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Global thinking

Local delivery

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intersol'2020

Congrès-Exposition International sur les Sols, les Sédiments et l'Eau

International Conference-Exhibition on Soils, Sediments and Water

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Case Studies in Normandy and Central France of DNBA for Groundwater Remediation : Polar & chlorinated solvents (TCE, PCE, DCE, VC, BTEX, etc.) & best Cost-effectiveness (Dynamized Natural Bio-Attenuation)

Etudes de cas en Normandie et Centre de la France de l'application de la BAND pour la dépollution des eaux souterraines : Solvants polaires & chlorés (TCE, PCE, DCE, VC, BTEX, etc.) et meilleurs coûts-avantages (BAND : Bio-Atténuation Naturelle Dynamisée)

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Project GW-Remediation Goals:

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- **Remediation under Buildings without Demolishing and ensuring no non-acceptable Health Risks (TERQ) via Indoor Air by PCE, TCE, DCE, VC, etc.**
- **Remediation in Alluvium Sediments and deeper fractured Carbonate Aquifer with Karst Conditions.**
- **Decontamination without P&T: Pump & Treat by more Cost-effective in-situ-Biotechnology.**
- **Remediation by use and Dynamization of Natural Bio-Attenuation forces (DNBA).**
- **Remediation for proved Elimination of non-acceptable Health Risks (TERQ: Toxicological Exposure-Risk-Quantification).**

Development of Remediation Strategy via a technical-economic Feasibility Study with Focus on in-situ Microbiological Decontamination (DNBA: Dynamized Natural Bio-Attenuation))

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Methodology of DNBA Feasibility Study:

NBA (Natural Bio-Attenuation) investigation Strategy for Determination of the natural Bio-Reactor Status

- **Characterization of Contaminants: HVOC and metabolites,**
- **Bio-geochemical parameters (Electron donors and acceptors, redox zones, availability of auxiliary substrates,...),**
- **PCR (Polymerase Chain Reaction): DNA Analyses for Identification of microbiological Degradation Potential of Enzyme Production and for Selection of best adapted in-situ Microorganisms,**
- **Isotope Fractioning for determination of in-situ Degradation Rates,**
- **Microbiological Ecotoxicity Tests and Identification of Zones with Constraints,**
- **Determination of Degradation Rates and different redox and microbiological Zones of the natural Bioreactor.**

Methodology of DNBA Feasibility Study:

NBA (Natural Bio-Attenuation) investigation Strategy for NBA Dynamization:

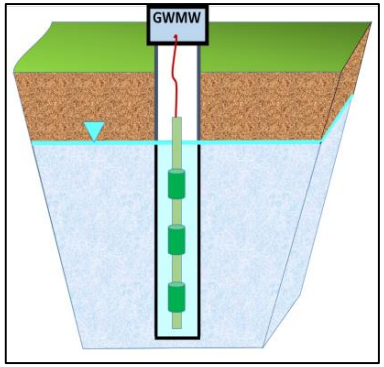
- **Mineralisation & Respiration Tests (Determination of Potential for enhancing & Dynamizing the NBA-Degradation),**
- **Challenge-Tests (Determination of Potential for max. degradation Capacities for max. Pollutant Concentrations by NBA),**
- **Remediation Pilot Testing (ONE®, BIOCLEAN®, KB1®, HRC®, ZVI, Oxygenation of the plume: Bio-Sparging, iSOC®,...),**
- **Evaluation of potential Dynamization Zones for enhancement and achievable degradation rates,**
- **Feasibility study (Technical & financial) and Groundwater Transport Modelling to compare MNBA, DNBA and P&T, etc.**

Aerobic & Anaerobic HVOC Degradation

Aerobic and anaerobic Degradation Reactions of Microbiological TCE Reduction of various Electron Acceptors.

Reaction	Reaction equation of the TCE-degradation and use of Electron-Acceptors	Eh Range in [mV]
Oxygen consumption	$C_2HCl_3 + O_2 \rightarrow C_2Cl_3O_2H \rightarrow$ $C_2Cl_3O_2H + O_2 + 2\ H^+ \rightarrow 2\ CO_2 + 3\ H^+ + 3\ Cl^-$	> 820 → 700
Nitrate reduction	$3\ C_2HCl_3 + 2\ NO_3^- \rightarrow 3\ C_2Cl_3O_2H + N_2 \rightarrow$ $3\ C_2Cl_3O_2H + 2\ NO_3^- + 6\ H^+ \rightarrow 6\ CO_2 + N_2 + 9\ H^+ + 9\ Cl^-$	< 740 → 500
Nitrate reduction	$3\ C_2HCl_3 + 2\ NO_3^- + 6\ H^+ \rightarrow 3\ C_2Cl_3O_2H + 2\ NH_3^+ \rightarrow$ $3\ C_2Cl_3O_2H + 2\ NO_3^- + 6\ H^+ \rightarrow 6\ CO_2 + 2\ NH_3^+ + 9\ H^+ + 9\ Cl^-$	< 600 → 520
Manganese redaction	$3\ C_2HCl_3 + 3\ MnO_2 \rightarrow 3\ C_2Cl_3O_2H + 3\ Mn^{2+} \rightarrow$ $3\ C_2Cl_3O_2H + 3\ MnO_2 + 6\ H^+ \rightarrow 6\ CO_2 + 3\ Mn^{2+} + 9\ H^+ + 9\ Cl^-$	< 520 → -50
Iron reduction	$3\ C_2HCl_3 + 3\ Fe^3O(OH) \rightarrow 3\ C_2Cl_3O_2H + 3\ Fe^{2+} \rightarrow$ $3\ C_2Cl_3O_2H + 3\ Fe^3O(OH) + 6\ H^+ \rightarrow 6\ CO_2 + 3\ Fe^{2+} + 9\ H^+ + 9\ Cl^-$	< -50 → -200
Sulfate reduction	$2\ C_2HCl_3 + SO_4^{2-} + 10\ H^+ \rightarrow C_2H_4 + 2\ CO_2 + H_2S + 6\ H^+ + 6\ Cl^-$	< -75→ -220
Methanogese	$2\ C_2HCl_3 + 4\ H_2O + 4\ H^+ \rightarrow 2\ CH_4 + 2\ CO_2 + 6\ H^+ + 6\ Cl^-$	< -220→ -500

Evaluation of the vertical and horizontal Pollutant Mass fluxes by Passive Samplers



Methodology:

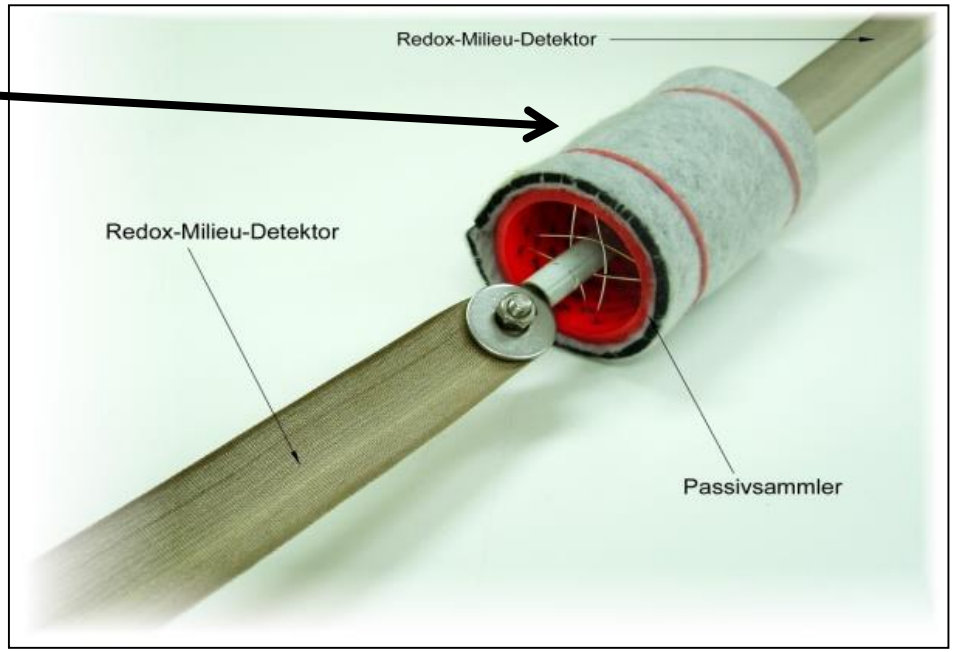
Organic pollutant accumulation useing adsorbent materials

(iron oxide and activated charcoal),

Passive sampler

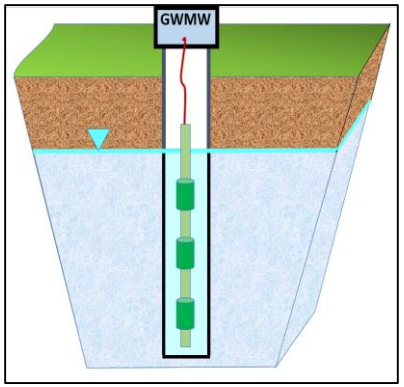
(Roll with adsorbent material at the inside)

in GWMW

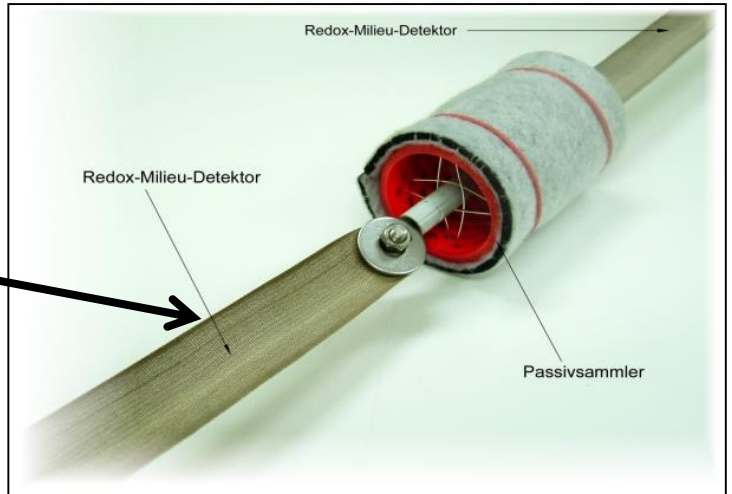


Evaluation of the vertical and horizontal Redox-zones / Distribution of Redox indicators (Mn^{IV}/Mn^{II}, Fe^{III}/Fe^{II}, H_2S/SO_4, etc.)

Methodology:

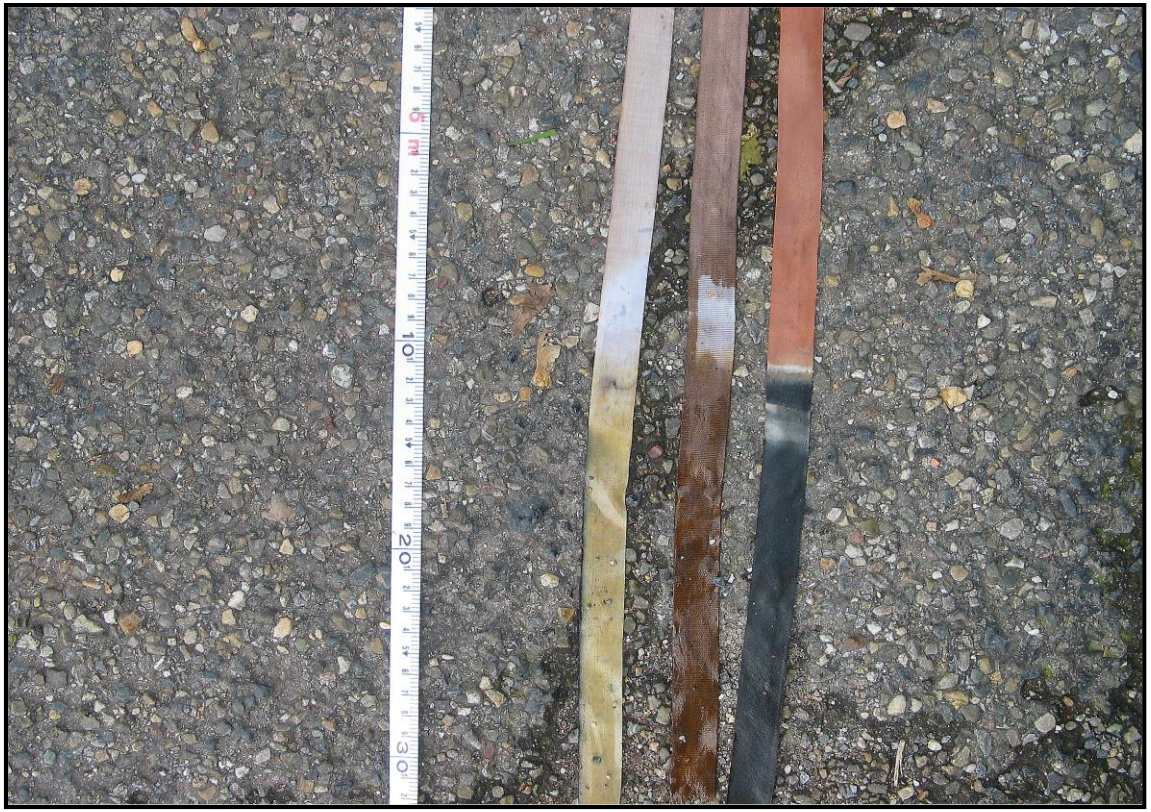


- Illustration of existing **redox zones** in Groundwater with redox detection band,
- **Color changes** indicate the presence or not of different **Electron Acceptors**,
- Detection of the available masses for oxydation or reduction of contaminants.



Evaluation of the distribution of vertical and horizontal Redox Zones with „In-Situ“ Redox indicators

Visual results of Redox test after removal from Groundwater: GWMW



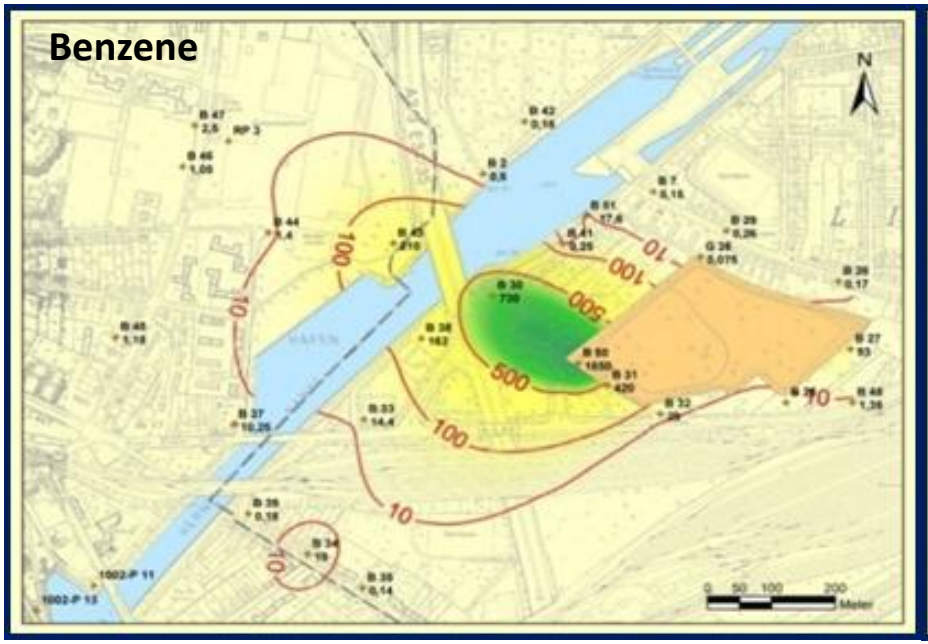
<u>Monitoring well B 32:</u>

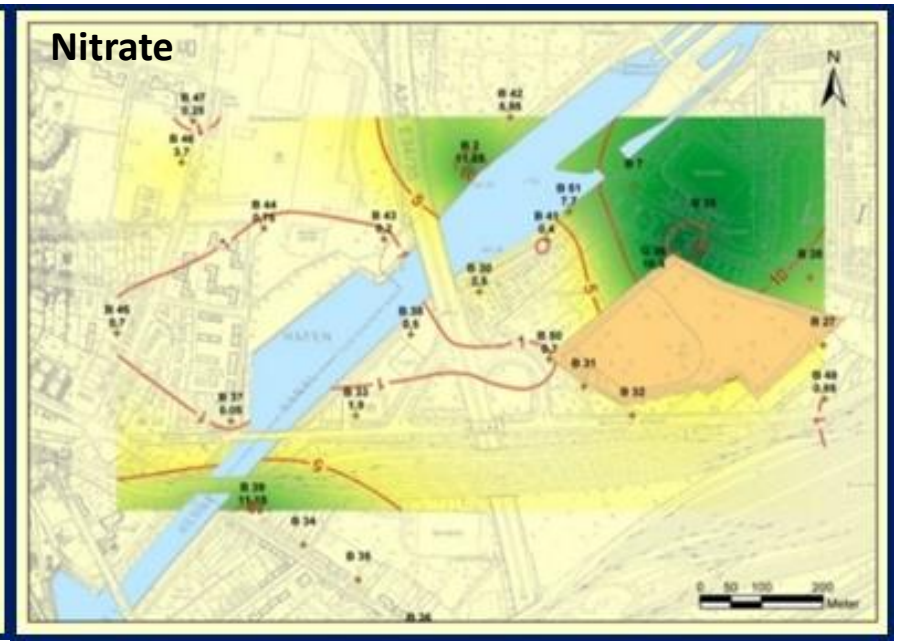
Manganese (left):	**transition layer from 5,07 to 5,12 m:**	**Manganese reduction (MnIV/MnII)**
Iron (middle):	**transition layer from 5,07 to 5,14 m:**	**Iron reduction (FeIII/FeII)**
Sulfate (right):	**transition layer from 5,07 to 5,11 m (black):**	**Sulfate reduction to sulfite**

Investigation Program M/DNBA-Study

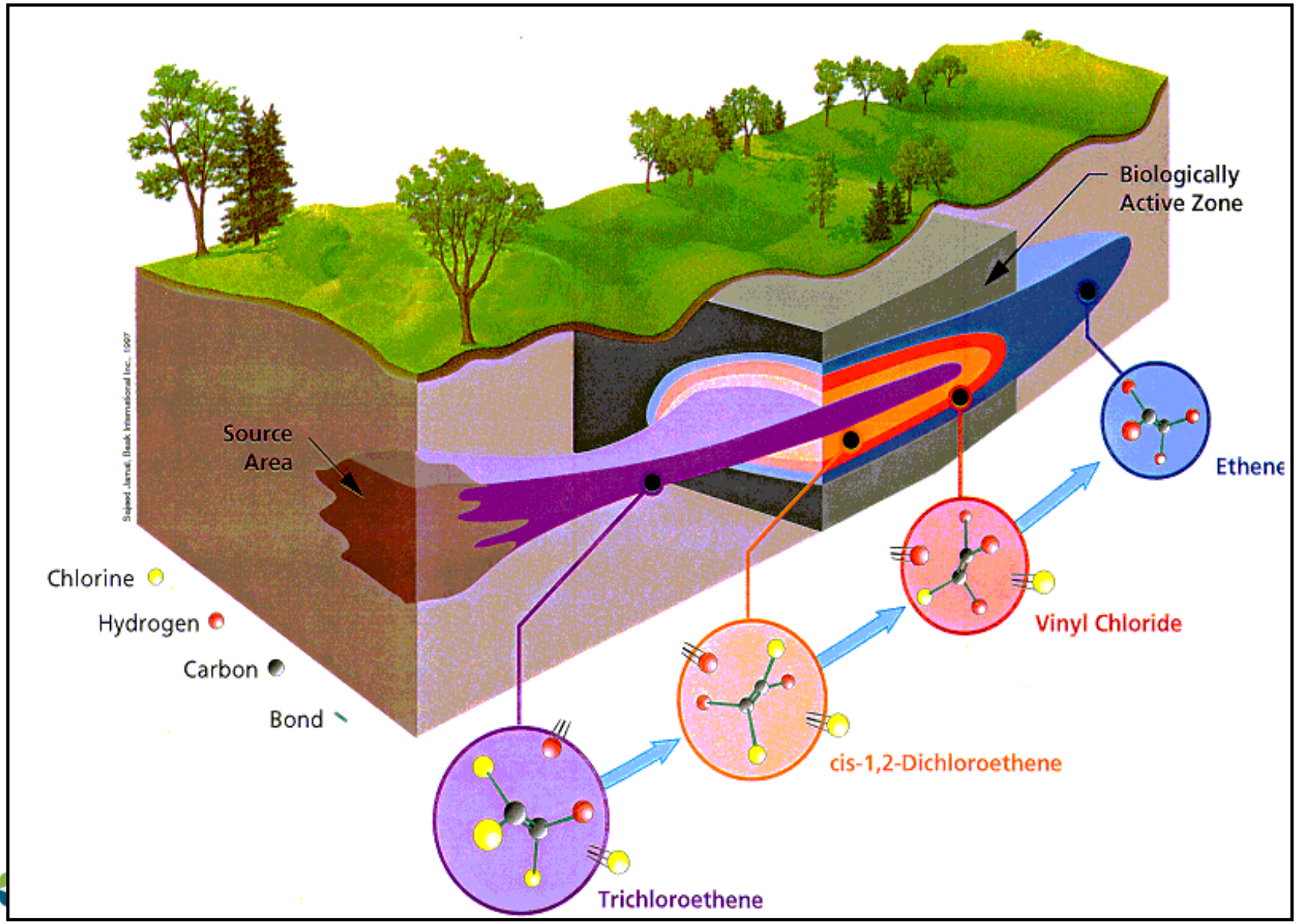
HPC-Reference case: **DNBA Studies & Application in Oberhausen, Rütgers Chemicals, Germany: Contaminants in Groundwater.**

- **Relationship of Pollutant presence in Groundwater and formation of a bioreactor with the use of electron-acceptors (microbiological Nitrate Reduction for pollutant degradation).**

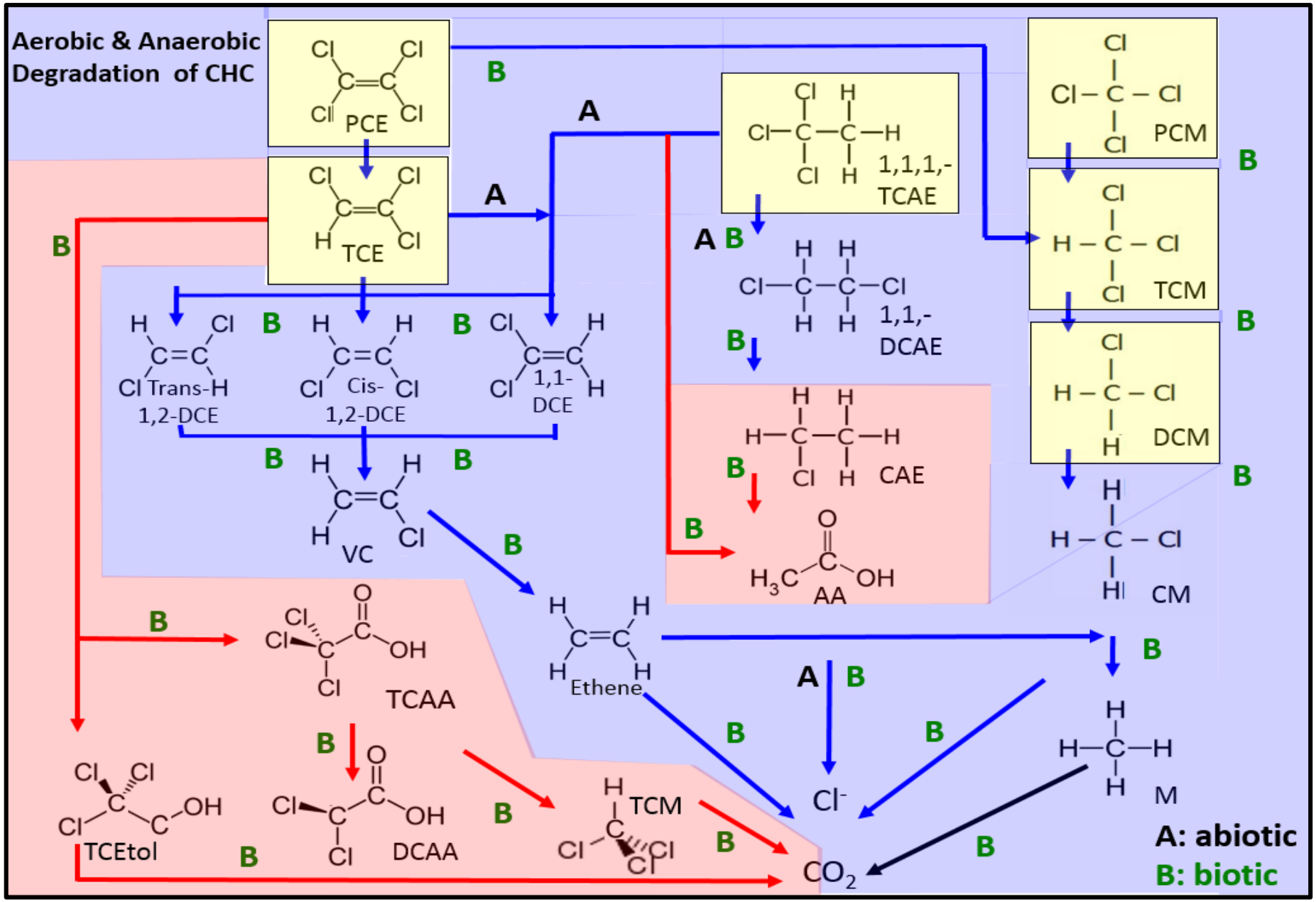




Schematic presentation of the TCE Plume in the bio-reactor

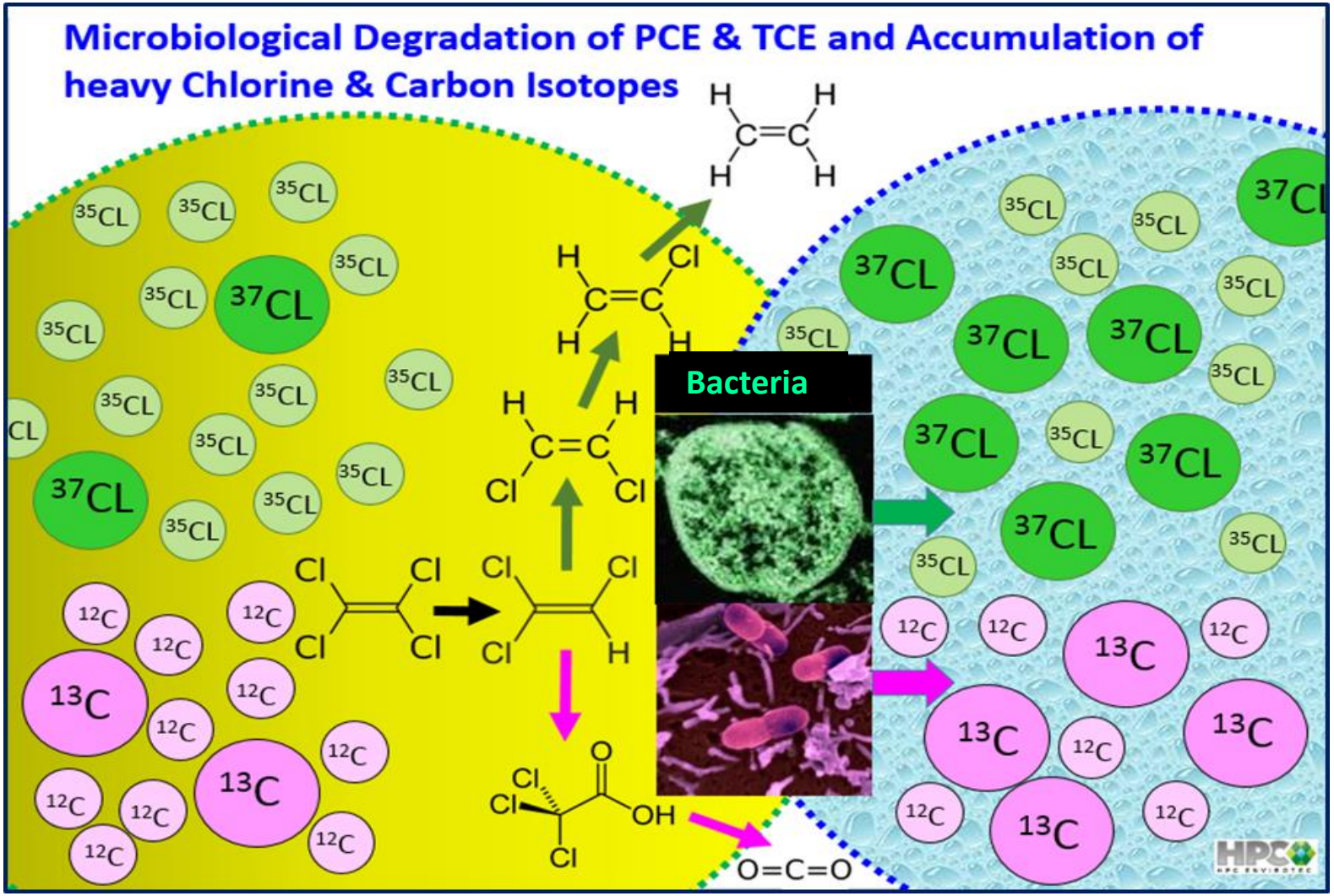


Aerobic & Anaerobic HVOC Degradation



Investigation Program M/DNBA-Study

Isotopic Fractioning to Identify in-situ Microbial Biodegradation



Investigation Program M/DNBA-Study

Determination of Microbiological Eco-toxicities to identify disruptive factors to the current microbiological degradation and to identify zones with DNBA measures required.

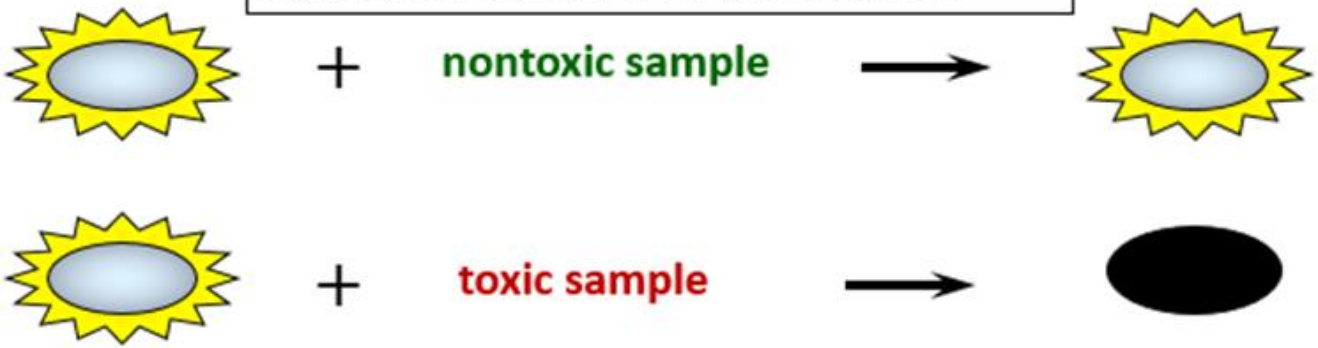
Microbiological ecotoxicity

Microbiological ecotoxicity tests (Investigation of disruptive factors of the pollution degradation), like:

Toximeter tests (Measurement of the metabolic activity of selected soil bacteria strains by using luminous soil bacteria)

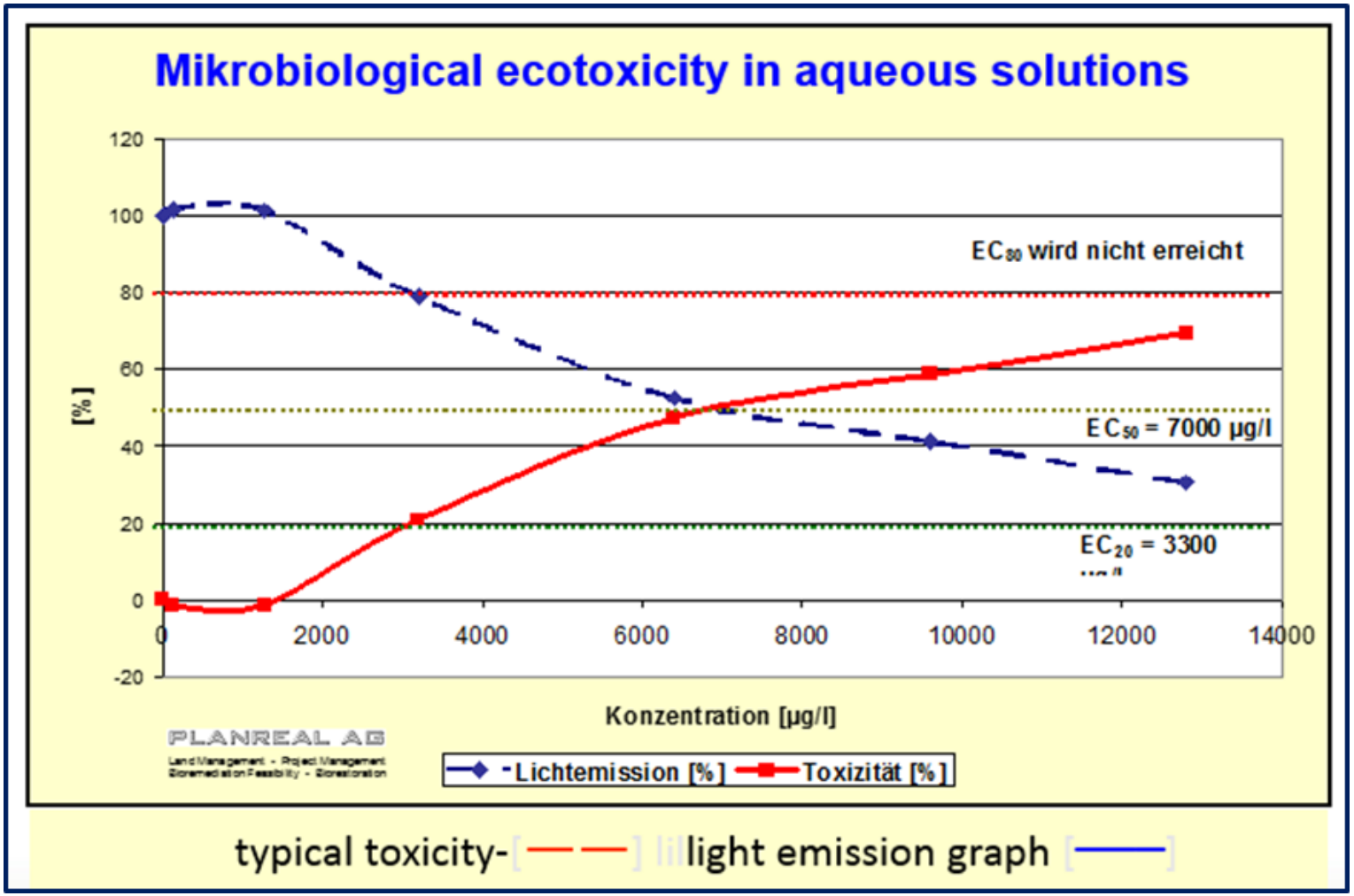
Determination of pollution toxicity for soil bacteria with gene-modified soil bacteria

e.g. *Ps. fluorescens* pUCD607 (non-degrader)



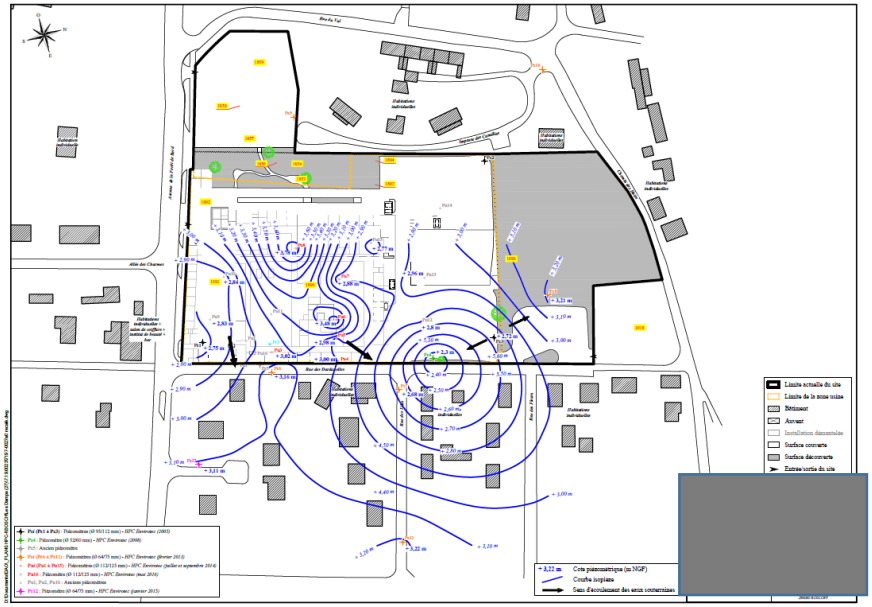
Investigation Program M/DNBA-Study

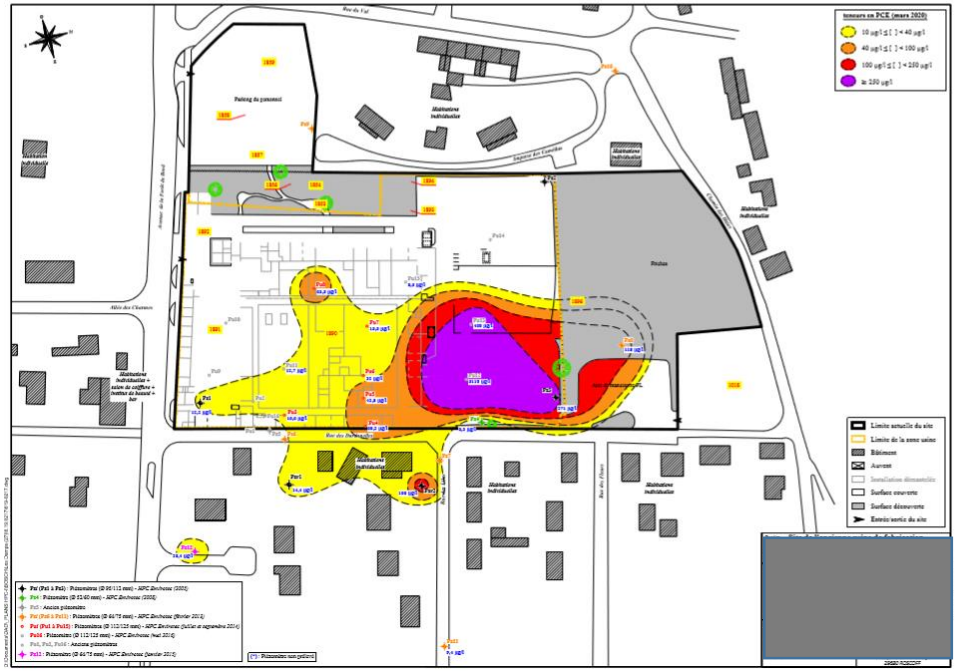
Calibrations & measurements of Microbiological Eco-toxicities in Groundwater by luminescent bacteria (soil bacterium Pseudomonas-tribe).



Case Study 1: Normandy / France
General Context

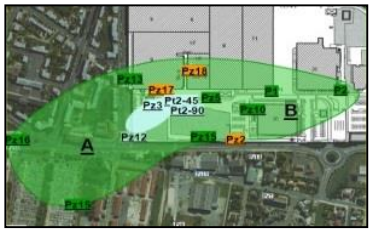
- **Site History:** Automotive Production,
  Today: Brownfield to be re-develloped
- **Geology - Hydrogeology:** Alluvial Sediments & Karst Limestone
- **Groundwater level:** 22 m (fluctuation +/- 3 m): → N
- **GW-pollution:** TCE, PCE, cDCE, VC, BTEX
- **Down gradient:** Sensitive Residential site use.
- **DNBA Application in Groundwater:** PCE, TCE, DCE, VC, BTEX





Full Scale Treatment: Starting 2015:
Zones A & B:
BC-228 + ONE

DNBA-Feasibility Study by Laboratory & Field Tests



After successful Laboratory Tests:

Realization of Field Tests with:

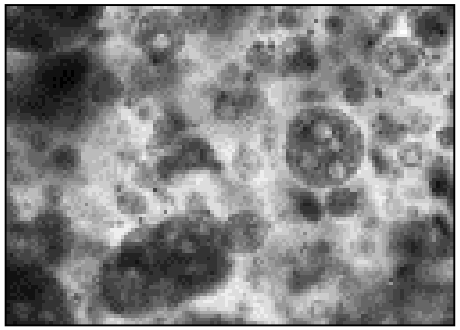
- **Site Specific Substratum for anaerobic Aquifer Conditions:**

  >Eh of about -200 mV: special Vegetable Oil Emulsion of 2,5 – 7 µm,

  >Lactates (site specific & Bacteria Consortium specific constituted),

  >bio-available degradable Tensides for Emulsion Stabilization,

  >special Nutriments & Oligo-elementes, to dynamize the Bio-degradation (constituted Site specificaly):



  → For reductive, anaerobic Dehalogenation of the HVOC and

  → For reductive Immobilization of CrVI to CrIII.

- **Site Specific Microbiological Consortium of:**

  Dehalococcoides restrictus, Dehalococcoides multivorans, Dehalococcoides ethenogenes:

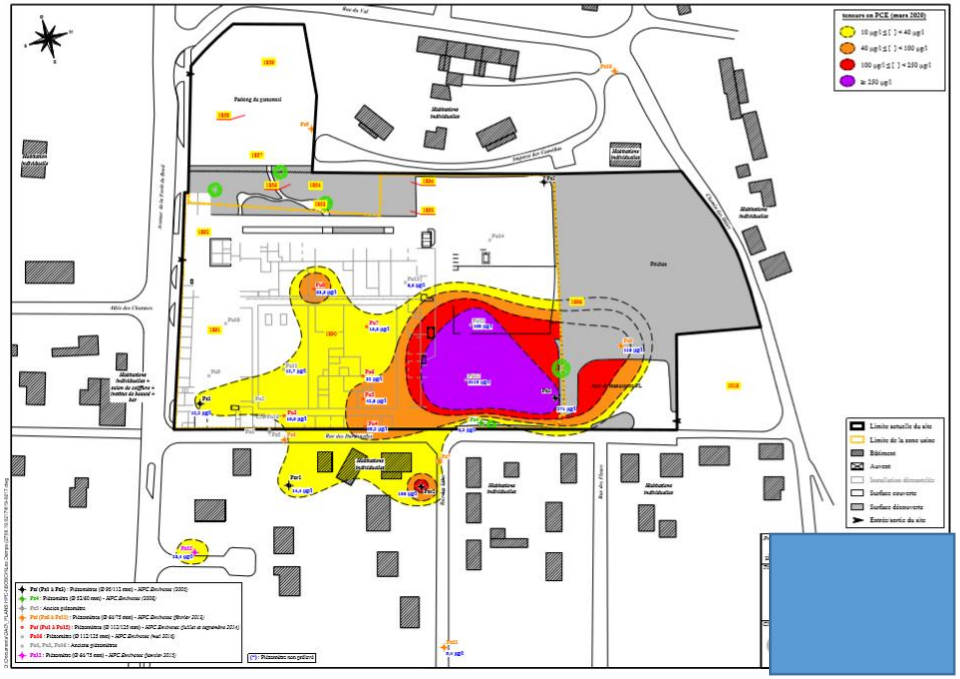
  → Anaerobic Degradation of PCE, TCE to cis-DCE

Case Study 1: Normandy / France

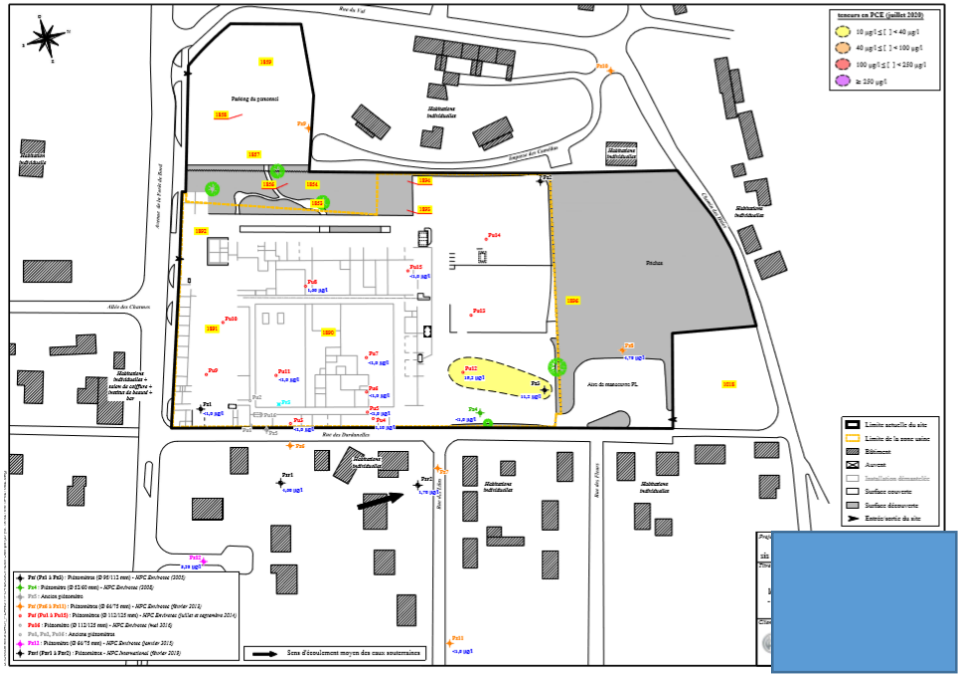
Anaerobic DNBA-Groundwater Remediation

➢ **PCE: Tetrachloroethylene**

03/2019



07/2020



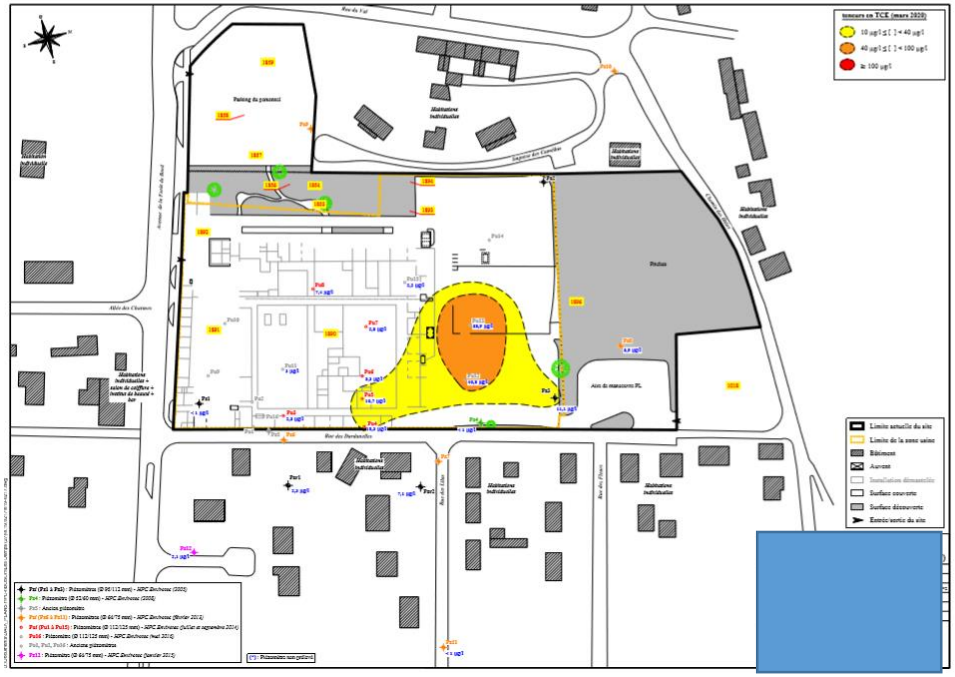
16 Months

Case Study 1: Normandy / France

Anaerobic DNBA-Groundwater Remediation

➢ **TCE: Trichloroethylene**

03/2019



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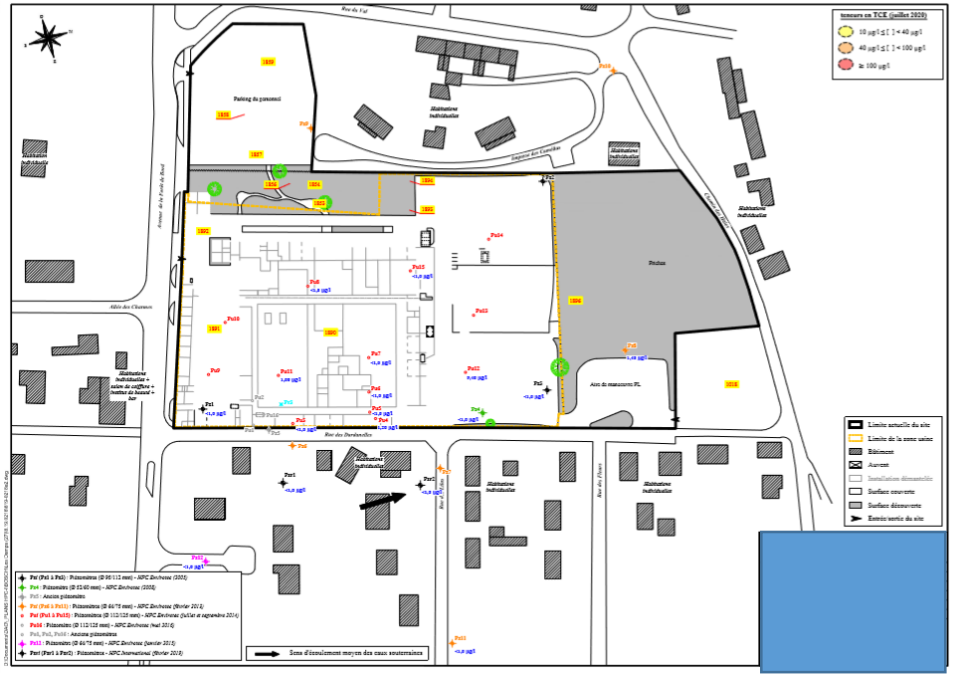


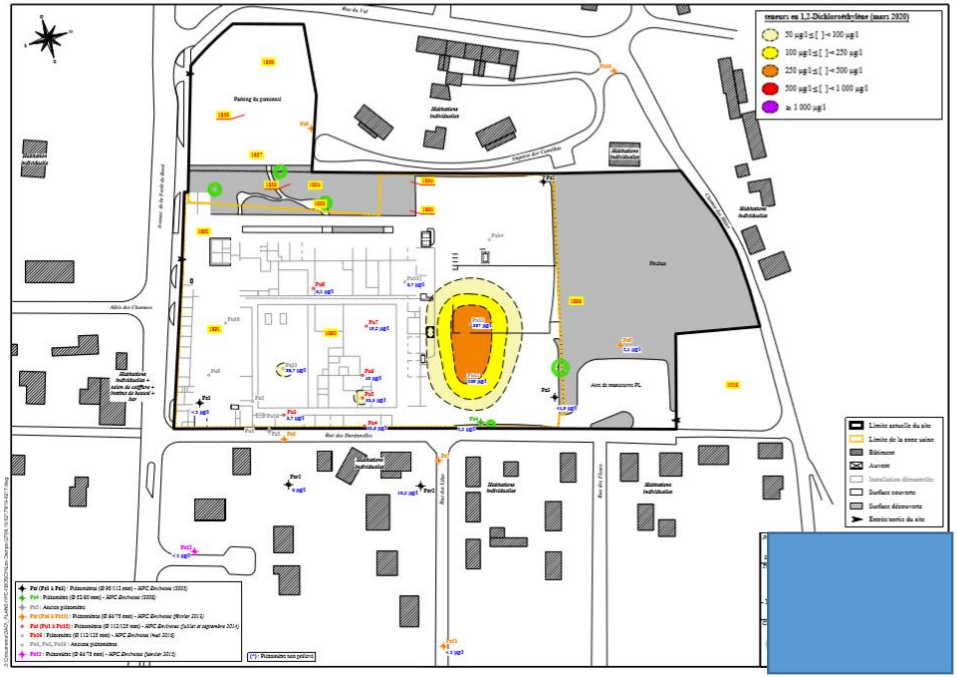
16 Months

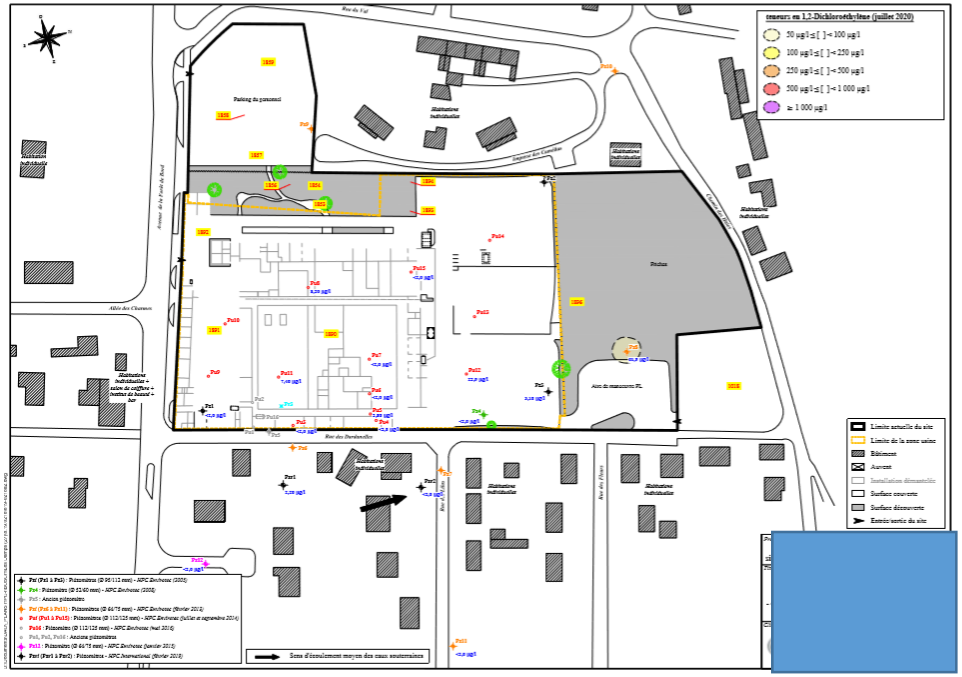
Case Study 1: Normandy / France

Anaerobic DNBA-Groundwater Remediation

- **DCE: cis-1,2-Dichloroethylene)**

03/2019 **07/2020**





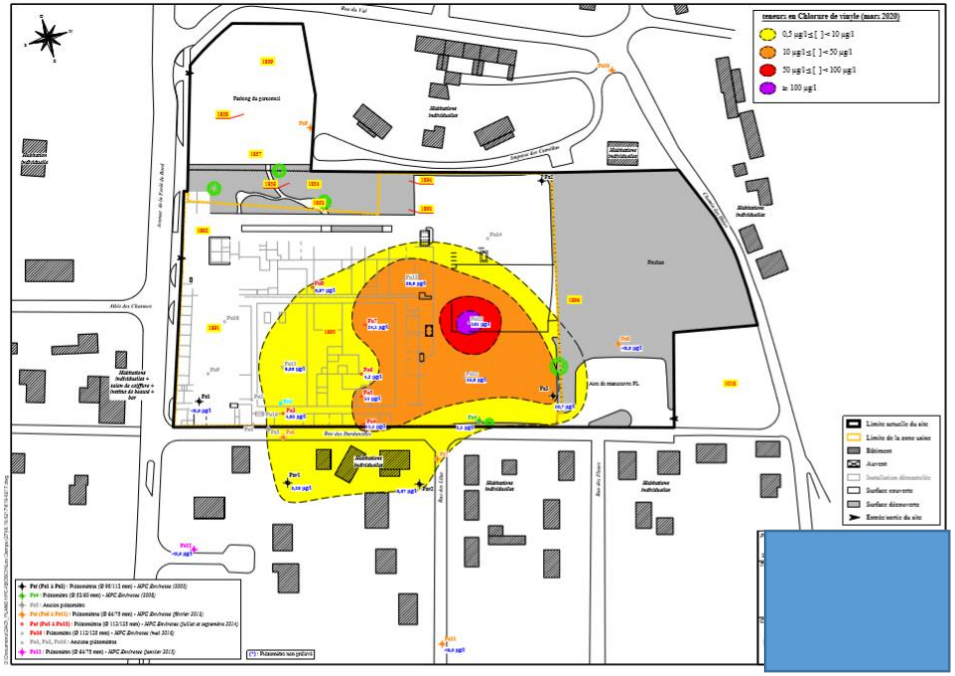
16 Months

Case Study 1: Normandy / France

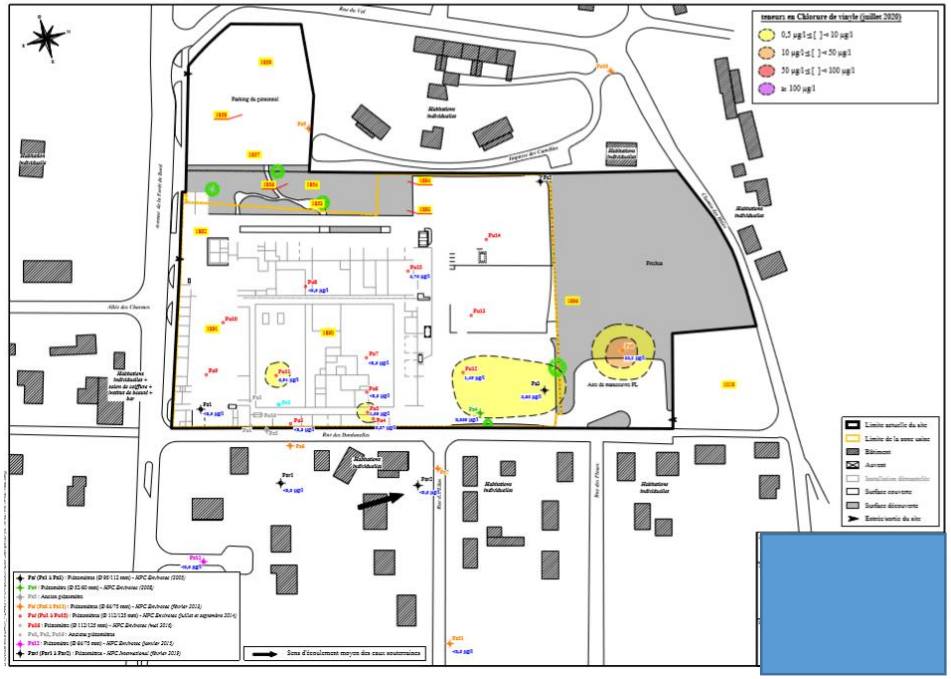
Anaerobic DNBA-Groundwater Remediation

- **VC: Vinyl chloride (Chloroethylene)**

03/2019



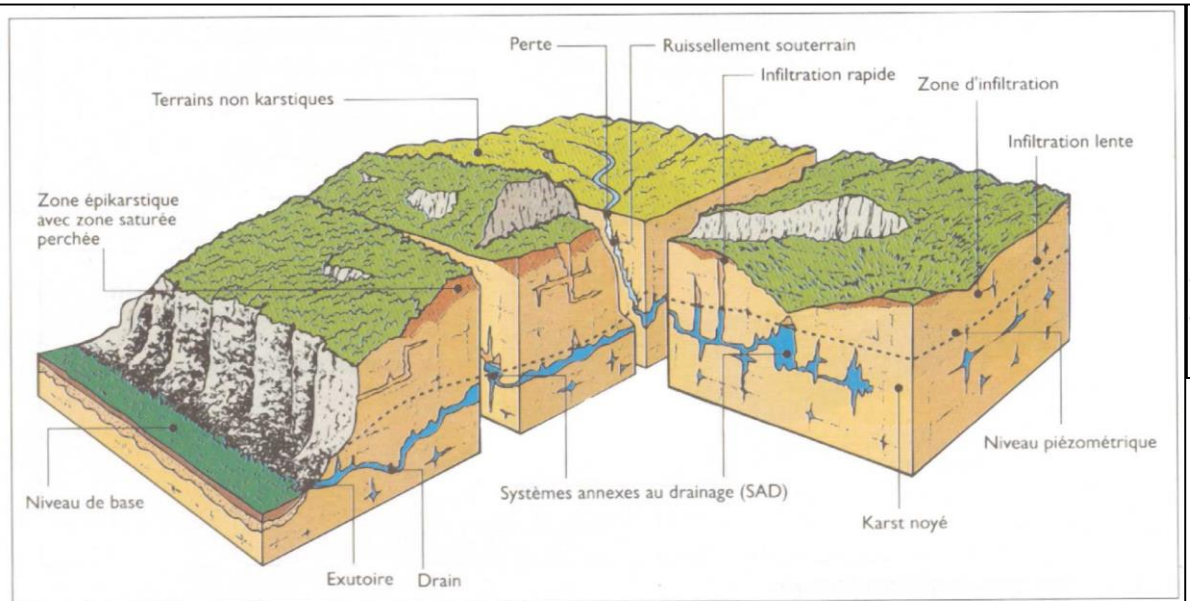
07/2020

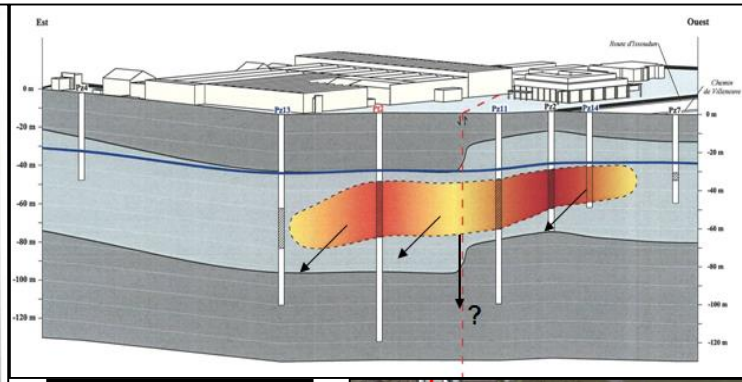


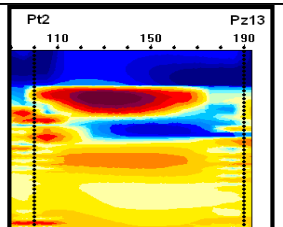
16 Months

Case Study 2: Central France
General Context

- **Site History:** Airport until the 2nd WW, Today: modern armament factory,
- **Geology - Hydrogeology:** Fractured limestone of the Jurrasic Oxfordian-Kimmeridgian Formations
- **Groundwater level:** 30 m (fluctuation +/- 10 m): → N (« Karstic »)
- **GW-pumping:** 7,5 m^3/hr for Industrial Process Water
- **GW-pollution:** TCE, PCE, cDCE, VC, Cr(VI) ⇩





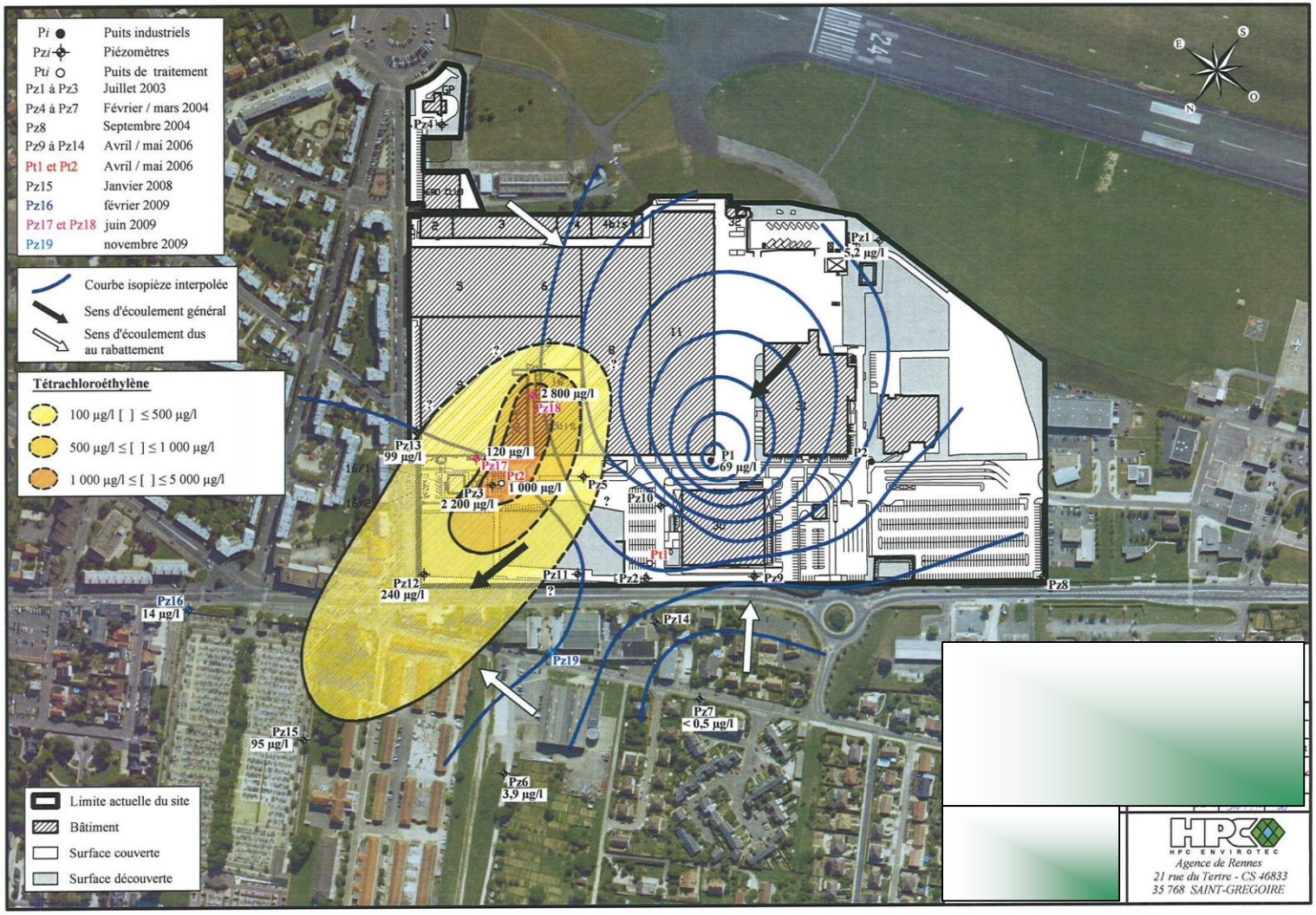


TCE in GW

max. 28 000 µg/l

PCE in GW:

max. 4 800 µg/l



Investigation Program M/DNBA-Study

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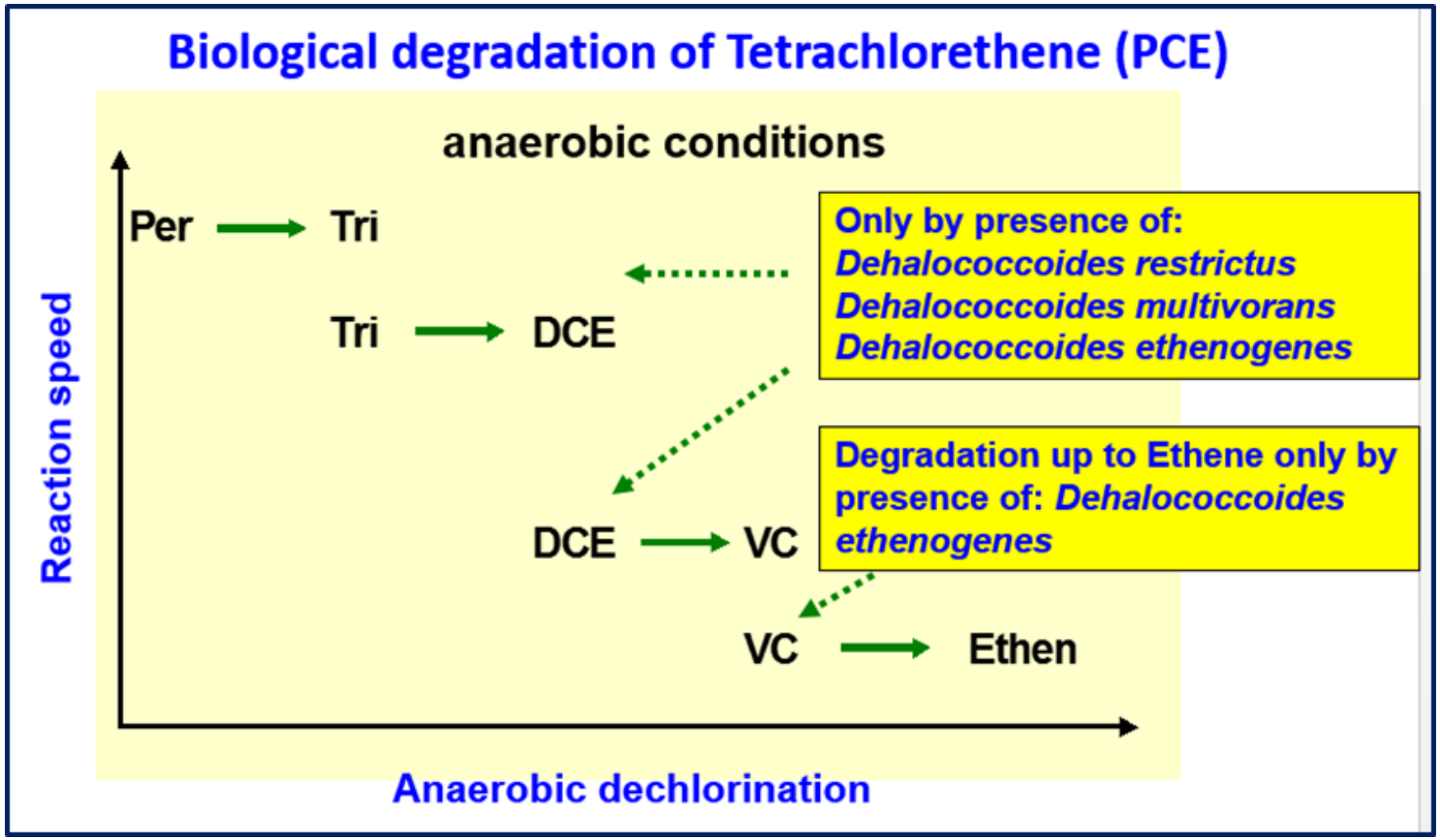
Anaerobic degradation of

Tetrachloroethene (PCE) & Trichloroethene (TCE).



The degradation of toxic Vinyl Chloride (VC) occurs only in the presence of the bacterium Dehalococcoides ethenogenes (which already exists or should be entered).

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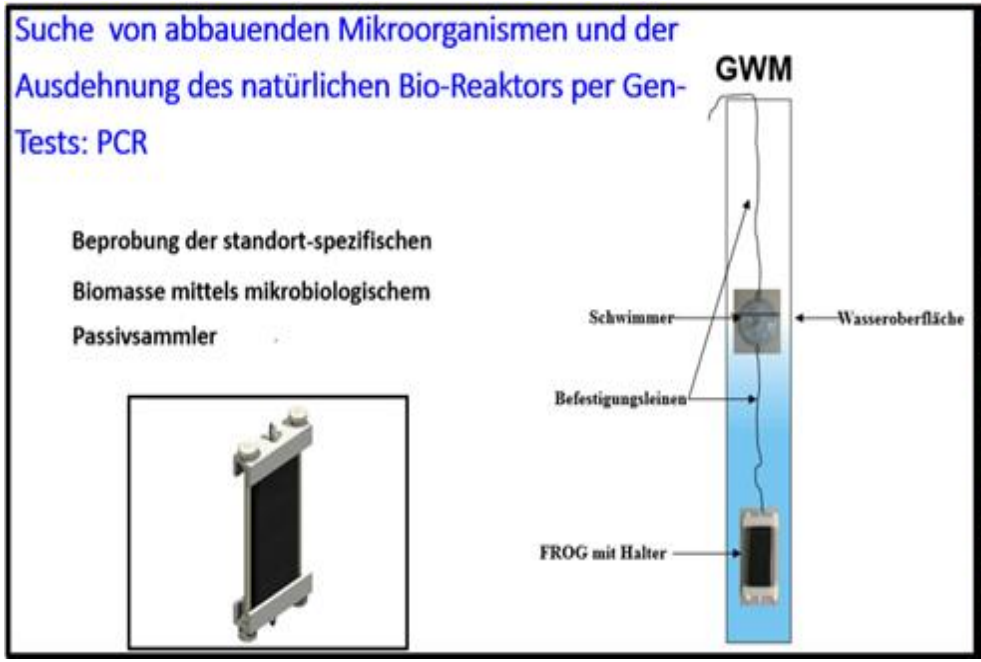
Investigation Program M/DNBA-Study

Sampling of Bacteria Consortia of groundwater with microbiological Passive Samplers (Frogs) by installing it in a Groundwater Monitoring Well (GWM) for PCR testing.



Investigation Program M/DNBA-Study

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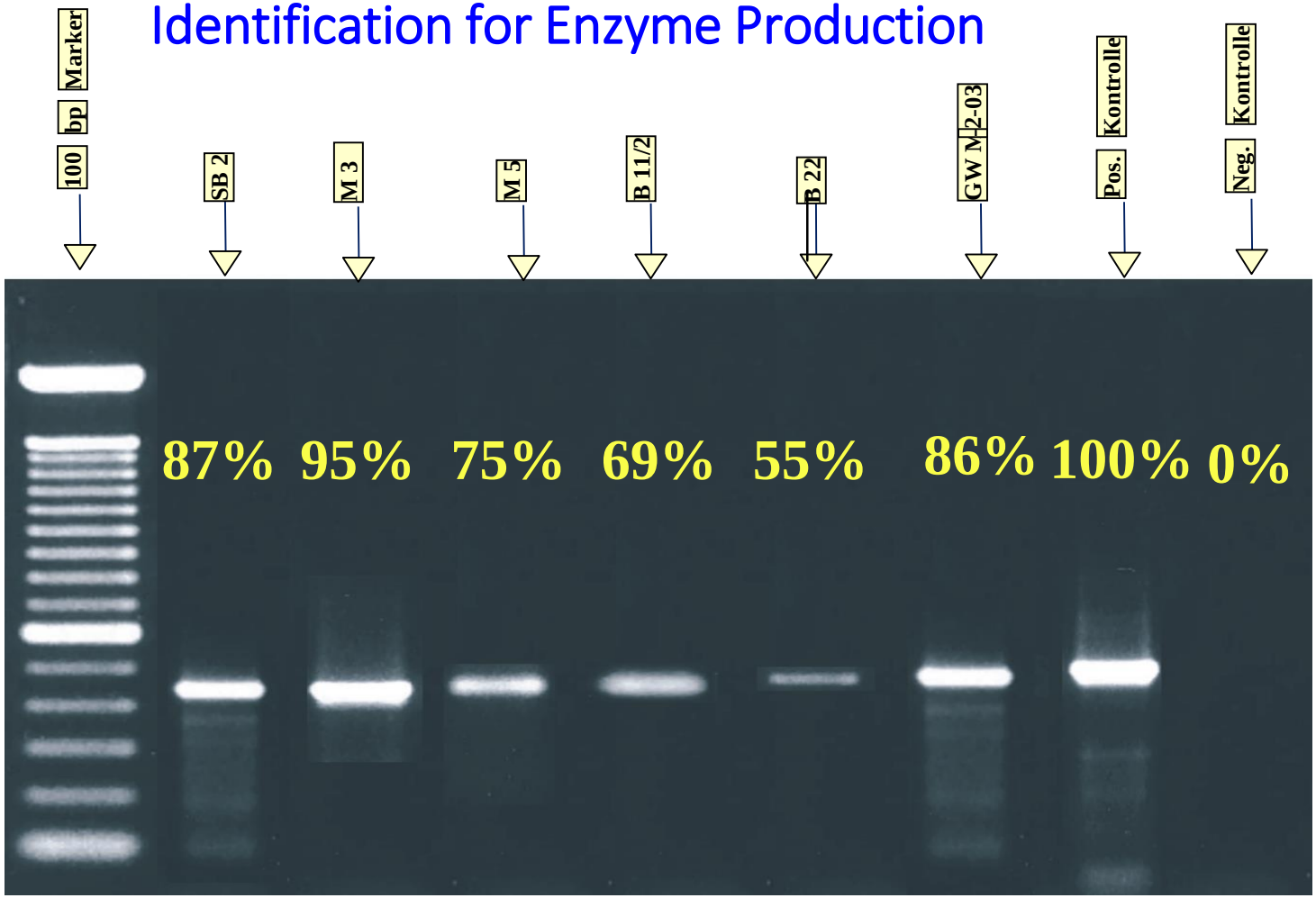
Competitive PCR and DNA Sequence Identification for Enzyme Production



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Investigation Program M/DNBA-Study

Mapping of microbiological iso-ecotoxicities and determination of two constraint zones of microbiological TCE & PCE degradation (on Zone "B“.). → ***Treatment of Zone B first !***

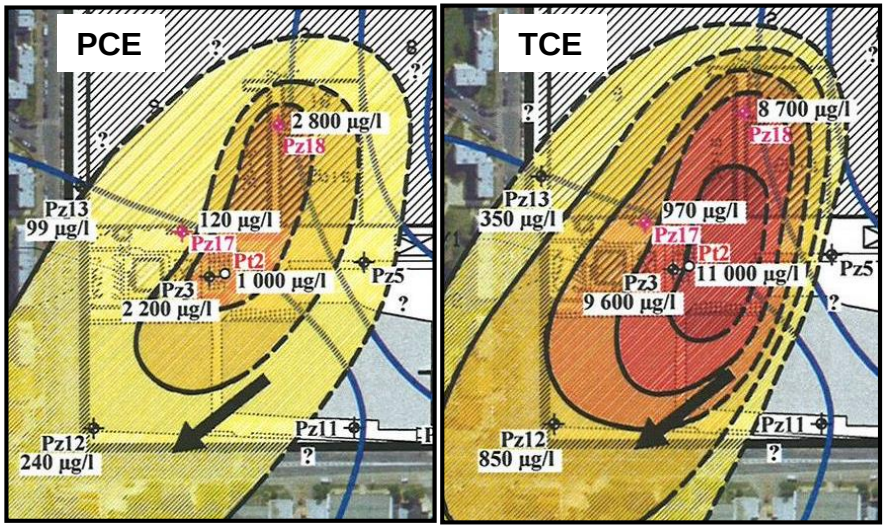
Stable Isotope Analysis

- **Stable Isotope Analyses allows to gather specific Information concerning Source Identification and Biodegradation.**
- **If biodegradation is responsible for the Decreasing of Contaminants concentration downstream of a Source this will lead to an increasing $^{13}C/^{12}C$-Ratio (Carbon stable Isotope Signature). → more ^{13}C Isotopes.**
- **In this case study we realized for the first time parallel Carbon- and Chlorine-Isotopes Analyses (2-dimensional Isotopemonitoring for TCE by GC-IRMS).**
- **This allows more detailed Statements regarding aerobic & anaerobic biodegradation processes.**
- **Determination of 50 Isotopic signatures** (Shift of values: ^{13}C-HCOV-Shift: + 0,5$^0/_{00}$, $^{37}Cl/^{35}Cl$ –HCOV-Shift: + 0,23$^0/_{00}$

Results of Isotope analysis

The folowing Isotopesignatures were identified:

- TCE-PCE-Source: in GWMW Pz18
- TCE-Source: in Pz2
- Aerobic TCE-Degradation: Downstream of Pz17, (Pt 2: 45 m↓ + 90 m↓)



Source:

Absence of Isotopeenrichement:

Potential Bio-Degradation:

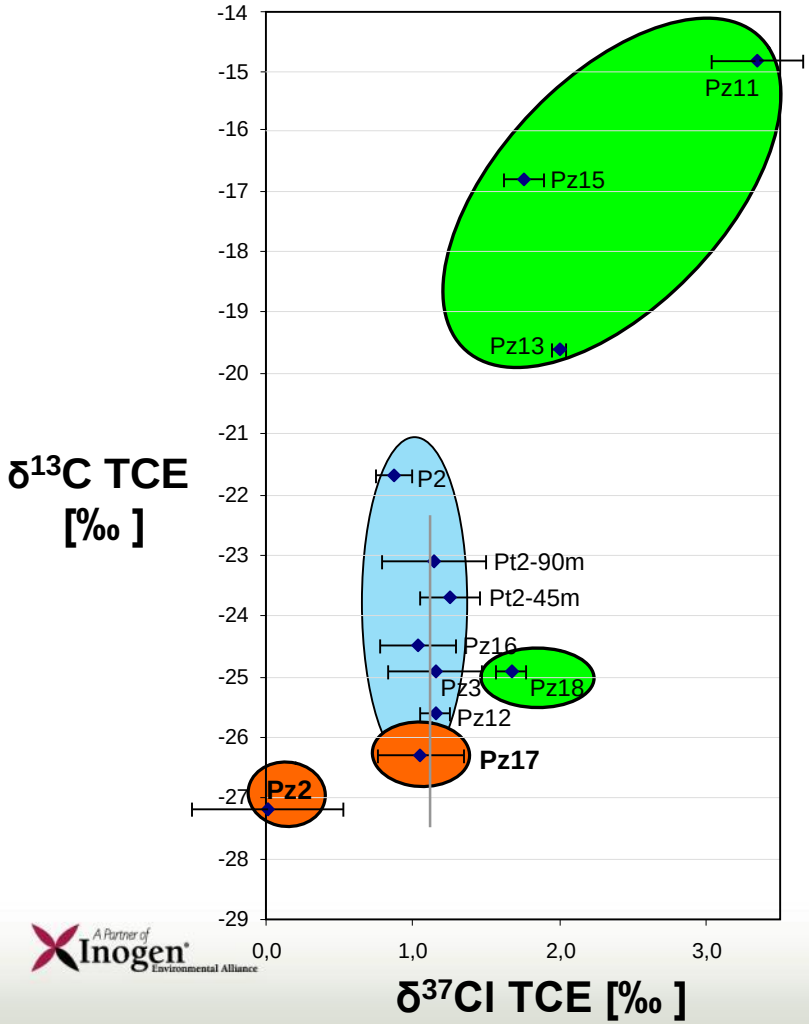
Identified Bio-Degradation:

		PCE		TCE		cDCE		VC		Σ LCKW	
		^{13}C [δ‰]	c [µg/L]	^{13}C [δ‰]	c [µg/L]	^{13}C [δ‰]	c [µg/L]	^{13}C [δ‰]	c [µg/L]	^{13}C [δ‰]	c [µM/L]
Pz18	Q	-30,3	4200	-24,9	3400	-19,7	110	n.d.	14	-27,2	53,2
Pz17	Q	-29,1	600	-26,3	2500	-23,4	710	-21,4	16	-26,6	38,6
Pt2-45m	A	-29,7	3300	-23,7	7500	-19,0	340	n.d.	80	-24,4	82,8
Pt2-90m	A	-30,1	1000	-23,1	12000	n.d.	99			-23,2	98,9
Pz3	A	-30,5	3300	-24,9	7500	-21,5	370	-1,7	80	-25,7	83,1
Pz12	A	-29,5	4600	-25,6	1200	-20,1	370			-27,6	43,3
Pz13	A	-27,9	31	-19,6	17	-17,0	24			-21,2	0,6
Pz15	A	-29,5	67	-16,8	54	-15,7	50			-20,9	1,6
Pz16	A	-27,6	20	-24,5	10					-26,4	0,2
Pz5	B	-27,9	110*								
Pz10	B	-27,6	110*								
Pz2	B	n.d.	43*	-27,2	270*						
P1	B	-27,3	69								
P2	B	-26,9	160*	-21,7	54*	-23,5	12*			-25,2	1,5*
Pz11	B	n.d.	25*	n.d.	18*	n.d.	17*				

A Partner of Inogen Environmental Alliance

Two dimensional Isotope monitoring ^{13}C and ^{37}Cl

Site:



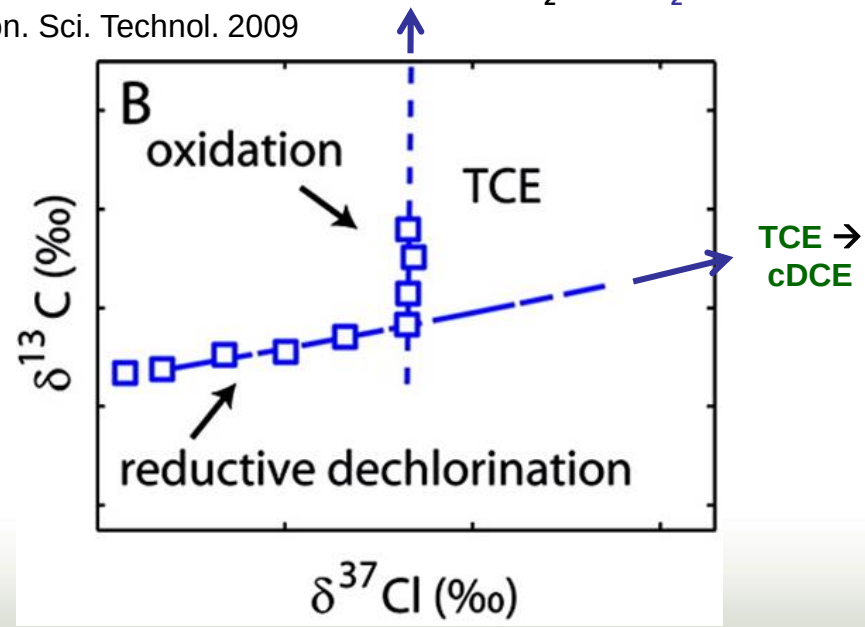
Oxidative TCE-Degradation:

Anaerobic reductive Dechlorination

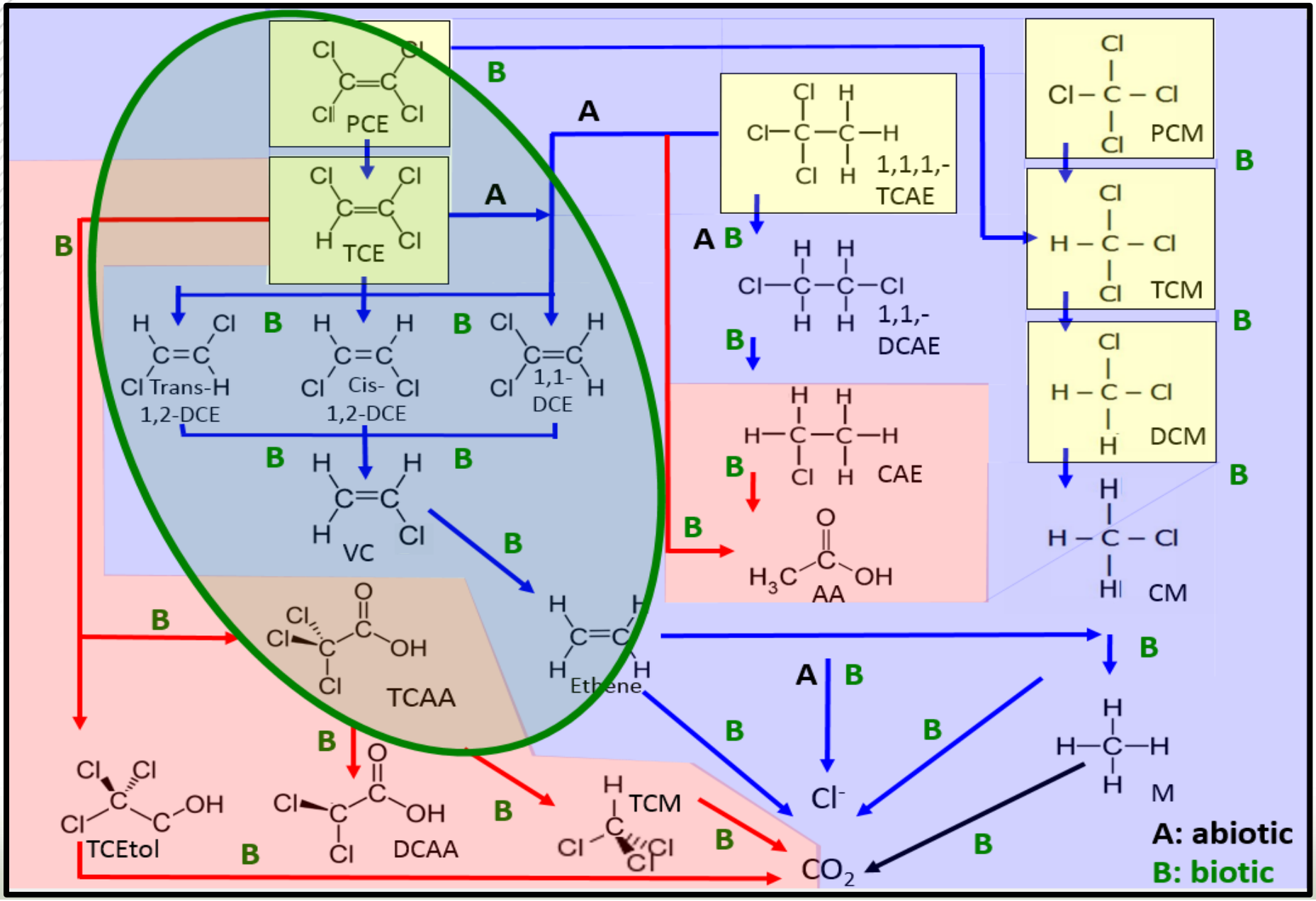
Adittional Sources zones

Hunkeler et al.
Environ. Sci. Technol. 2009

TCE + $2O_2$ → 2 CO_2 + 3 Cl^- + H^+



Aerobic and anaerobic HVOC Degradation at Site



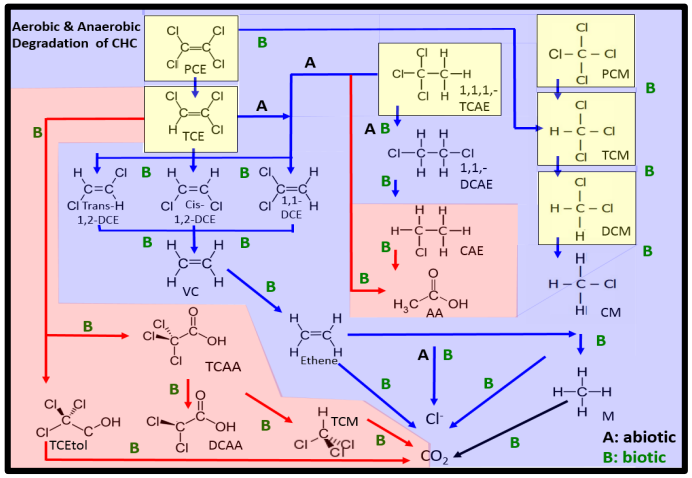
Investigation Program M/DNBA-Study

Mapping of iso-degradation rates (in-situ) of TCE (max: 2.13 µg/l/day TCE), determined by isotope Fractioning.

Investigation Program M/DNBA-Study

The ***Challenge Test*** is used to **identify the highest level of degradable Pollutant Concentration** (compatible for micro-organisms).

The ***Mineralisation Tests*** are performed to quantify the actual metabolism and **maximum achievable degradation rates** (in µg/l/day and microbiological half-lives) by microorganisms. There is a complete mineralization, if all organic pollutants are converted into CO_2 (in aerobic medium), CH_4 or C_2H_4 (in anaerobic medium) are converted.

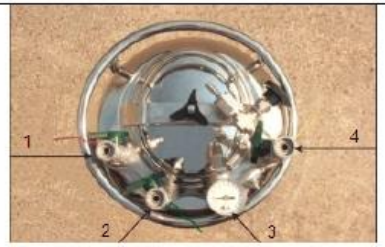


DNBA Feasibility Study: Production of site specific Bacteria Consortium after Selection by PCR:

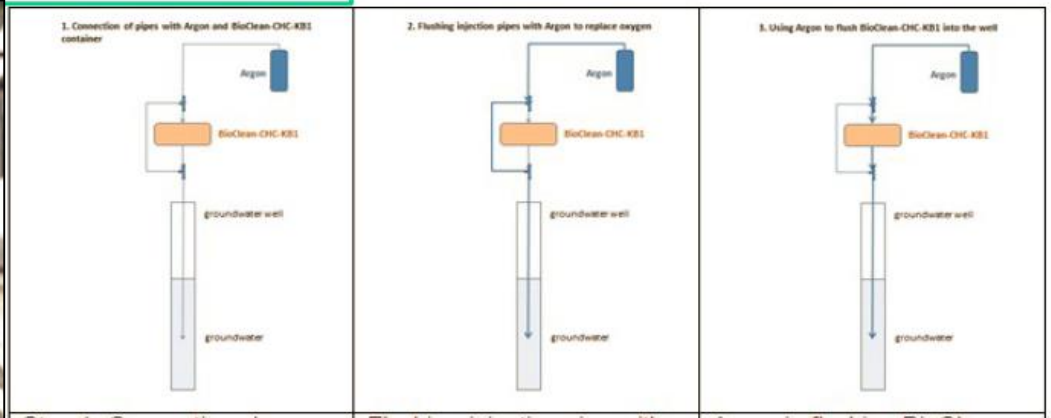
Injection of site specific Bacteria Consortium: SS-BC 83

How is BioClean-CHC-KB1 injected?

BioClean-CHC-KB1 is delivered in a stainless steel container with several in- and outlets. The organisms are very sensitive to oxygen. Therefor it is best practice to transport BioClean-CHC-KB1 under inert Argon into the groundwater.

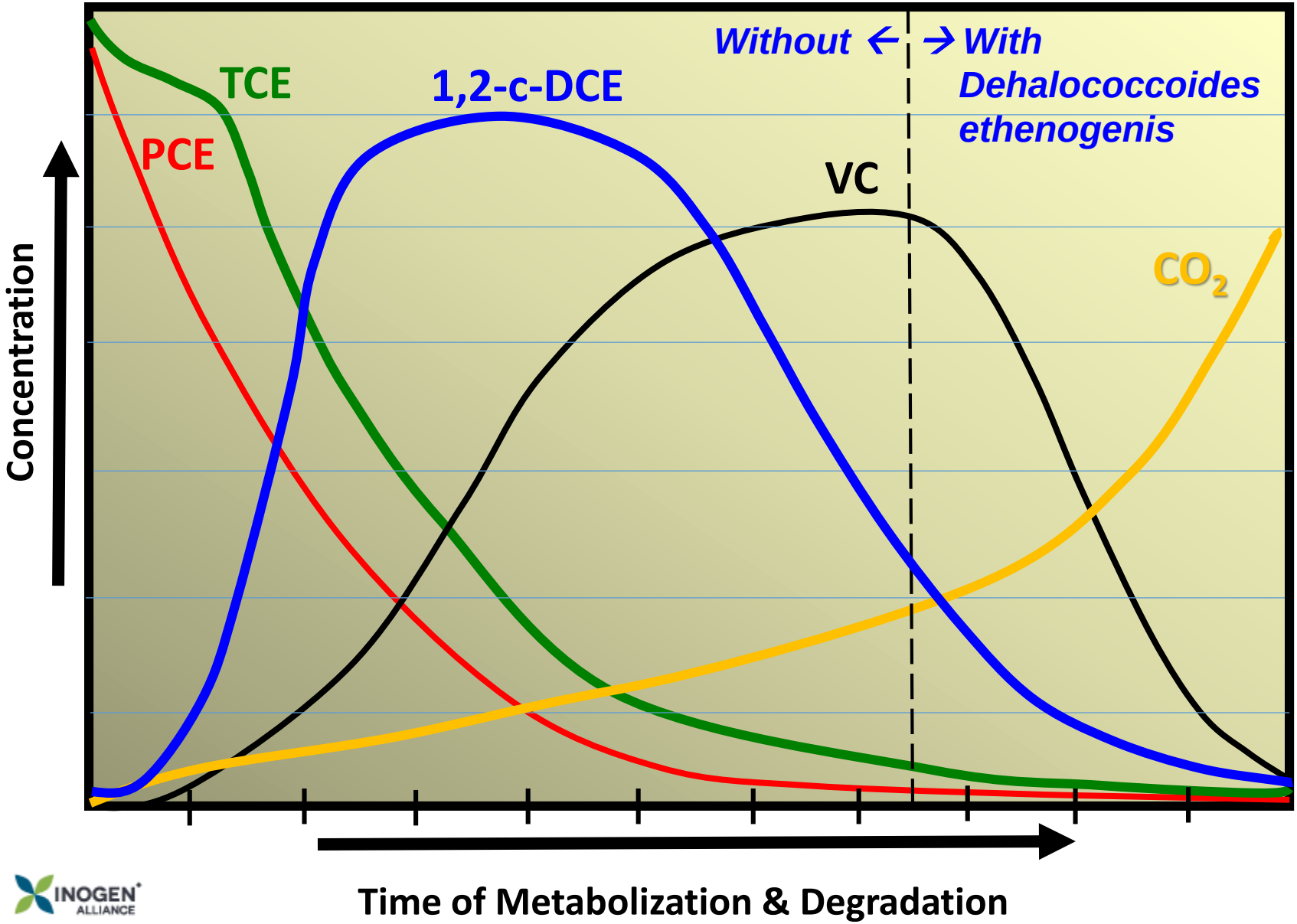
	
BioClean-CHC-KB1 Vessel	1 Pressurization Port 2 Purge Port 3 Pressure Relief and Pressure Gauge Port 4 Inoculation Port

Injection of site specific Bacteria Consortium: SS-BC 83



Step 1: Connecting clean pipes with Argon gas container, BioClean-CHC-KB1 and the injection pipe into the groundwater well	Flushing injection pipe with Argon	Argon is flushing BioClean-CHC-KB1 out of the container directly into the groundwater

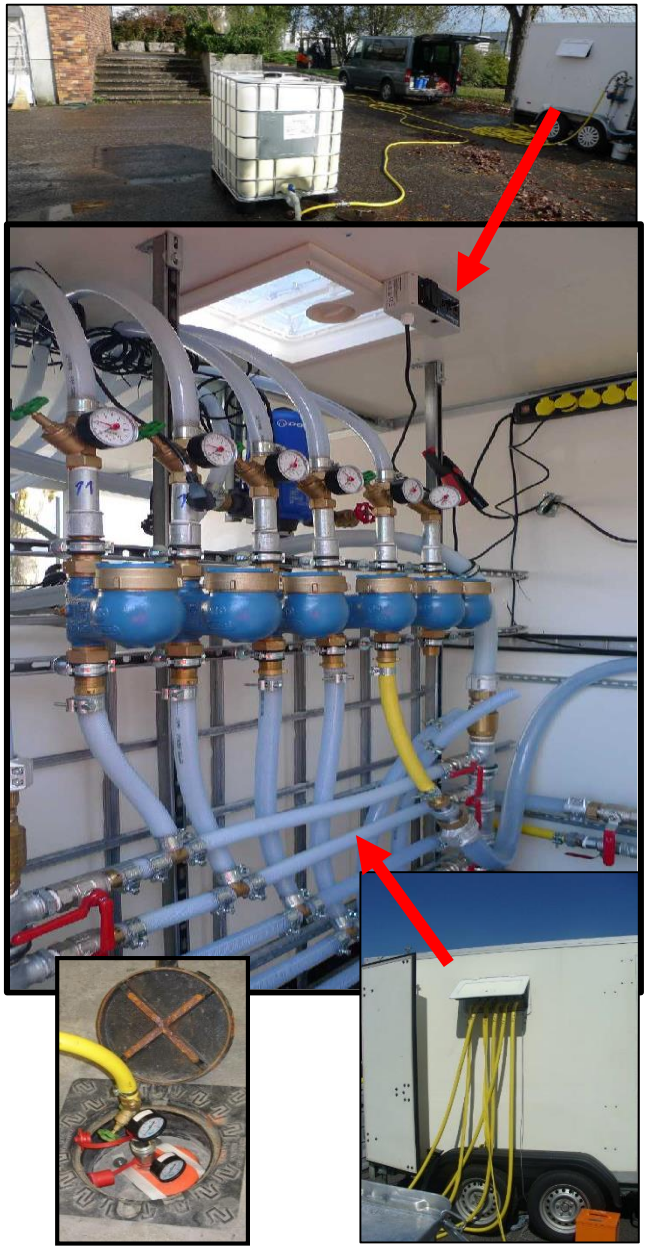
Optimal Degradation of PCE & TCE to DCE & VC



Full Scale Treatment: Starting 2015: Zones A & B

CrVI & HVOC:

- BIOCLEAN -CrVI + KB1 +
- ONE-228-9



CrVI:

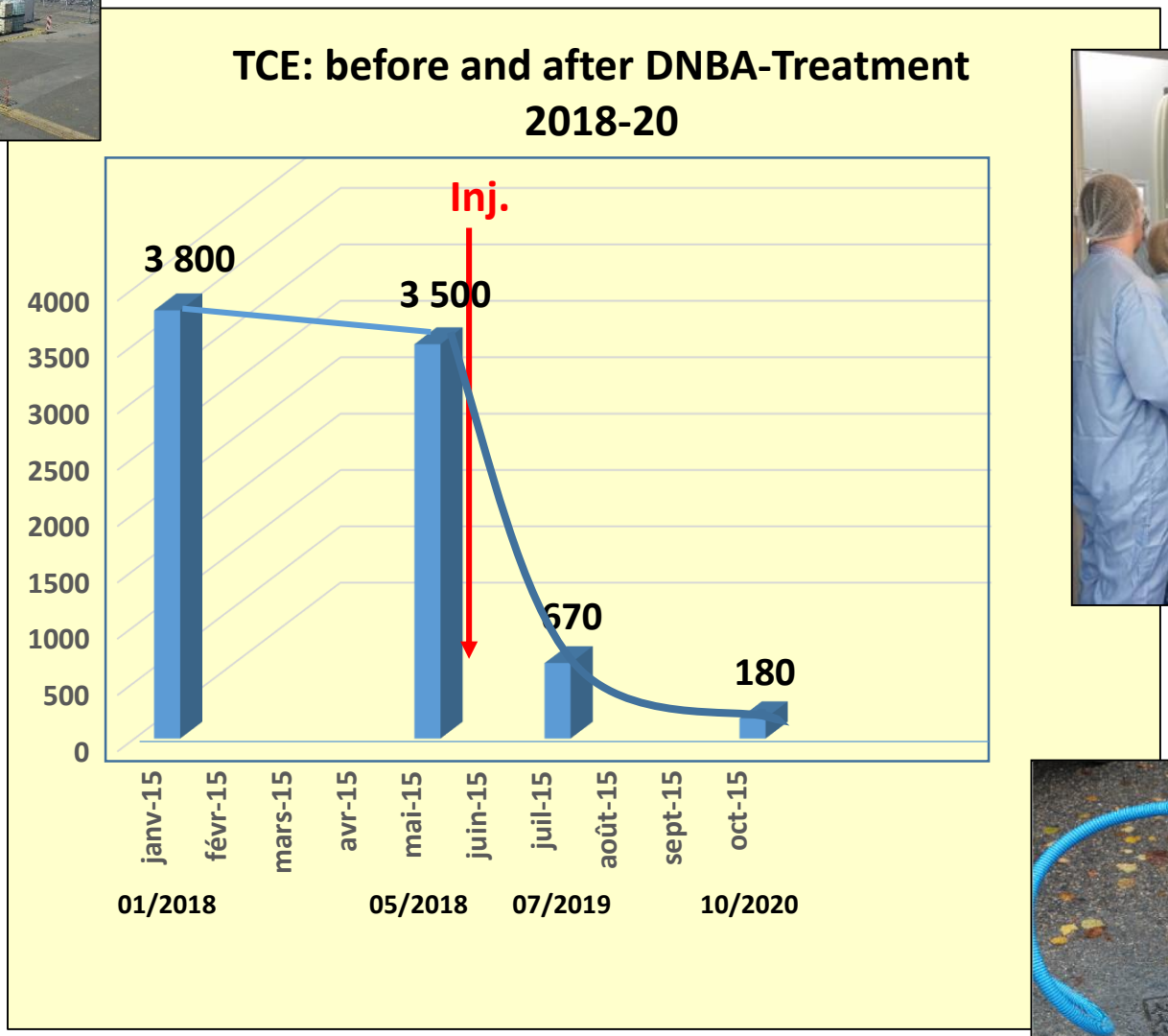
- Zone A & B: 11/2014 – 01/2015 + 06/2015

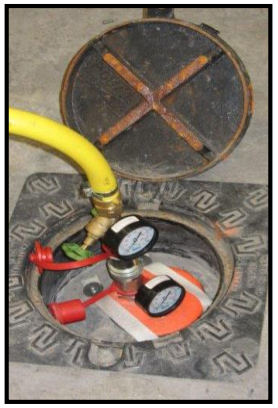
LHKW:

- Zone A: 06/2015

Full Scale DNBA Treatment: ONE & BC Injection

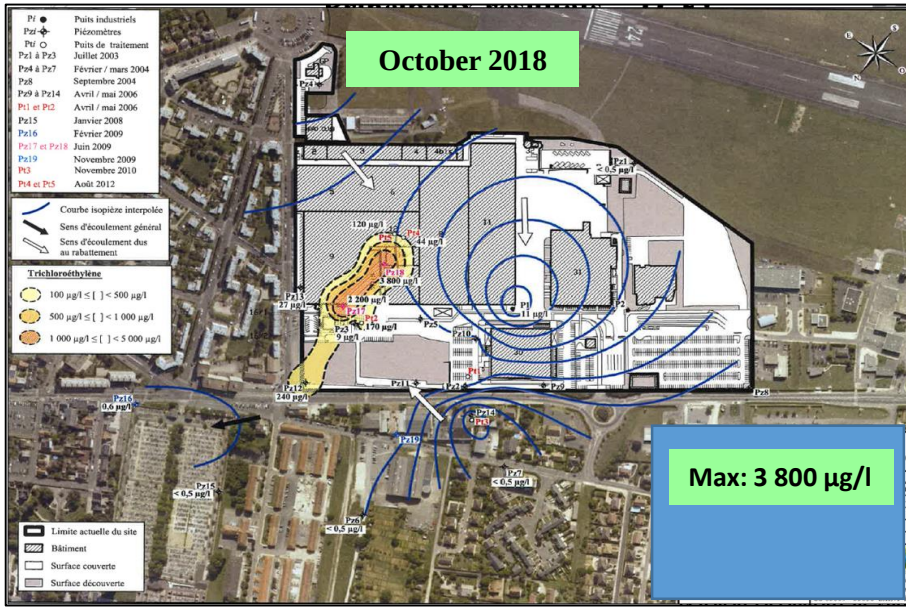
TCE (10/2018 – 10/2019)

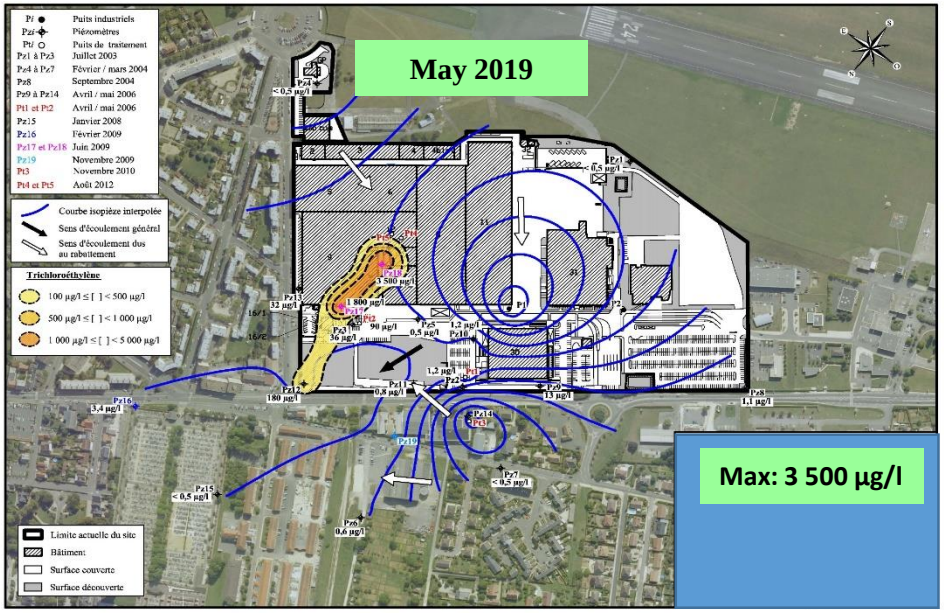


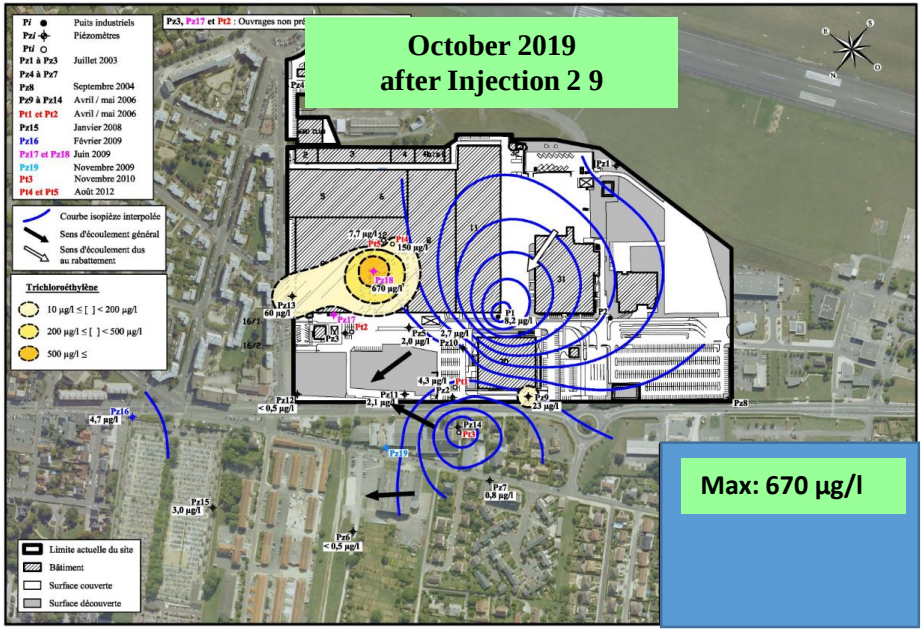


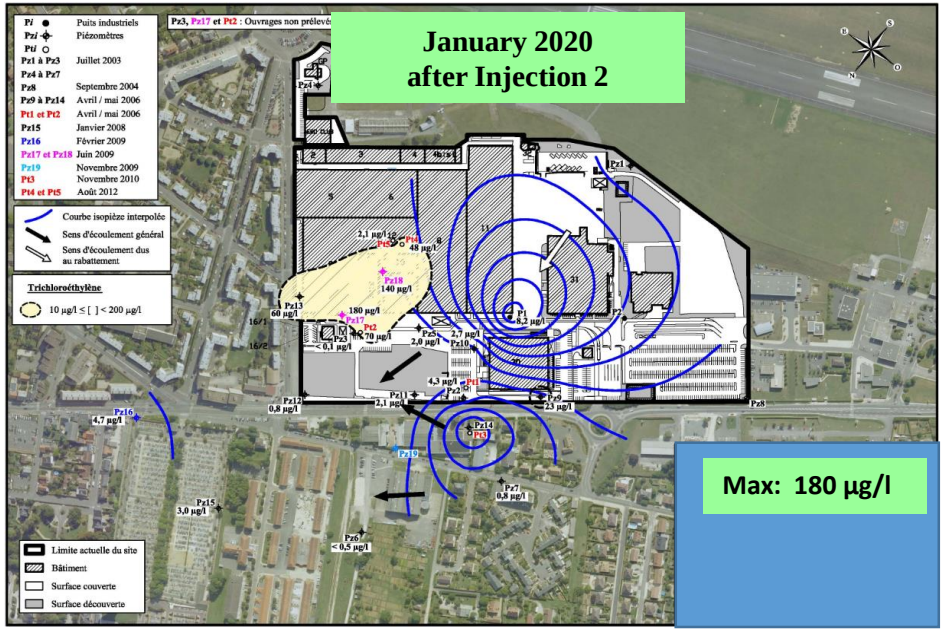
Full Scale DNBA Treatment: ONE & BC Injection

TCE (10/2018 – 01/2020)



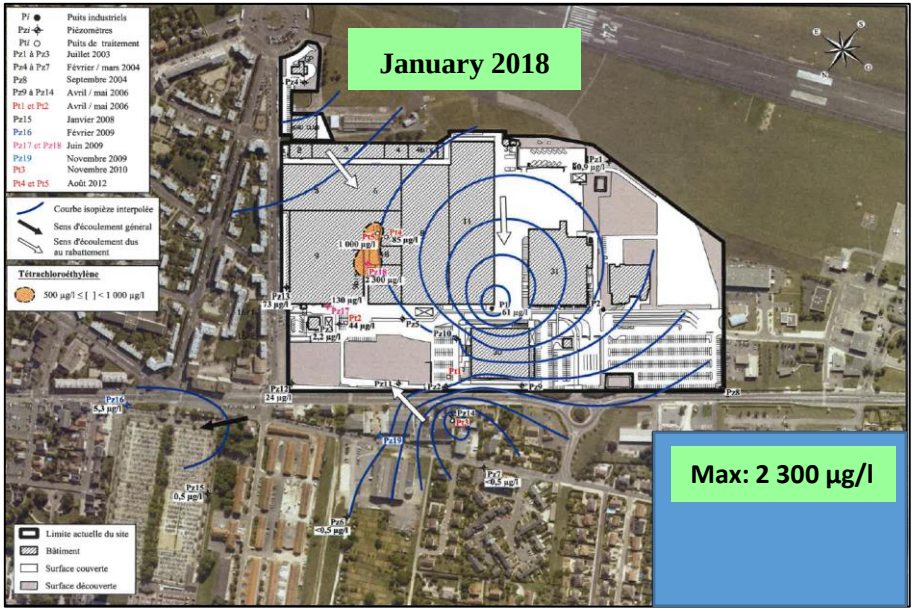


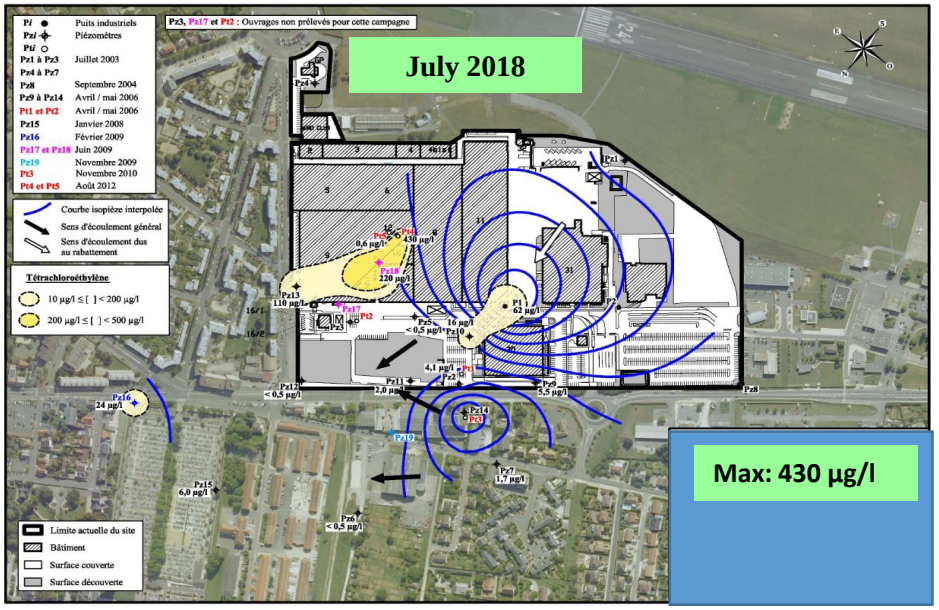


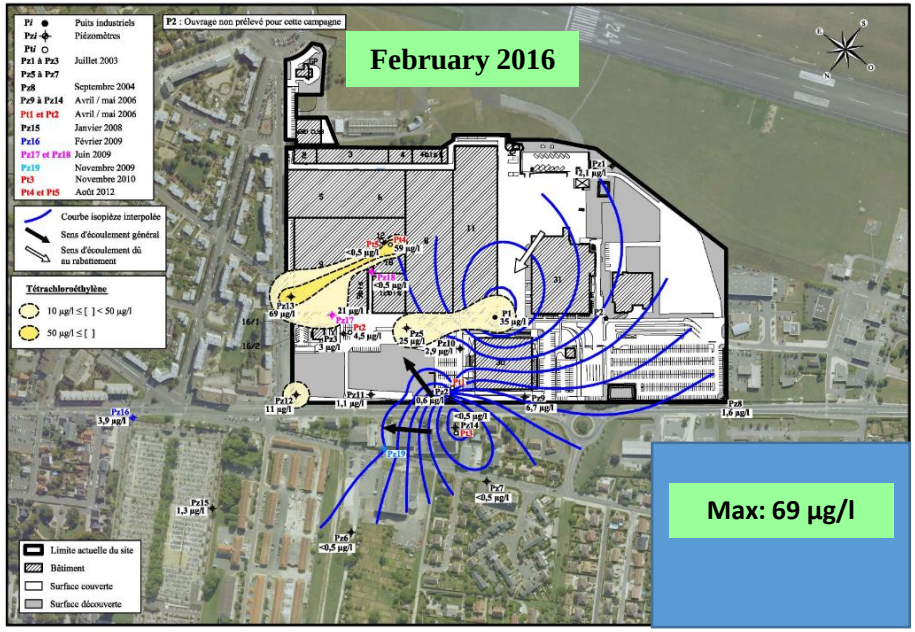


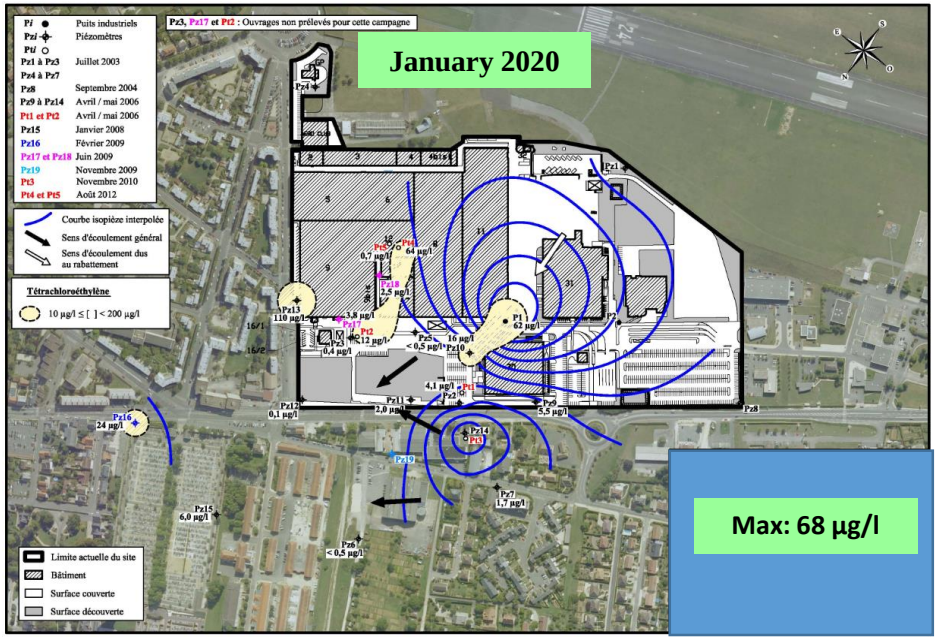
Full Scale DNBA Treatment: ONE & BC Injection

PCE (10/20184 – 01/2020)

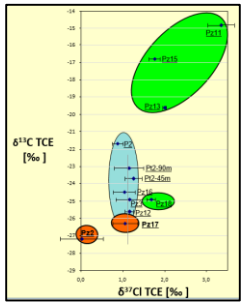


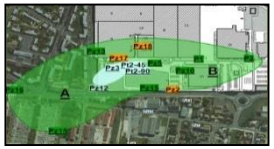




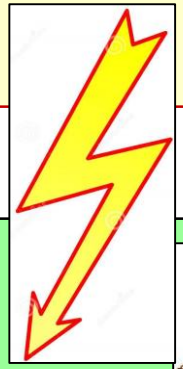


Conclusion :





The technical-economic Feasibility Study and detailed DNBA Investigations was the Basement for the Successfully microbiological DNBA-Remediation by NBA Dynamization.



→ **<u>Cost Saving</u> in Comparison to P&T: Pump & Treat: Min.: 7,8 Million €uros**

→ **<u>Time Saving:</u> Remediation Time of 2 -3 Years in Comparison to P&T: 22 – 35 Years.**