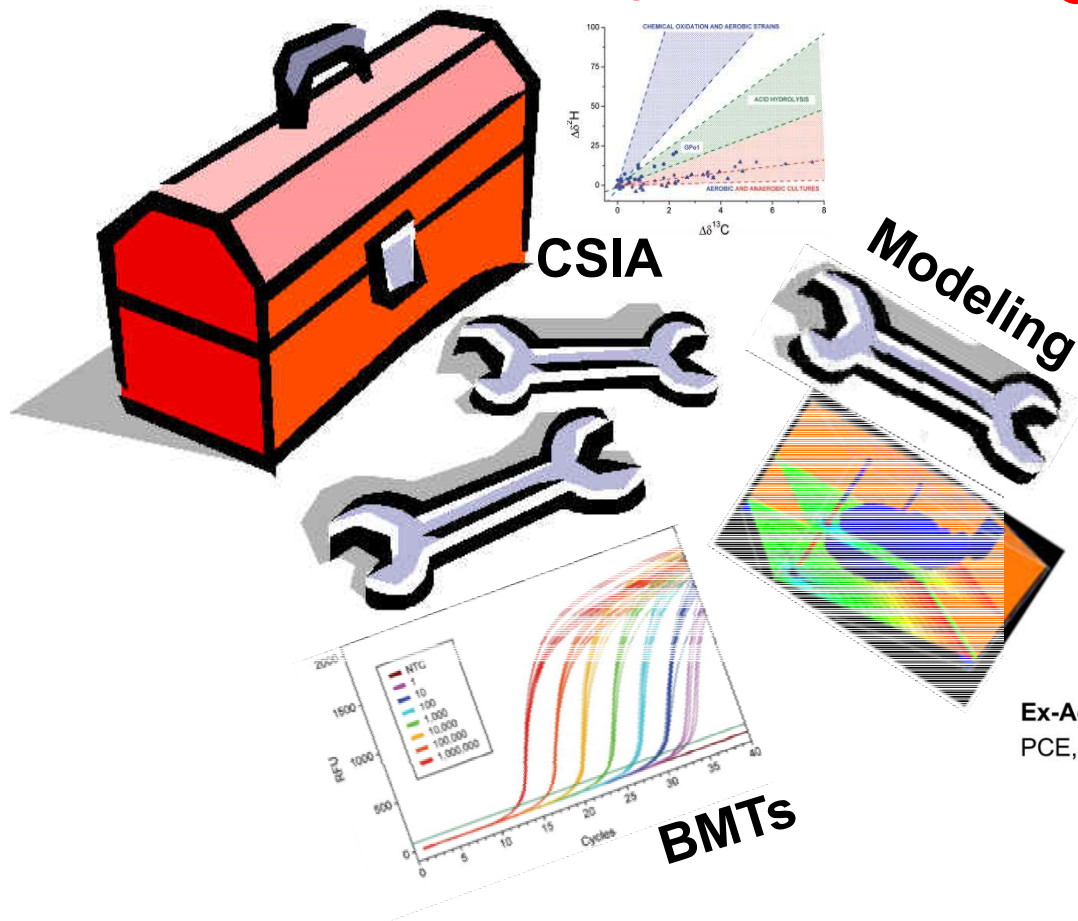


To develop a tool-box

CSIA

MBTs

Modeling





TEAM



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D. I. C. A.

**DIPARTIMENTO DI INGEGNERIA
CIVILE E AMBIENTALE**

Luca Alberti, Massimo Marchesi



IST. DONEGANI - TEAMB:

Francesca de Ferra
Giovanna Carpani
Ilaria Pietrini

GEOLAB (E&P)

Corrado Barbieri
Roberto Galimberti

SYNDIAL

Danilo Antonellini
Luciano Zaninetta



Massimo.marchesi@polimi.it



Andrea Franzetti, Tatiana Stella



Prof. Ramon Aravena

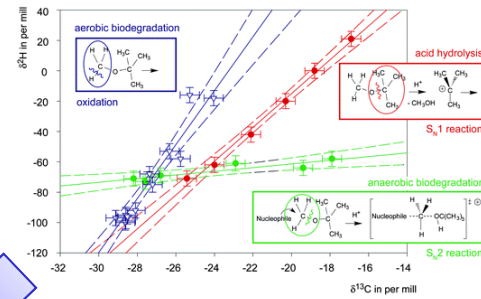
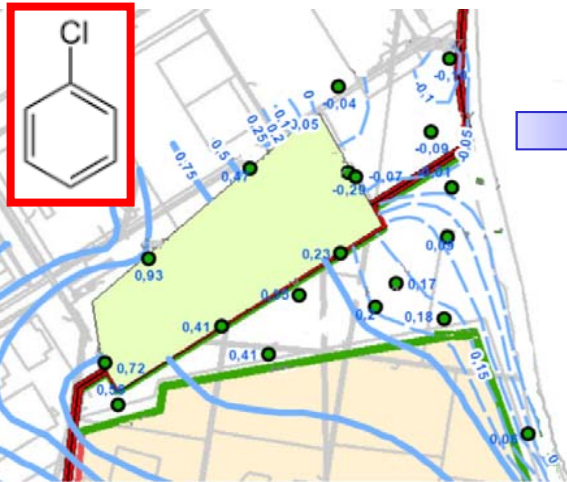
Prof. Orfan Shouakar-Stash



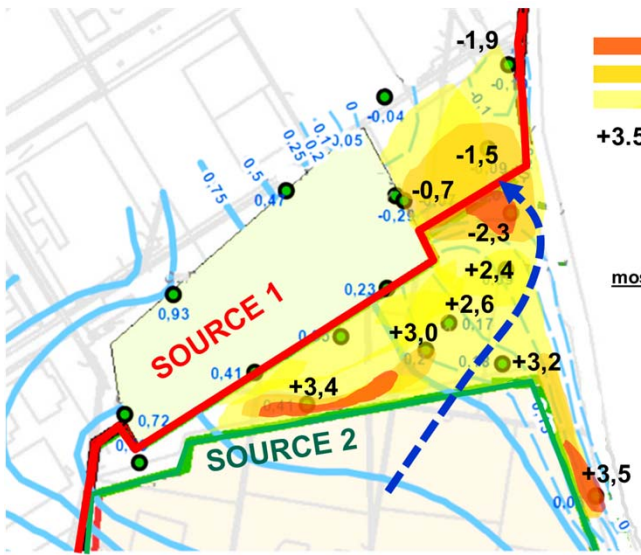
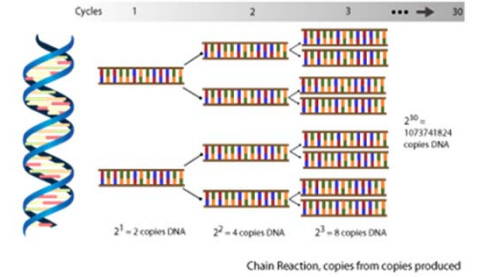
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APPROACH

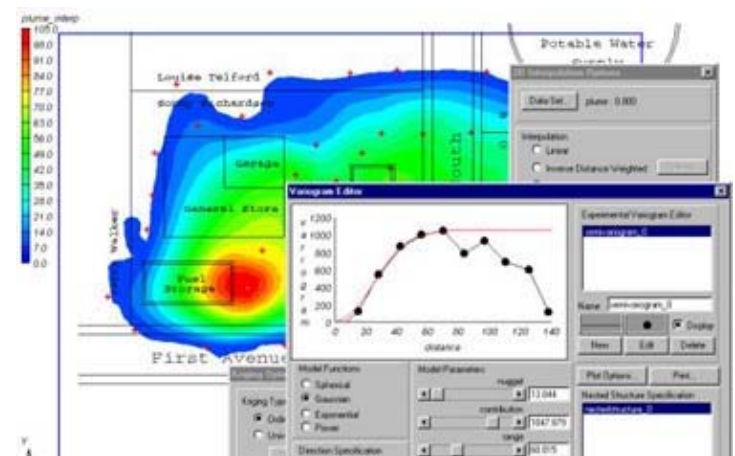


PCR amplification



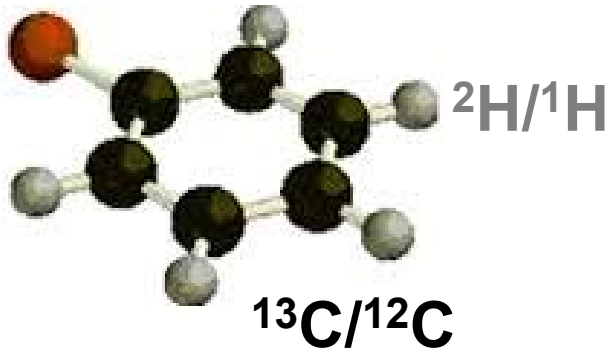
> 1000 ug/L
 100 – 1000 ug/L
 10 – 100 ug/L
 +3.5 $\delta^{37}\text{Cl}$ ‰

mostly anaerobic cond.
 High CH_4 and Fe





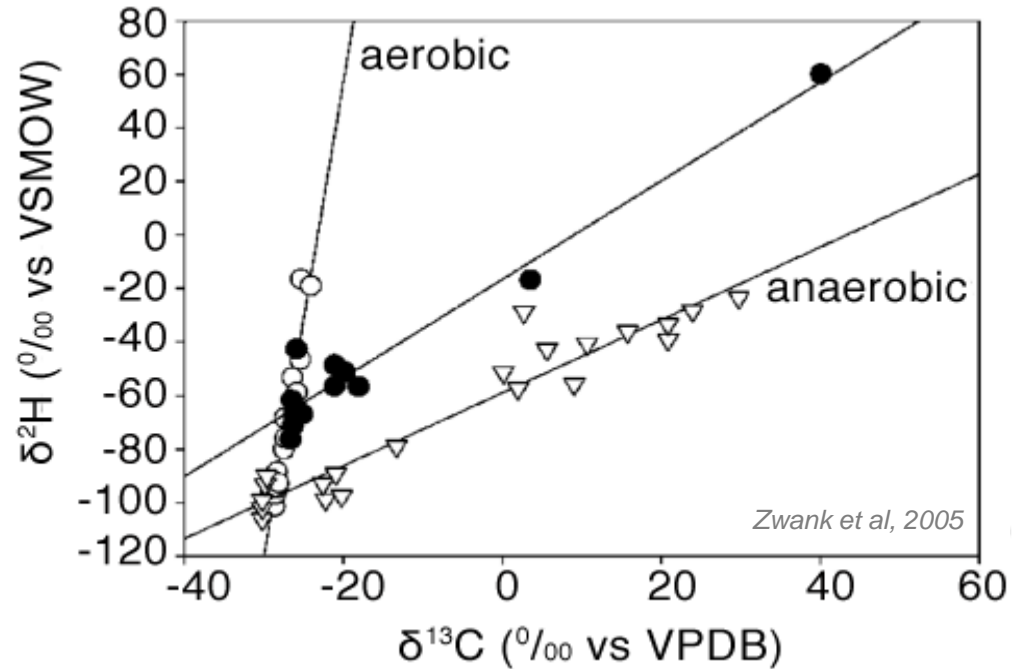
$^{37}\text{Cl}/^{35}\text{Cl}$



AEROBIC $\text{C}_6\text{H}_5\text{Cl} (\text{O}_2) \rightarrow \text{CO}_2$
no ^{13}C enrichment

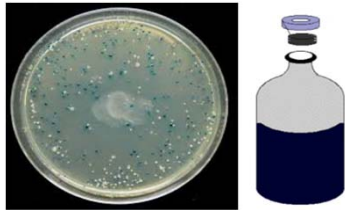
DEHALOGENATION $\text{C}_6\text{H}_5\text{Cl} \rightarrow \text{C}_6\text{H}_6$ (benzene)
 ^{13}C enrichment approx. -5 ‰

PROCESSES DISTINGUISHAL



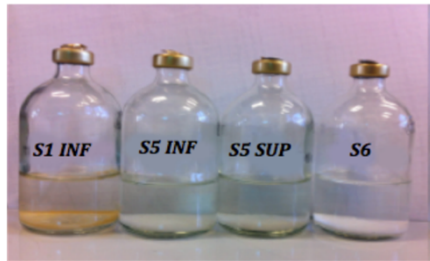


RESULTS (1): Aerobic biodegradation



pure culture

Sphingomonas



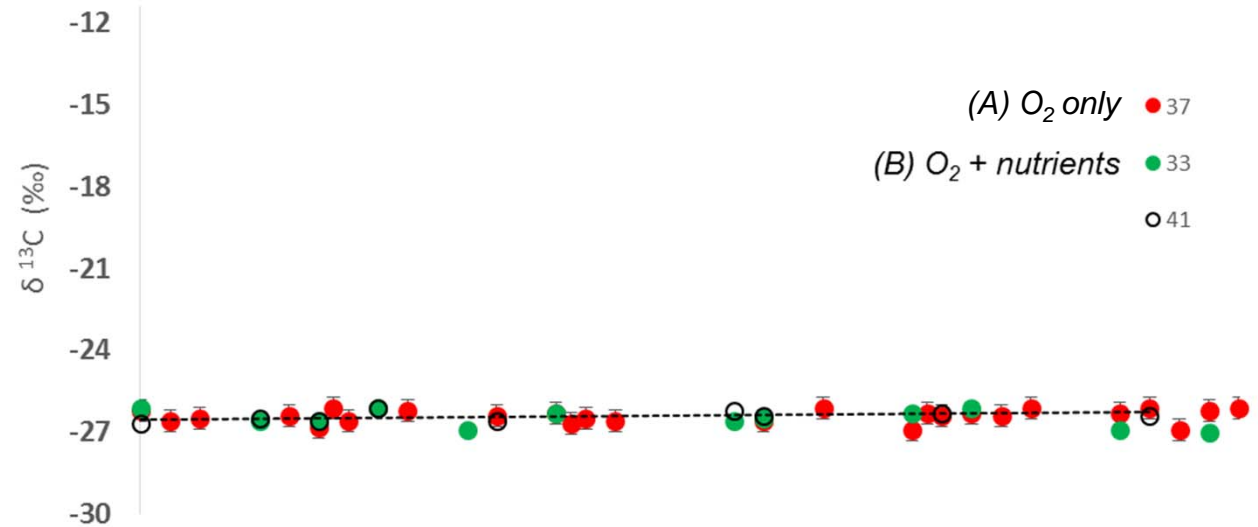
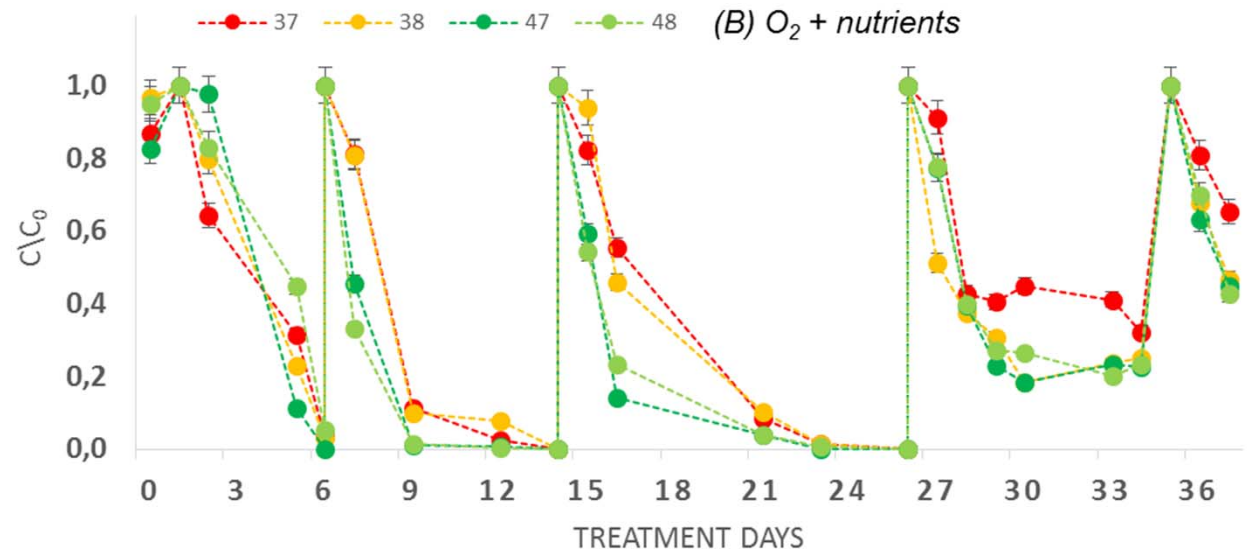
groundwater

(A) O₂ only / (B) O₂ + nutrients



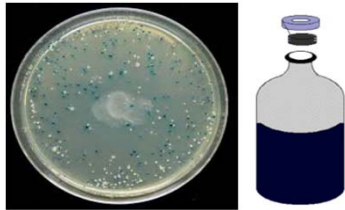
soil slurry

(A) O₂ only / (B) O₂ + nutrients



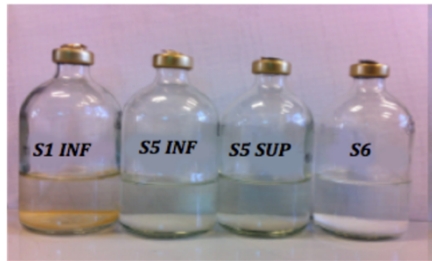


RESULTS (2): Anaerobic biodegradation



pure culture

Dehalococcoides



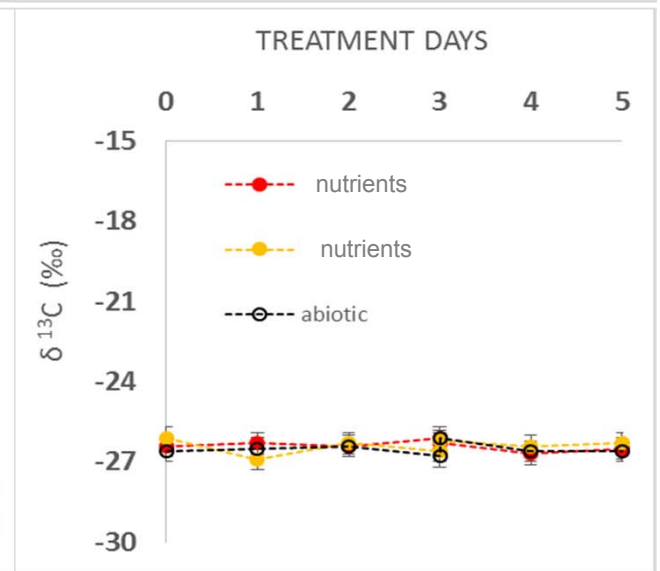
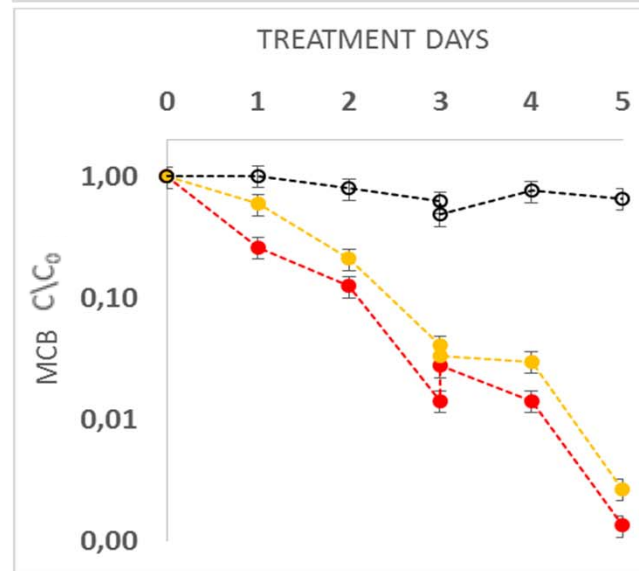
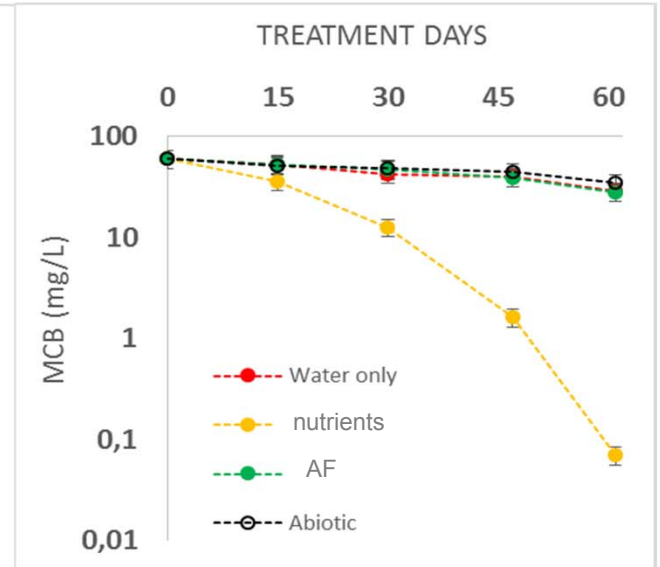
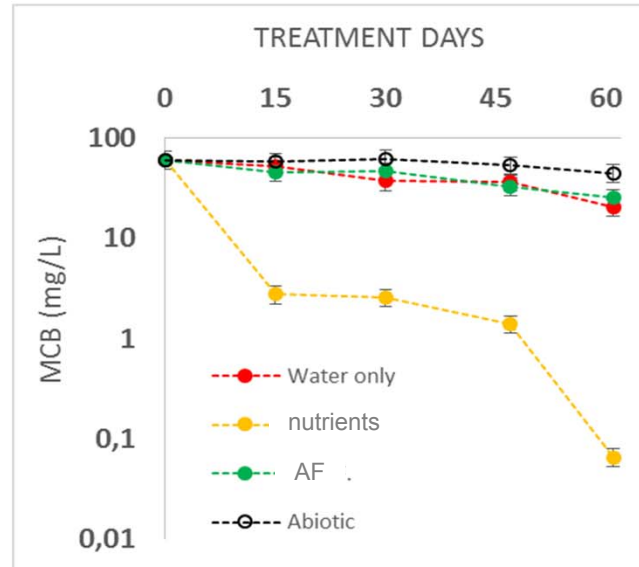
groundwater

(A) anoxic / (B) nutrients / (C) AF



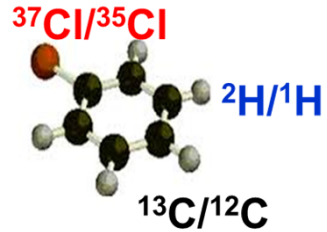
soil slurry

(A) anoxic / (B) nutrients / (C) AF





ANALYTICAL ADVANCEMENTS



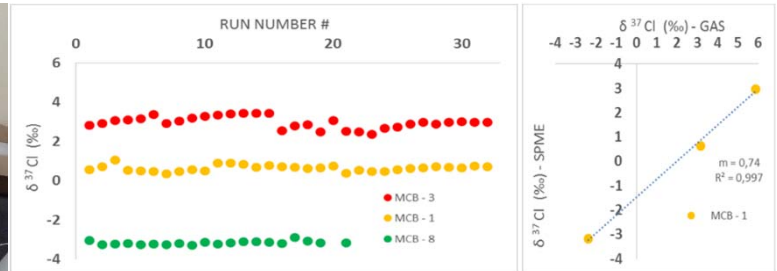
AEROBIC $\text{C}_6\text{H}_5\text{Cl} (\text{O}_2) \rightarrow \text{CO}_2$ no ^{13}C enrichment

ANAEROBIC no ^{13}C enrichment

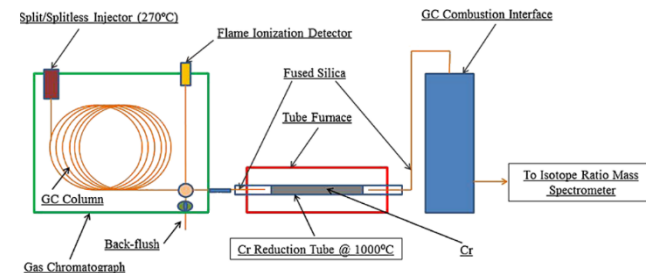
No infos about:

^{37}Cl or ^2H

2) $^{37}\text{Cl}/^{35}\text{Cl}$ – CSIA for MCB at IT² (CANADA)

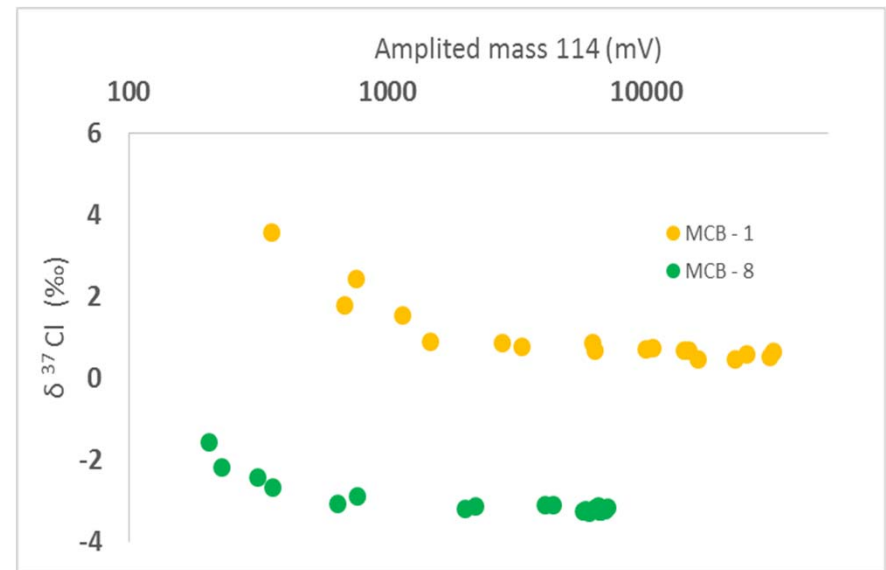
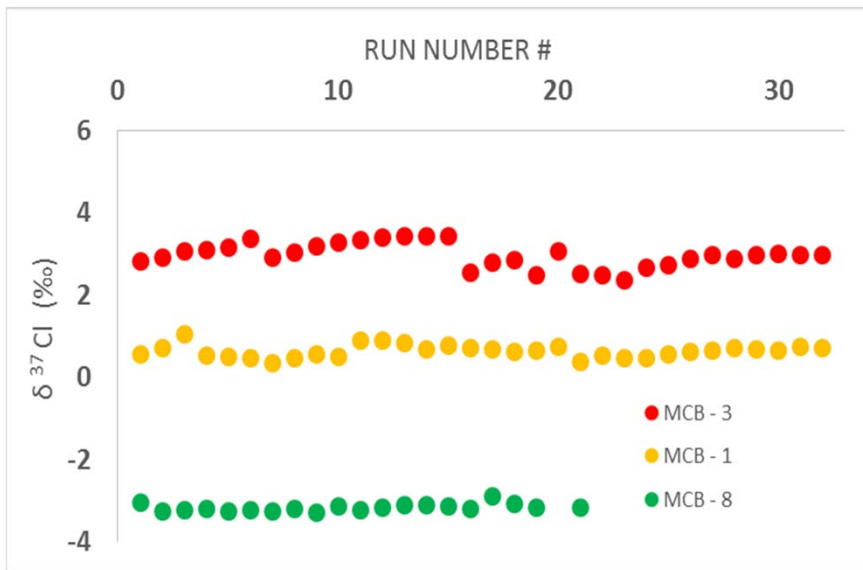
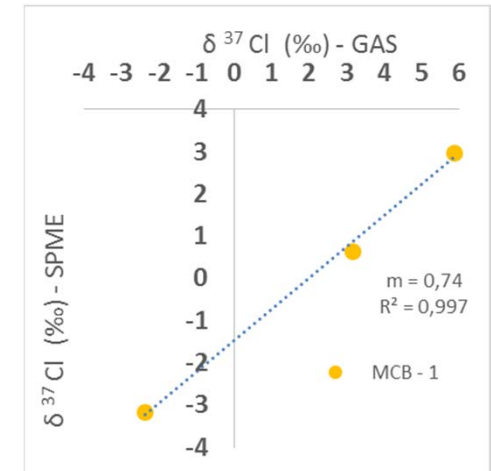
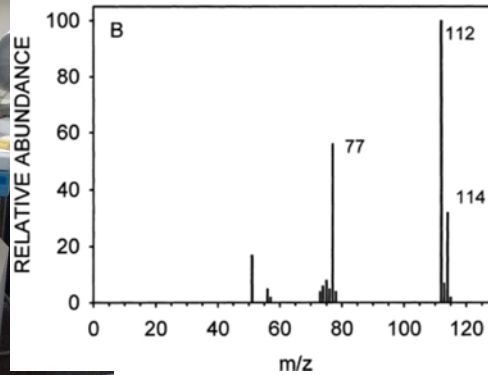


3) A continuous-flow method was developed for ^2H -CSIA; $\delta^2\text{H}$ values were measured with an analytical error of ± 4 ‰ (similar method as for Shouakar-Stash and Drimmie, 2013).





³⁷Cl CSIA method development (Waterloo, Canada)



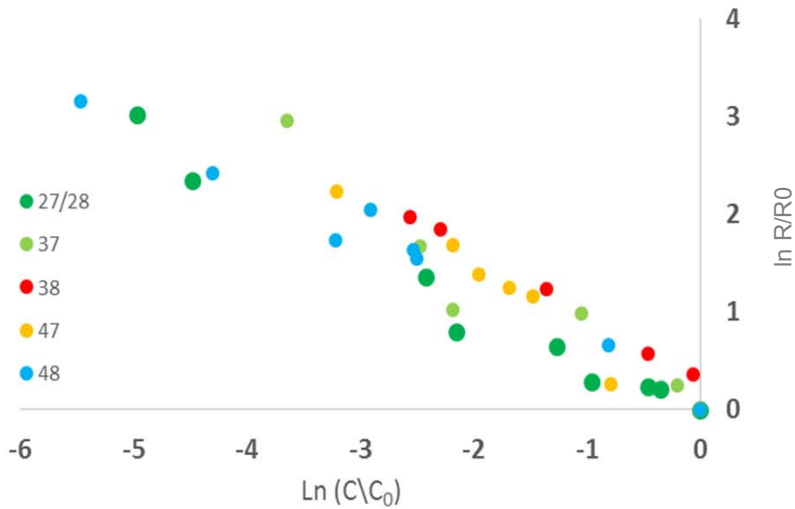
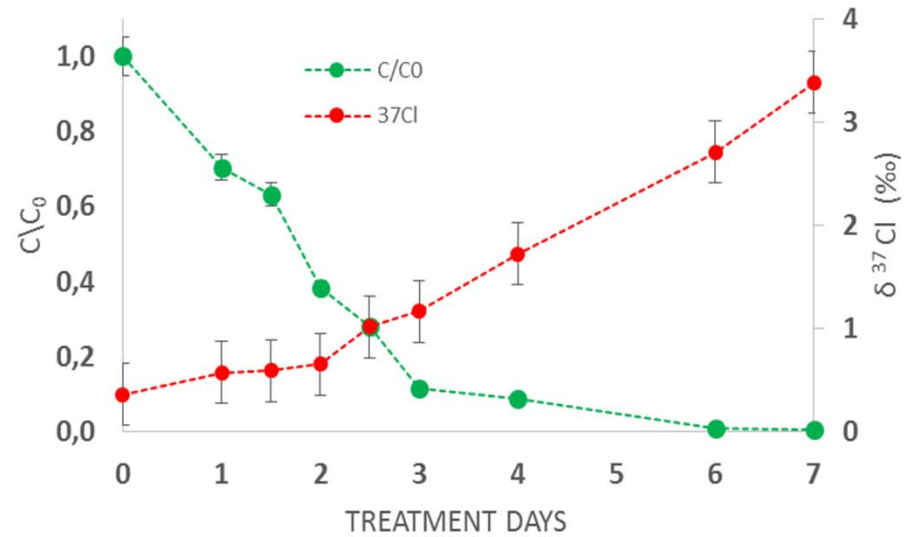


³⁷Cl results for aerobic



soil slurry

(A) O₂ only / (B) O₂ + nutrients



ε in between 0.6 – 0.7 ‰



^{37}Cl and ^{13}C (dual isotope approach) and BMTs

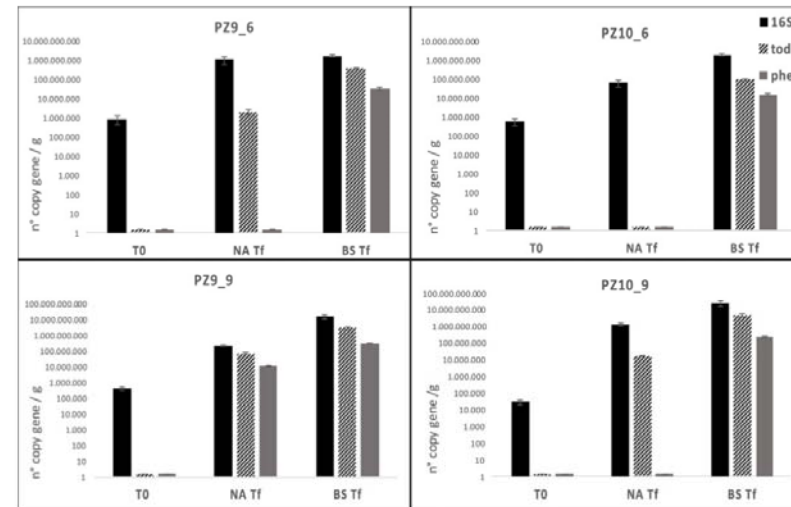
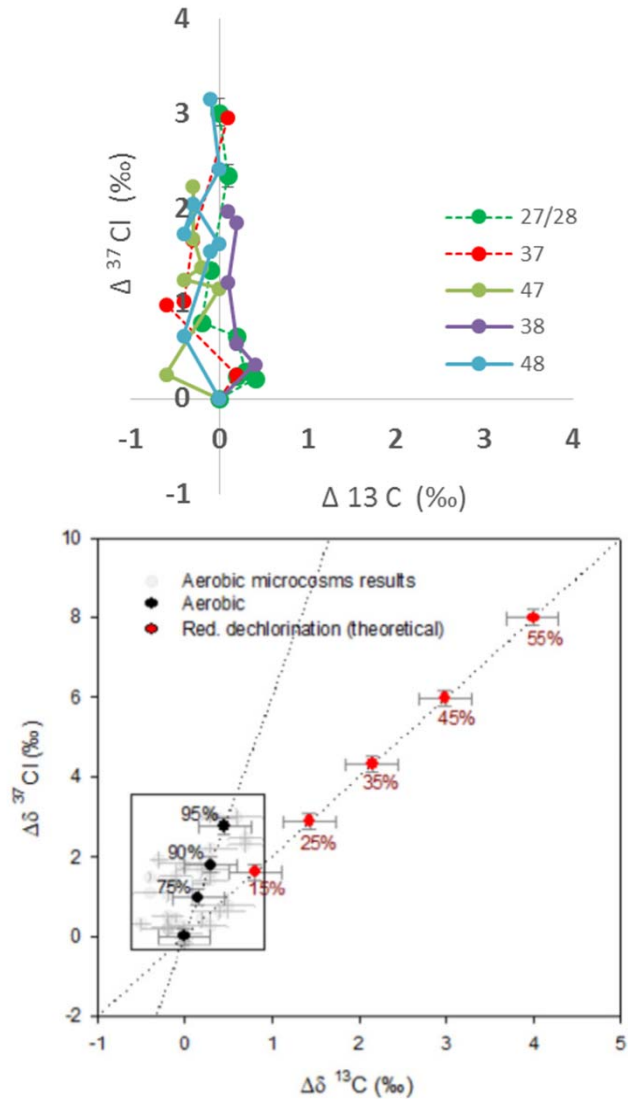
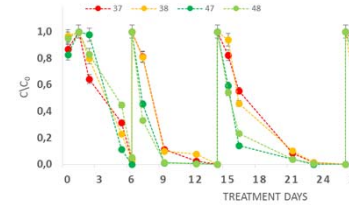


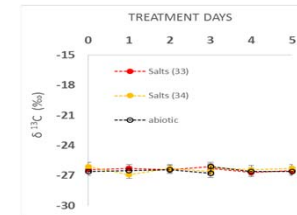
Fig. 6. Copy number of 16S rRNA, toluene dioxygenase (*todC*) and phenol hydrolase (*phe*) genes in natural attenuation (NA) and biostimulation (BS) slurry microcosms set up using groundwater collected from piezometers PZ9 and PZ10 and sediments collected at 6 and 9 m of depth at the beginning (t_0) and at the end of the incubation period (t_f). The number of gene copies below the detection limit (b.d.L) was of $5.47 \cdot 10^2$.



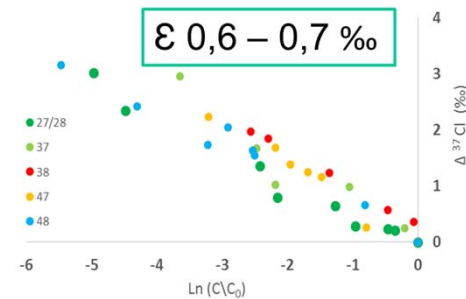
Potential for aerobic biodegradation



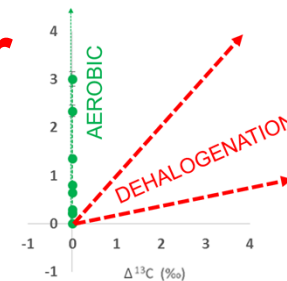
Potential for anaerobic biodegradation
BUT No dehalogenation processes



Potential for using $\delta^{37}\text{Cl}$ to evaluate natural attenuation

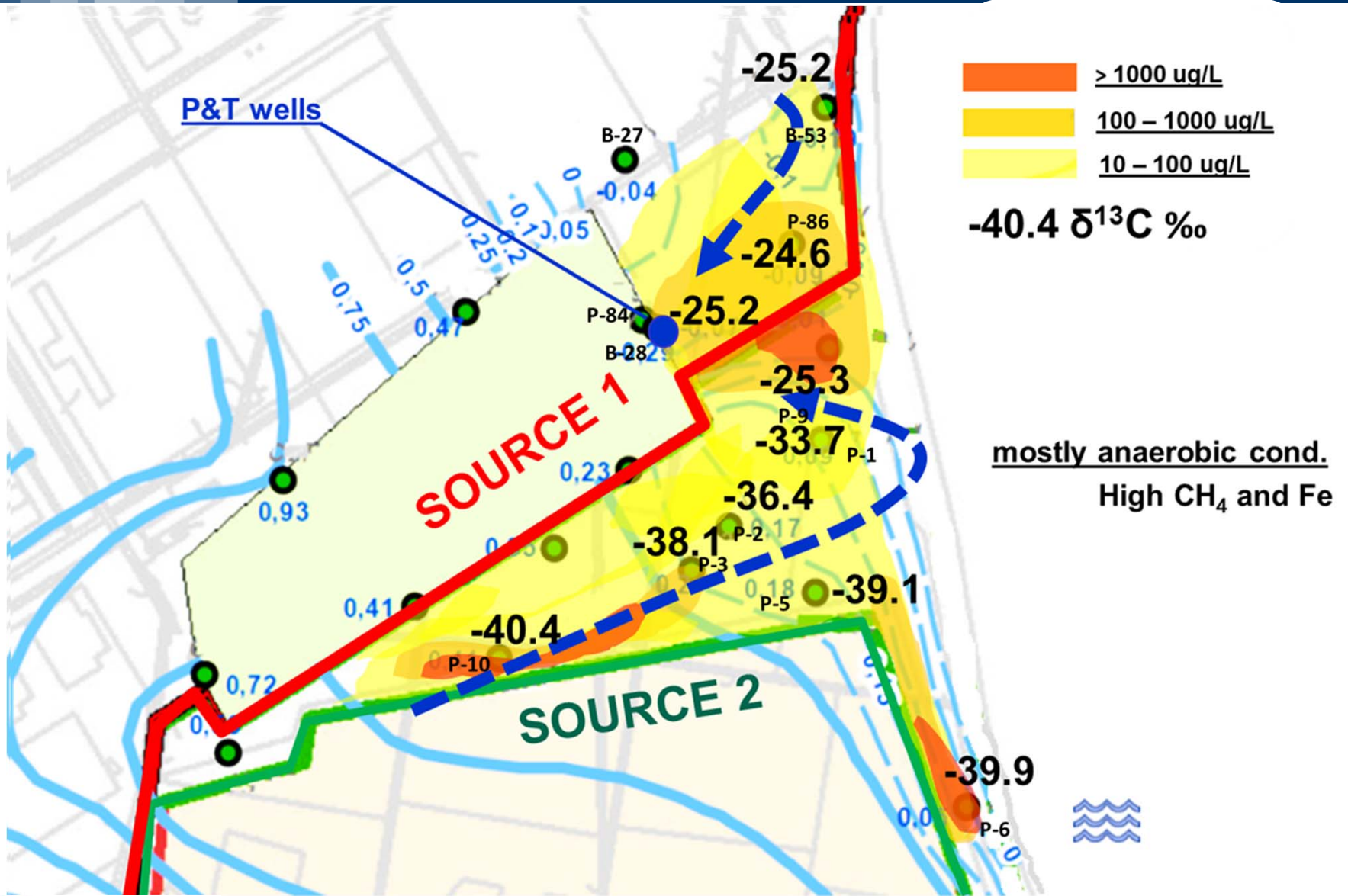


Potential for using $\delta^{37}\text{Cl}/\delta^{13}\text{C}$ for processes distinguishal



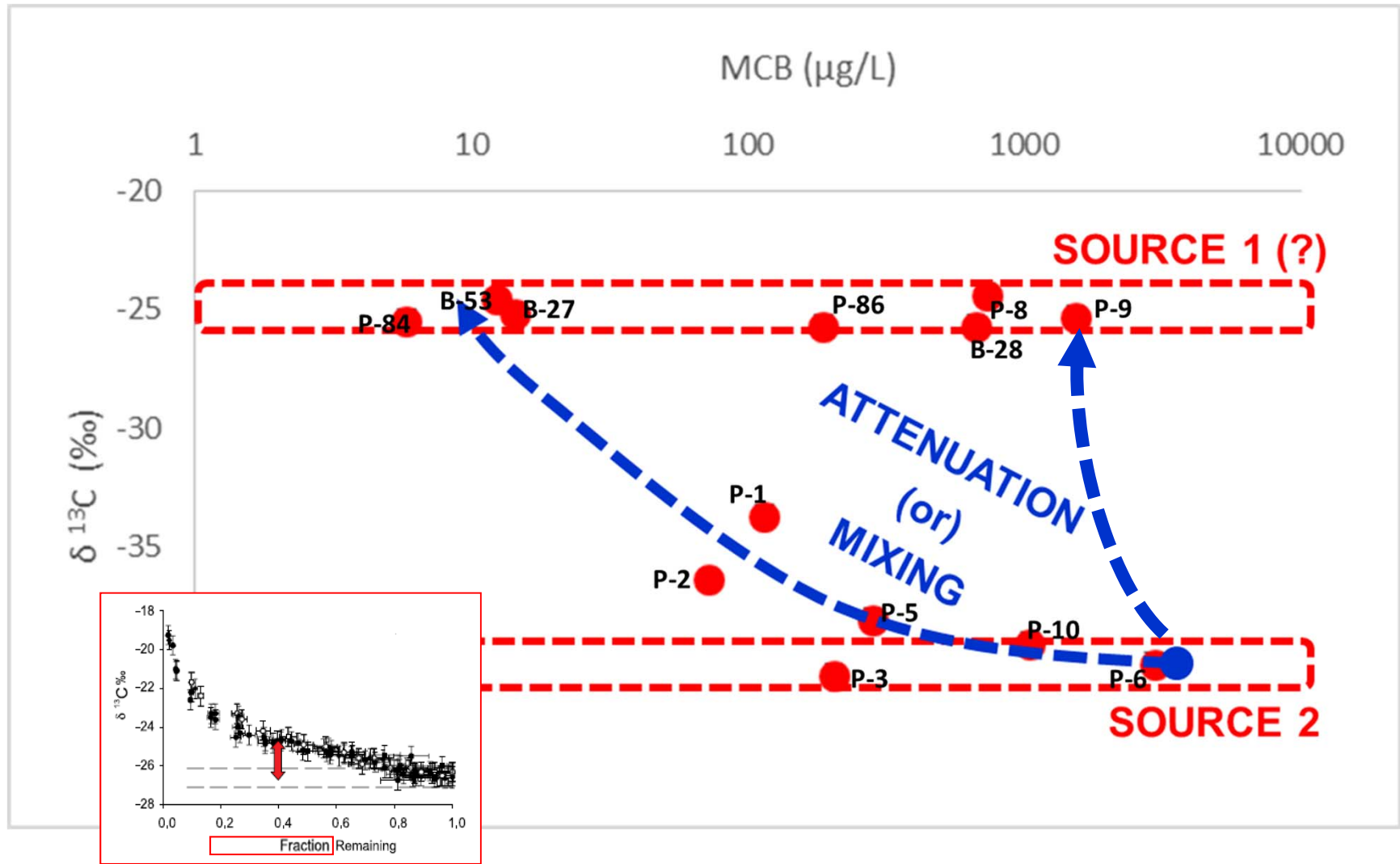


FIELD RESULTS



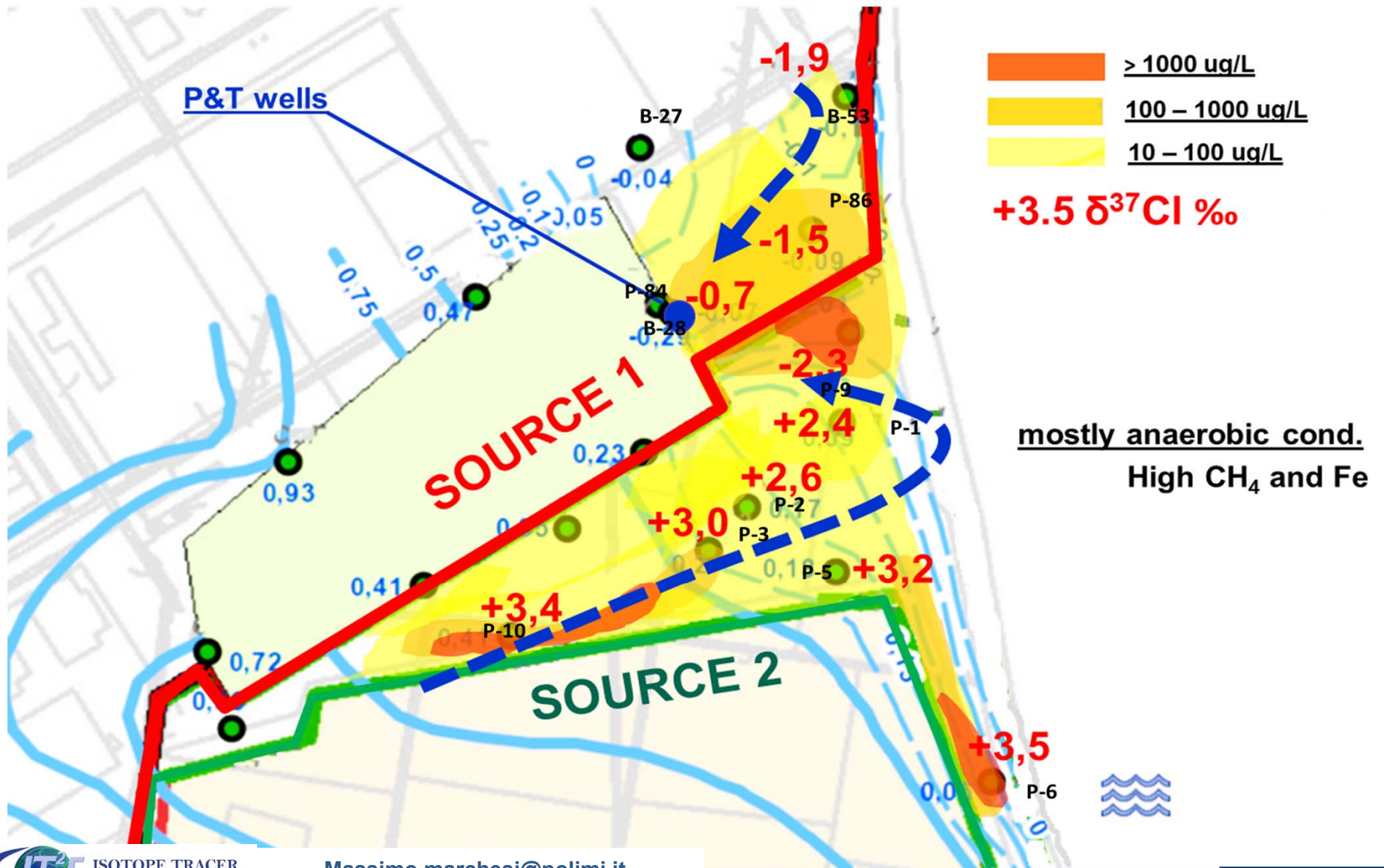


^{13}C -CSIA results



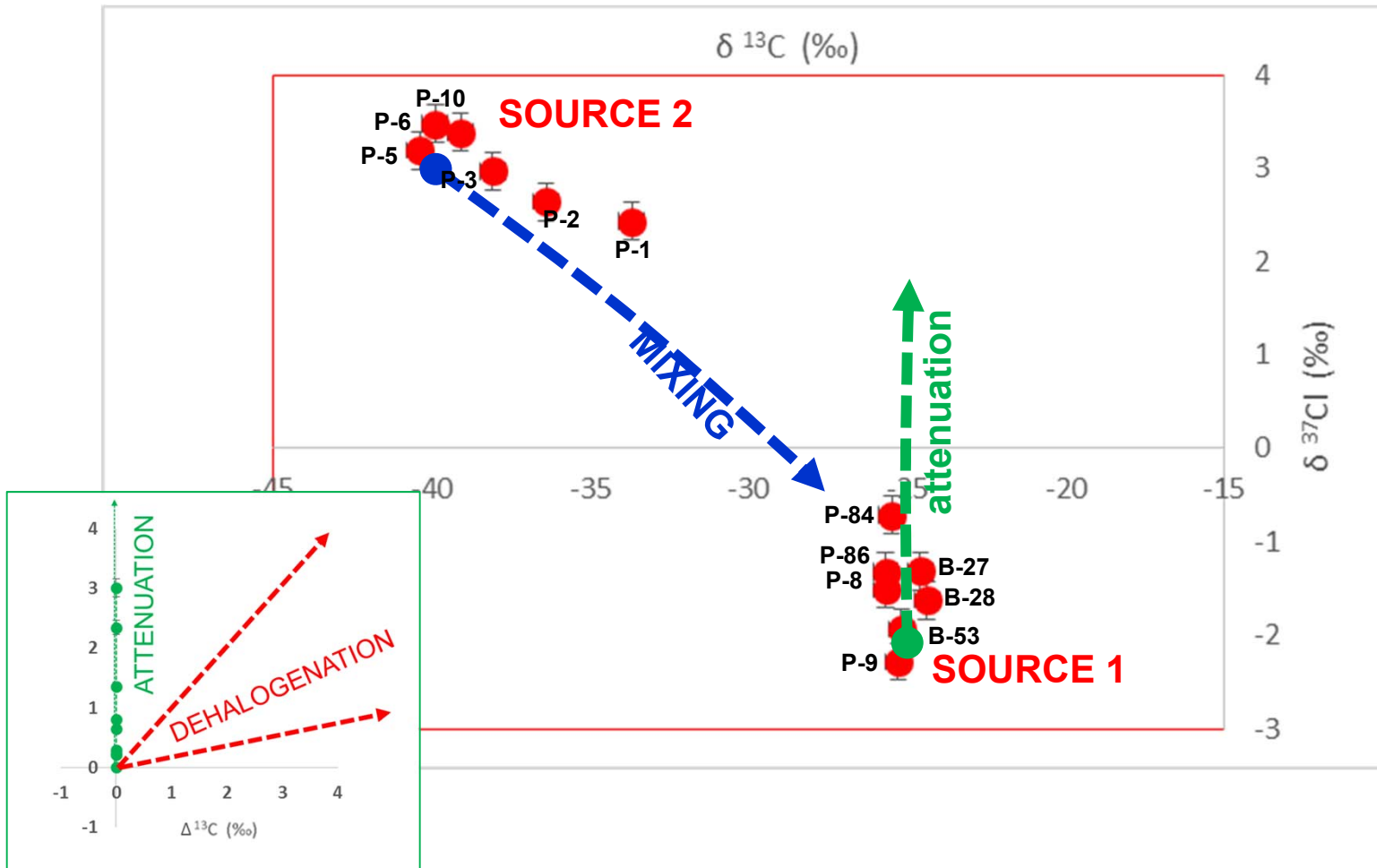


³⁷CI-CSIA results





³⁷Cl-CSIA results



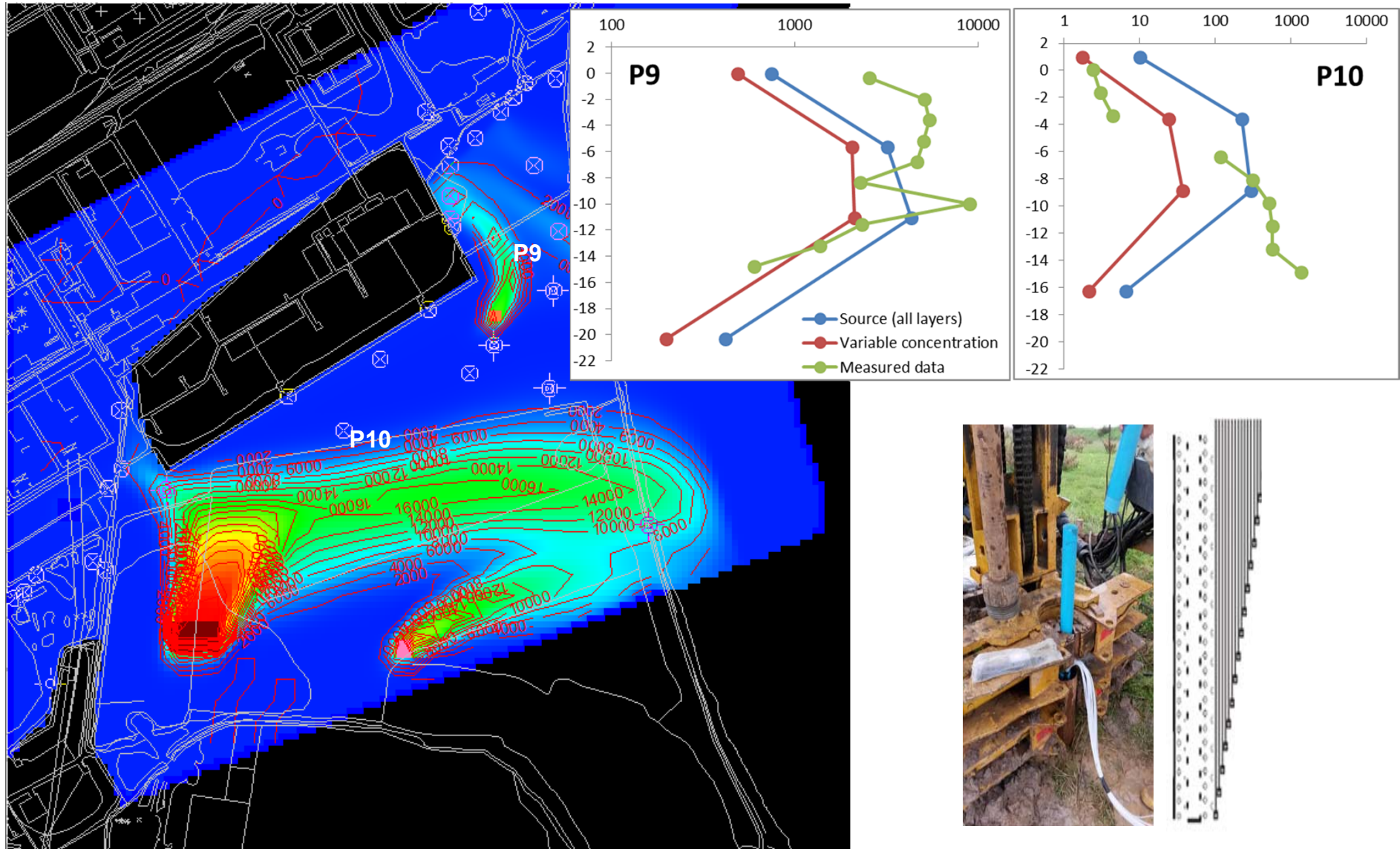


CSIA results

	ug/L	$\delta^{13}\text{C}$ ‰	$\delta^{37}\text{Cl}$ ‰	$\delta^2\text{H}$ ‰	Source 1 %	Source 2 %	Biodegradation %
P-10	1050	-39,1	3,4	63	100		-
P-6	2950	-39,9	3,5	59	100		-
P-5	205	-40,4	3,2	60	100		-
P-3	282	-38,1	3,0		90	10	-
P-2	72	-36,4	2,6	55	80\95	5\20	-
P-1	115	-33,7	2,4	25	60\80	20\40	40 - 85 %
P-8	735	-24,4	-1,6	-91		100	65 %
P-9	1530	-25,3	-2,3	-91		100	-
P-86	188	-25,7	-1,3			100	75 %
B-28	670	-25,7	-1,5	-96		100	70 %
B-53	14	-25,2	-1,9			100	45 %
B-27	12	-24,6	-1,3			100	75 %
P-84	5	-25,5	-0,7			100	95 %

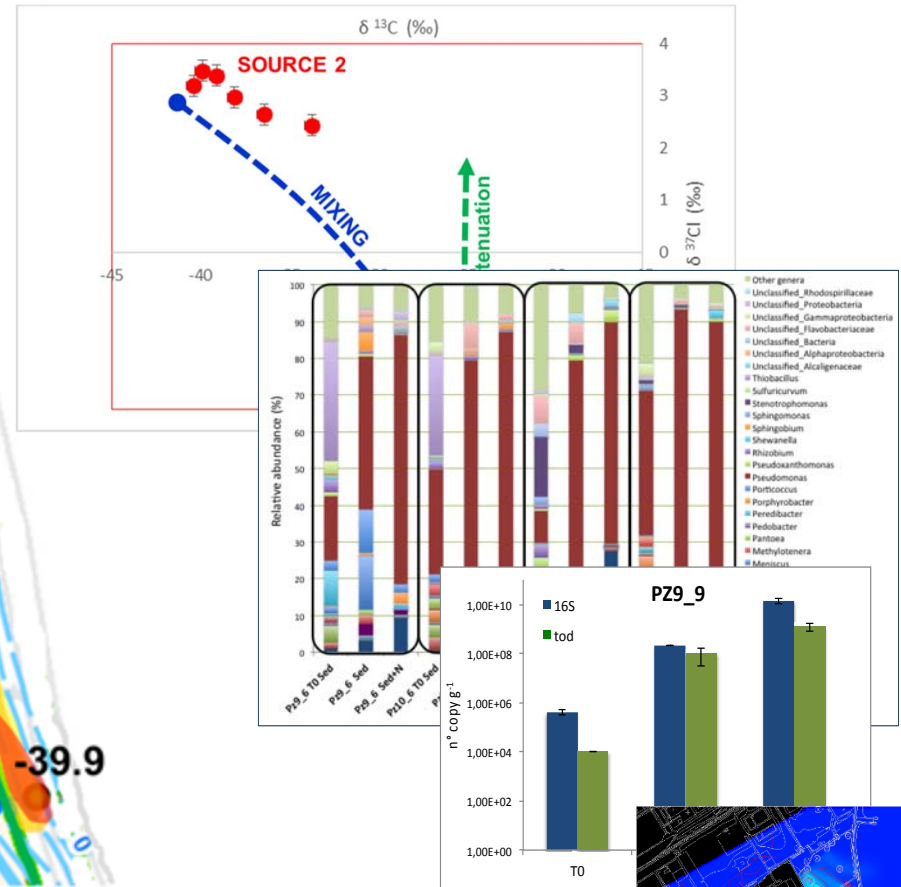
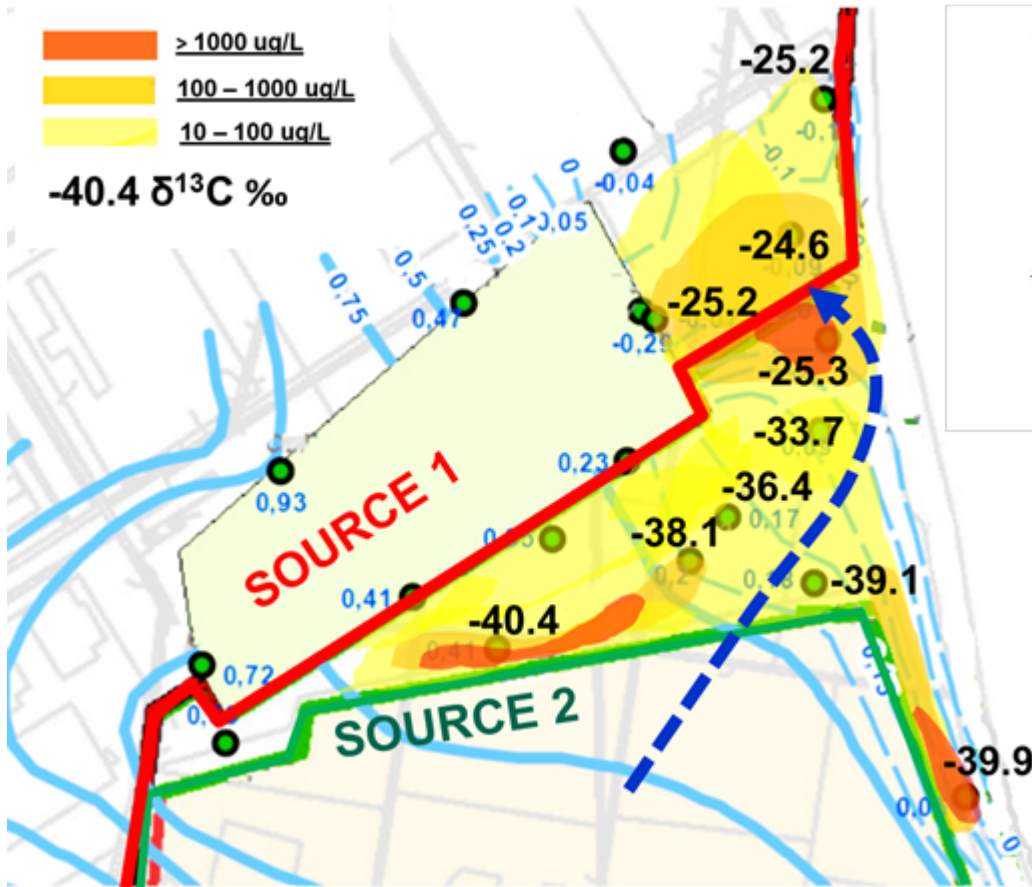


MODELING

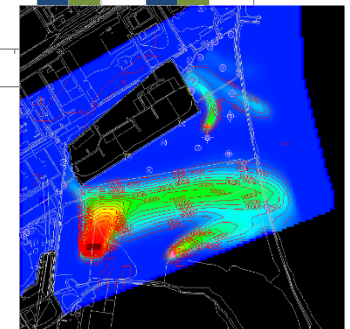




MULTIDISCIPLINARY APPROACH



- ³⁷Cl, ¹³C e ²H – CSIA for chlorinated solvents**
- DGGE, qPCE ecc. – MBTs in different conditions, aerobic, anaerobic...**
- Numerical modeling**
- Innovative sampling techniques**





CONCLUSIONS

- 1) ^{37}Cl and ^2H CSIA methodology was developed for MCB**
- 2) measurable ϵ for ^{37}Cl were estimated for aerobic biodegradation of MCB**
- 3) ^{37}Cl coupled with ^{13}C and ^2H was applied for sources apportionment and natural attenuation evaluation at a contaminated field site**
- 4) Additional tools such BMTs, modeling, multilevel sampling were also developed**



Thanks for your attention



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Syndial



Massimo.marchesi@polimi.it



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Prof. Orfan Shouakar-Stash

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