

## Dépollution du sous-sol par traitement in-situ BAND des HET-NSO, HAP, Solvants chlorés, etc.



HPC INTERNATIONAL SAS



**Dr. Frank KARG / CEO (PDG) HPC INTERNATIONAL SAS / France**

Scientific Director of HPC-Group International

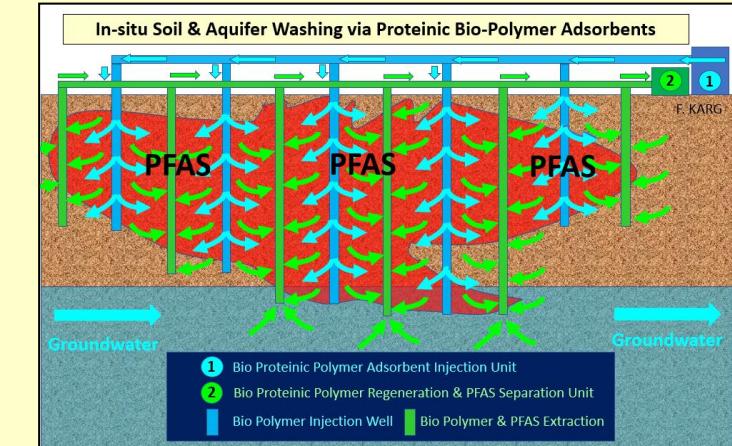
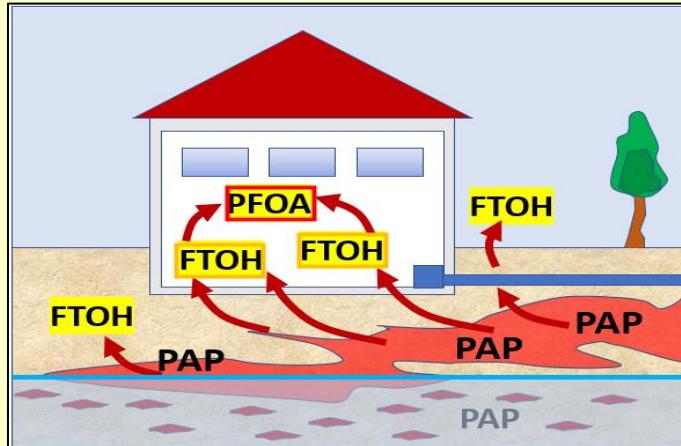
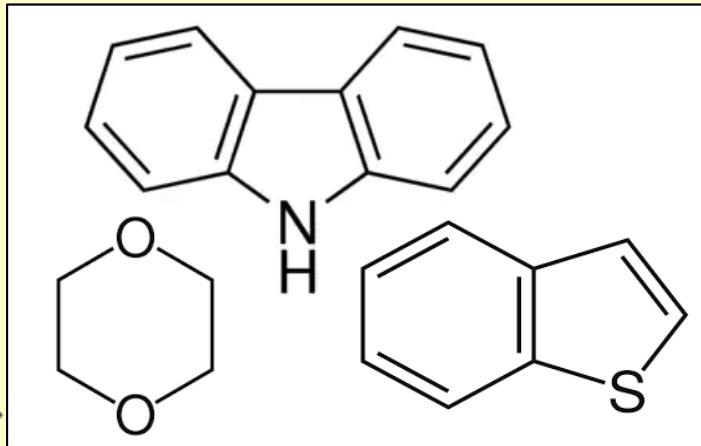
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**Micropolluants toxiques et les biotechnologies des traitements de dépollution in-situ microbiologiques et biochimiques applicables : Exemples des BAND  
(Bio-Atténuation Naturelle Dynamisée) /  
L'Approche au meilleur bilan Coût-avantages**

**Toxic Pollutants and Application of Bio-Technologies by microbiological & bio-chemical in-situ-Treatments: Examples DNBA (Dynamized Natural Bio-Attenuation) /  
The most Cost-effective Approach**

Dr. (es. Sc.) Frank Karg / Scientific Director of HPC-Group (INOGEN JV) &  
CEO-President of HPC INTERNATIONAL / France, Germany, Suisse, Hungary, Balkan, etc.

Email: [frank.karg@hpc-international.com](mailto:frank.karg@hpc-international.com) / Tél: +33 607 346 916



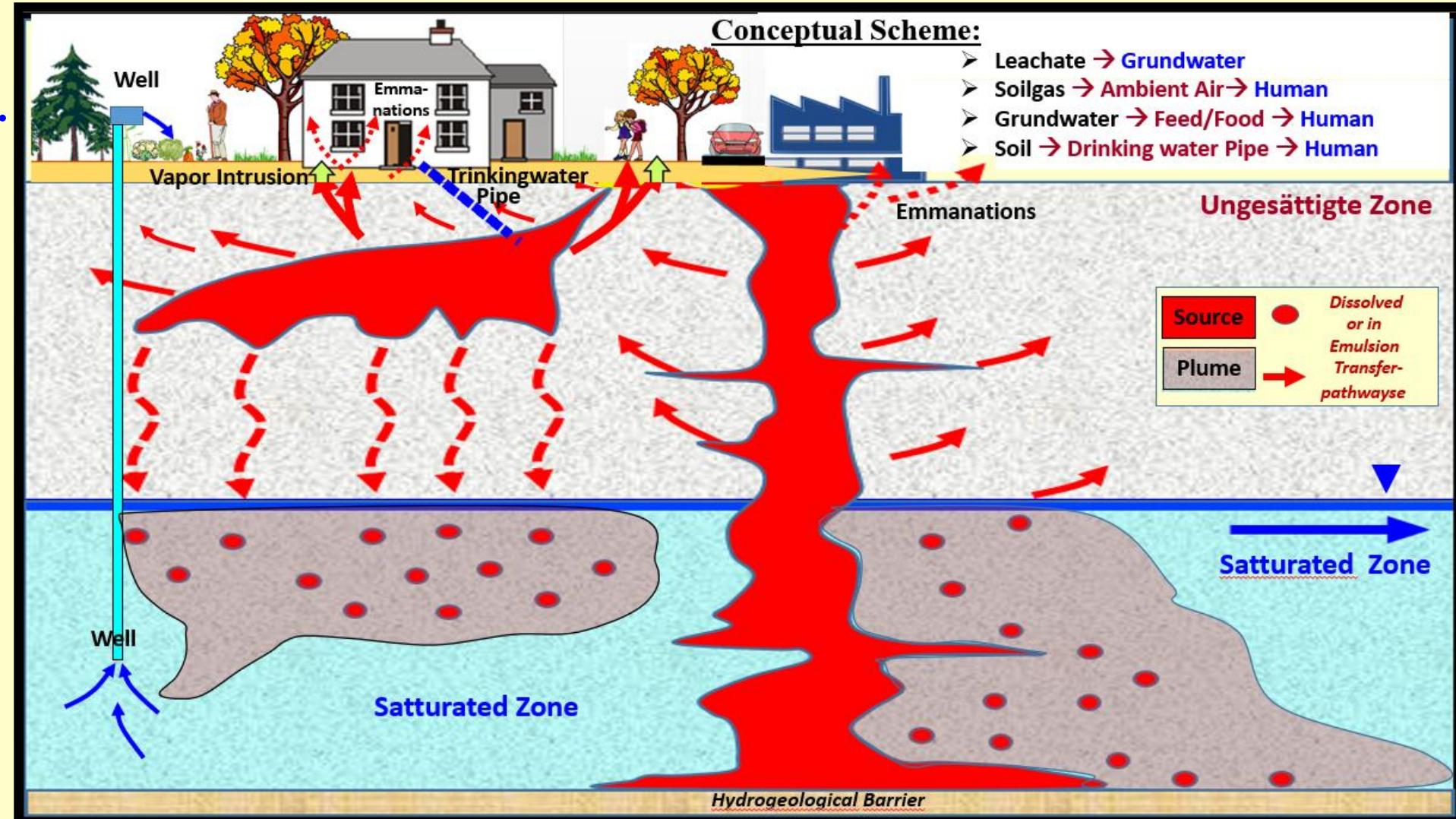
## Polluants : Solvants chlorés, HET, HAP, etc.

HET: Hétérocycliques & Amines aromatiques.

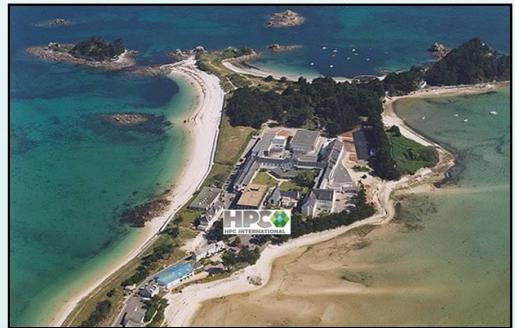
Solvants chlorés (PCE, TCE, DCE, CV, etc.).

HAP (Hydrocarbures Aromatiques, Polycycliques)

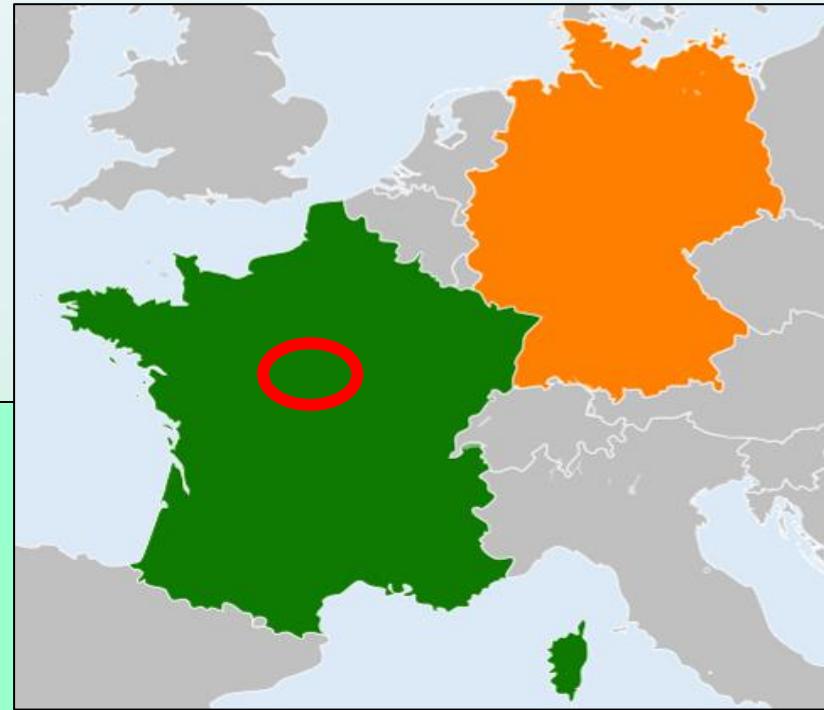
Pollutions sous forme de LNAPL, DNAPL (huiles de goudron et dissoutes).



Dépollution du sous-sol par traitement in-situ  
BAND des HET-NSO, HAP, Solvants chlorés, etc.



## In-situ DNBA: Dynamized Natural Bio-Attenuation



Project Example: Aerospatiale/

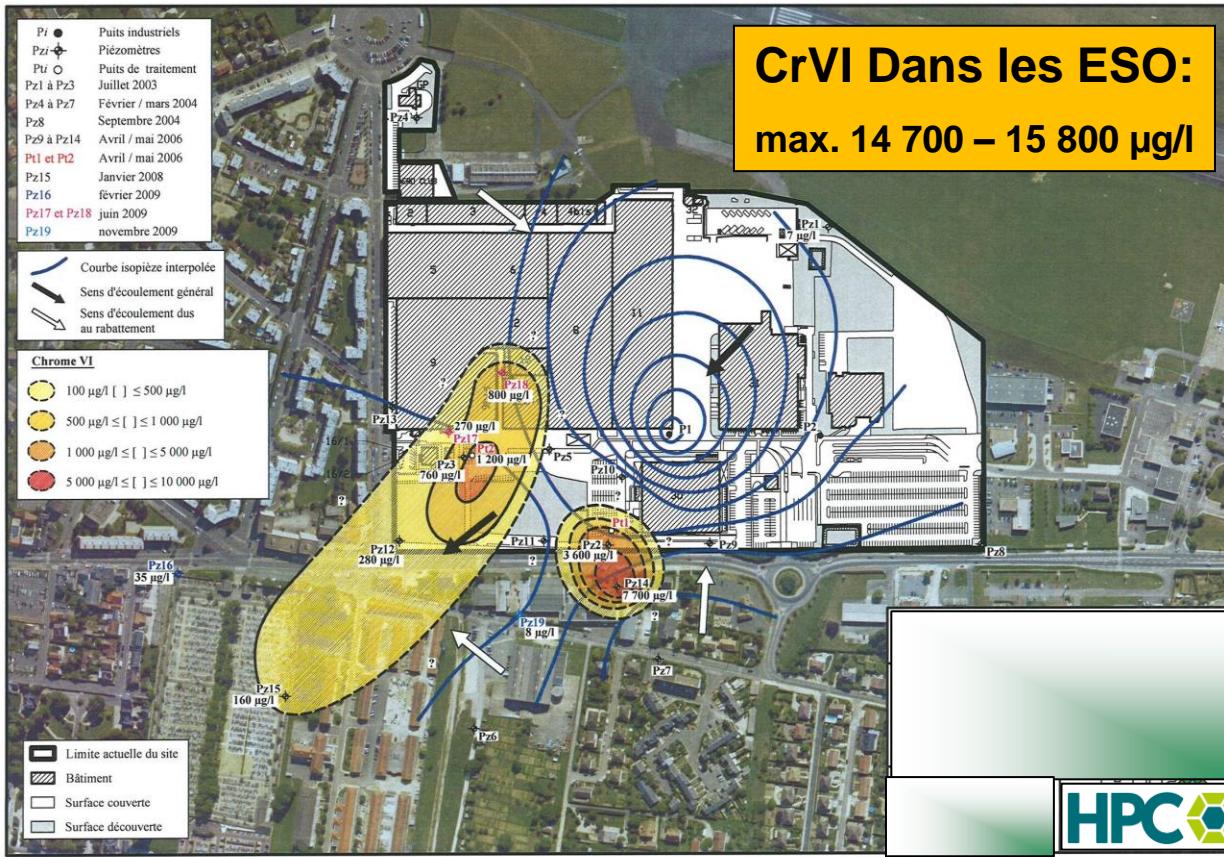
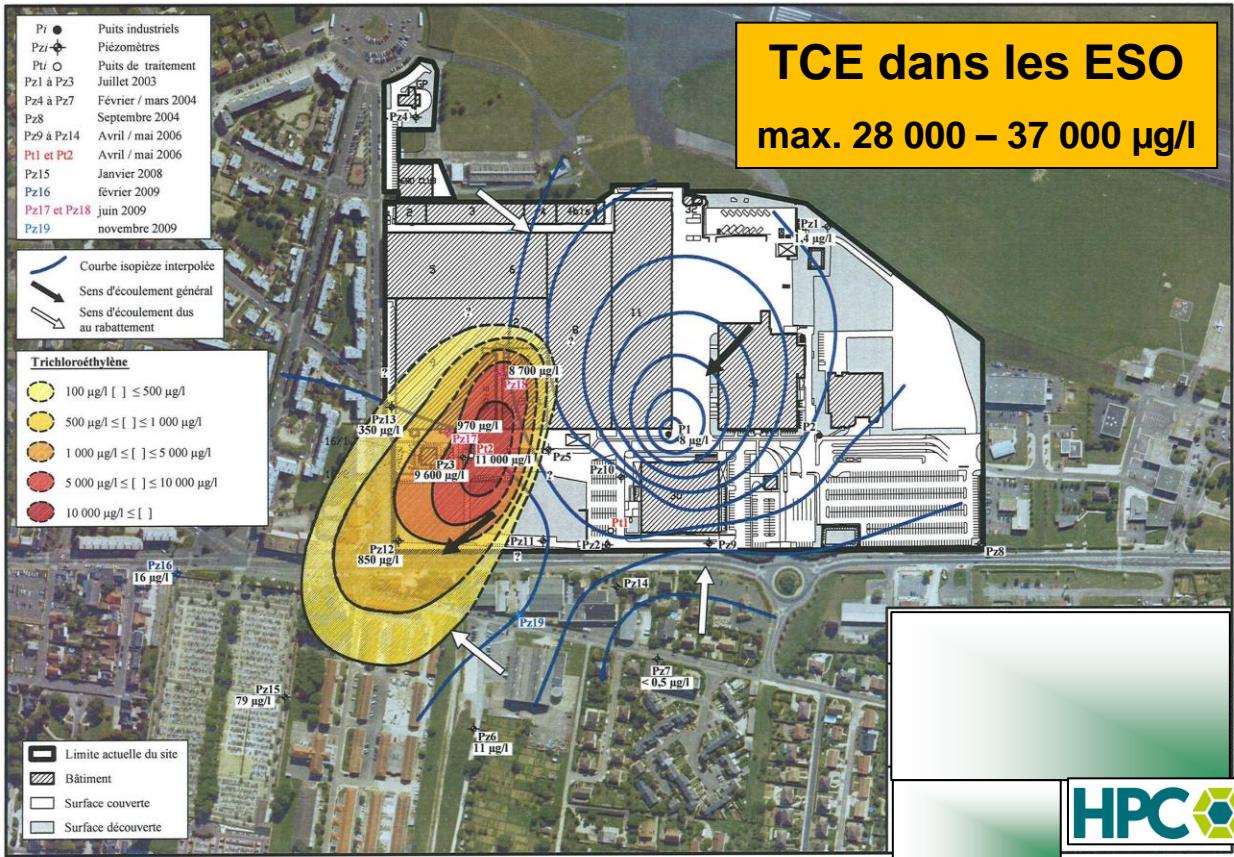
Chlorinated Solvents + Cr6:

Microbiological In-situ-Treatment

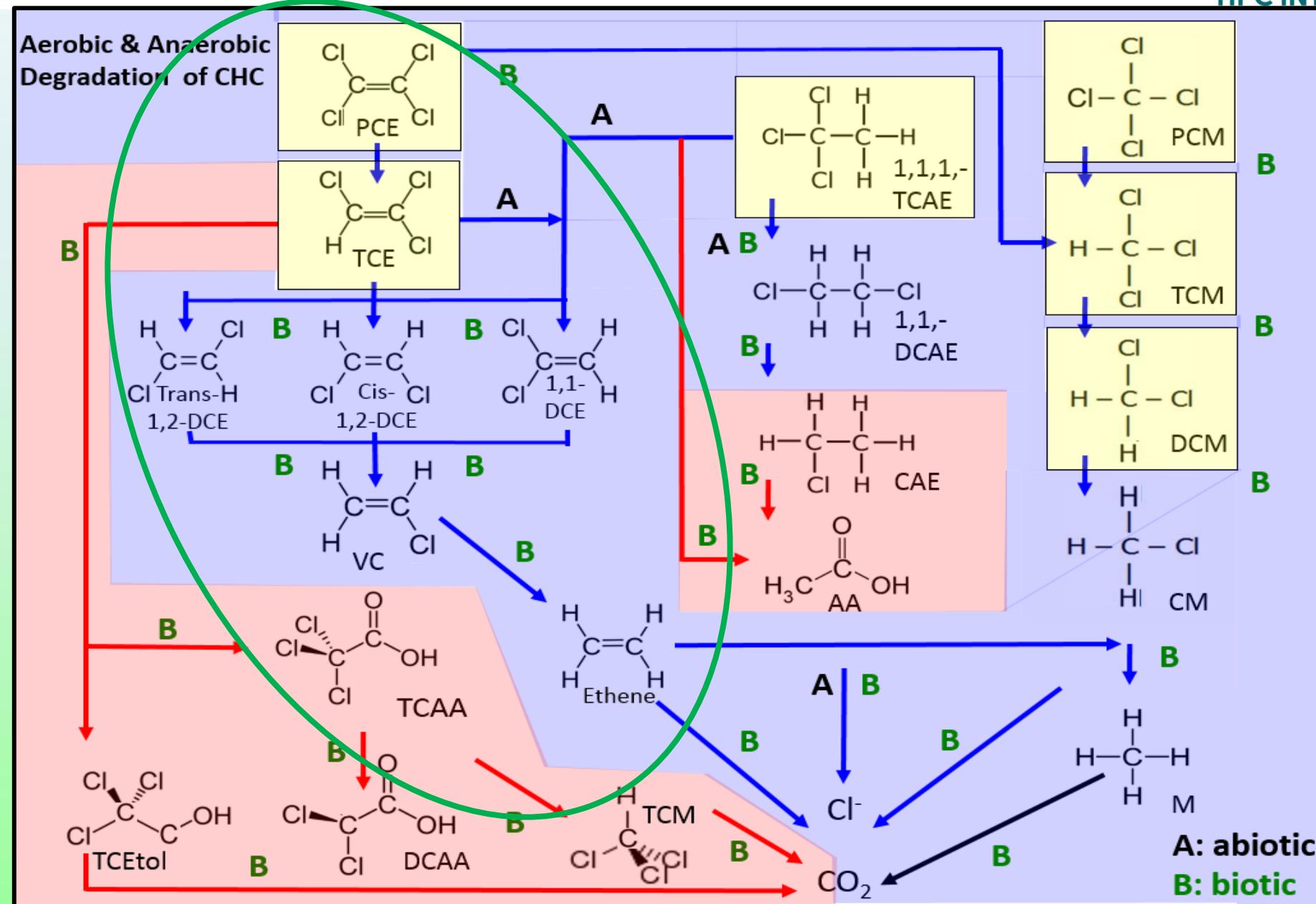
Bio-Technologies & Genetic Bacteria Selection



# Project Example: DNBA: Chlorinated Solvents + Cr6: Microbiological In-situ-Treatment via DNBA (France)



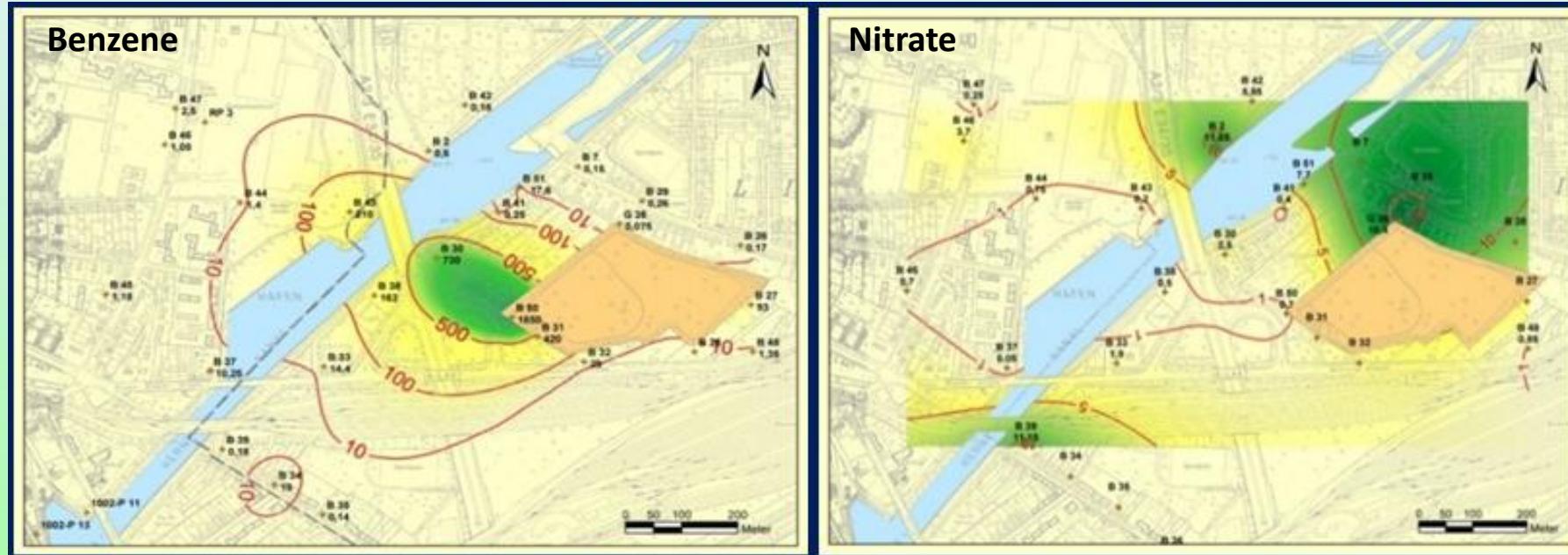
Project Example: DNBA: Chlorinated Solvents + Cr6:  
Microbiological In-situ-Treatment via DNBA



## Aerobic and anaerobic Degradation Reactions of TCE Reduction of various electron acceptors. PCE Degradation only in anaerobic conditions.

Reaction equation of the TCE Degradation		Eh [mV]
Oxygen consumption	$C_2HCl_3 + O_2 \rightarrow C_2Cl_3O_2H$ $C_2Cl_3O_2H + O_2 + 2 H^+ \rightarrow 2 CO_2 + 3 H^+ + 3 Cl^-$	> 740
Nitrate reduction	$3 C_2HCl_3 + 2 NO_3^- \rightarrow 3 C_2Cl_3O_2H + N_2$ $3 C_2Cl_3O_2H + 2 NO_3^- + 6 H^+ \rightarrow 6 CO_2 + N_2 + 9 H^+ + 9 Cl^-$	500 → 740
Nitrate reduction	$3 C_2HCl_3 + 2 NO_3^- + 6 H^+ \rightarrow 3 C_2Cl_3O_2H + 2 NH_3^+$ $3 C_2Cl_3O_2H + 2 NO_3^- + 6 H^+ \rightarrow 6 CO_2 + 2 NH_3^+ + 9 H^+ + 9 Cl^-$	0 → 500
Manganese reduction	$3 C_2HCl_3 + 3 MnO_2 \rightarrow 3 C_2Cl_3O_2H + 3 Mn^{2+}$ $3 C_2Cl_3O_2H + 3 MnO_2 + 6 H^+ \rightarrow 6 CO_2 + 3 Mn^{2+} + 9 H^+ + 9 Cl^-$	-50 → 0
Iron reduction	$3 C_2HCl_3 + 3 Fe^3O(OH) \rightarrow 3 C_2Cl_3O_2H + 3 Fe^{2+}$ $3 C_2Cl_3O_2H + 3 Fe^3O(OH) + 6 H^+ \rightarrow 6 CO_2 + 3 Fe^{2+} + 9 H^+ + 9 Cl^-$	-240 → -50
Sulfate reduction	$2 C_2HCl_3 + SO_4^{2-} + 10 H^+ \rightarrow C_2H_4 + 2 CO_2 + H_2S + 6 H^+ + 6 Cl^-$	< -240
Methanogese	$2 C_2HCl_3 + 4 H_2O + 4 H^+ \rightarrow 2 CH_4 + 2 CO_2 + 6 H^+ + 6 Cl^-$	

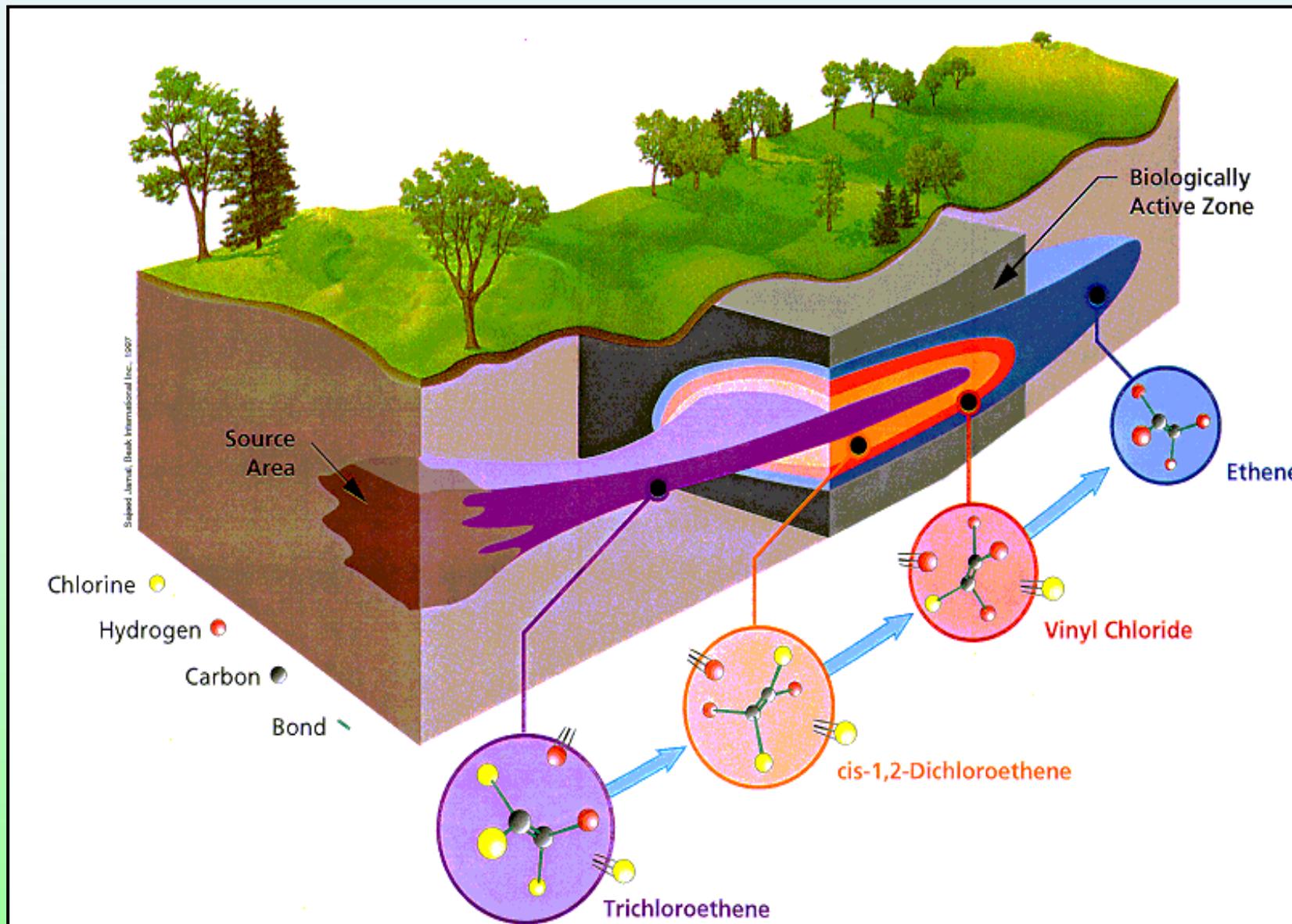
# Microbiological Pollutant Degradation and Electron Acceptor use : Nitrate



Project Example: DNBA: Chlorinated Solvents + Cr6:  
Microbiological In-situ-Treatment via DNBA



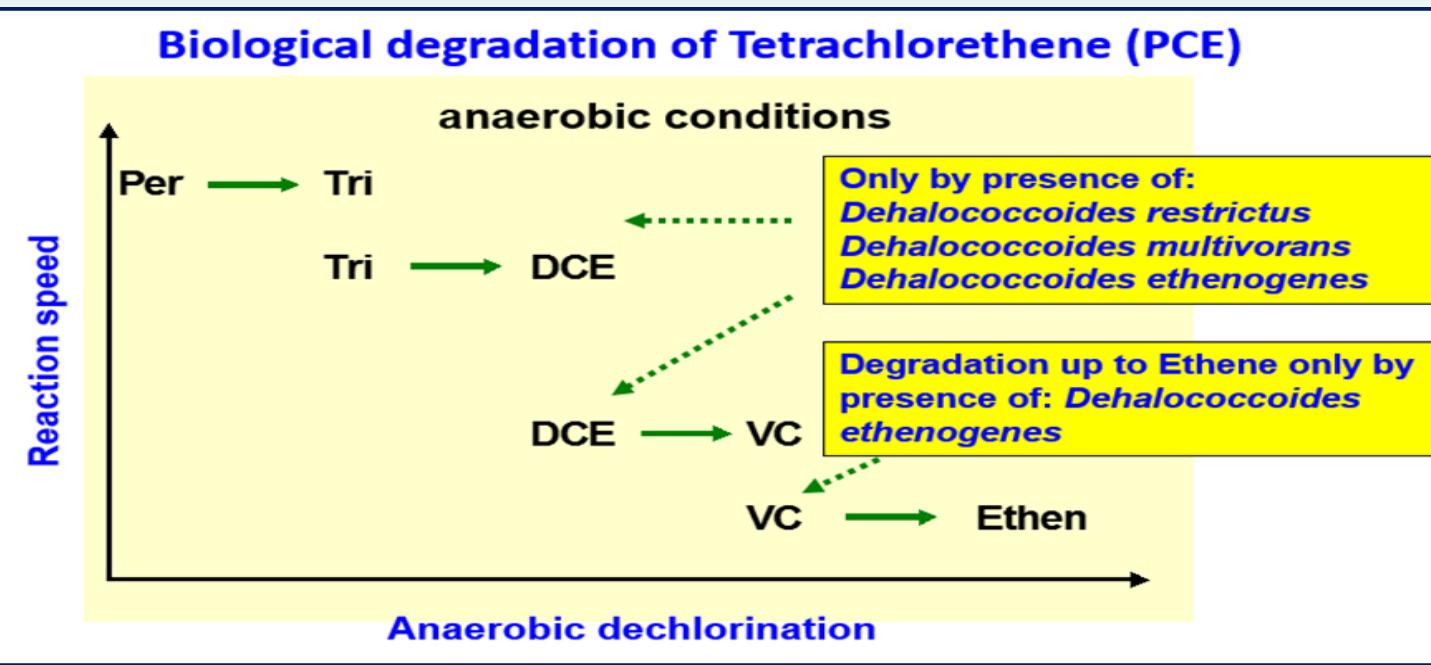
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## Project Example: DNBA: Chlorinated Solvents + Cr6: Microbiological In-situ-Treatment via DNBA



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Suche von abbauenden Mikroorganismen und der Ausdehnung des natürlichen Bio-Reaktors per Gen-Tests: PCR

Beprobung der standort-spezifischen Biomasse mittels mikrobiologischem Passivsammler

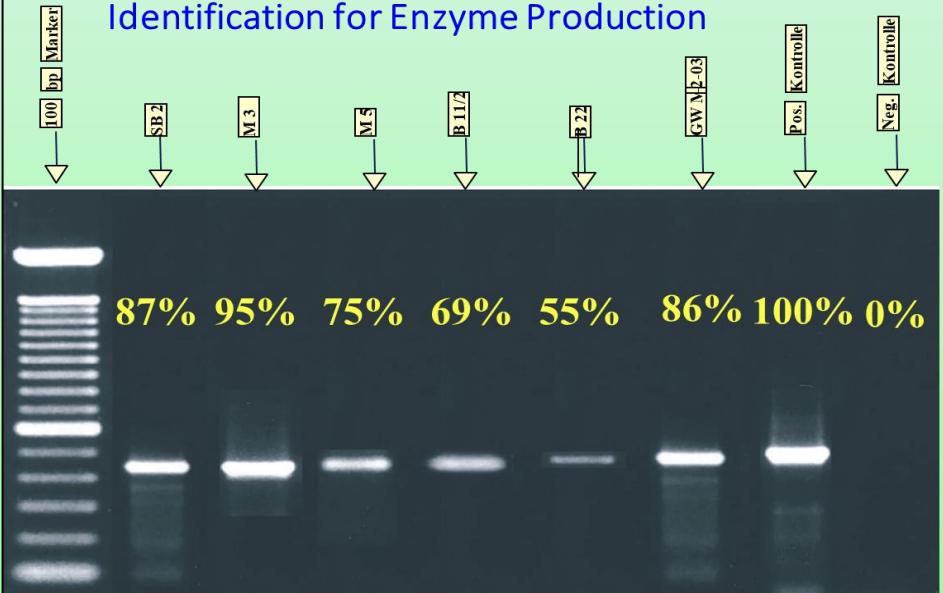


**PCR**

## PCRq-based Bacteria Selection:

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

Competitive PCRq and DNA Sequence Identification for Enzyme Production



## Project Example: DNBA: Chlorinated Solvents + Cr6: Microbiological In-situ-Treatment via DNBA



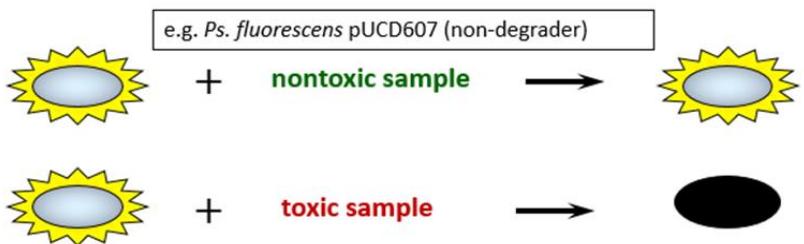
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### Microbiological ecotoxicity

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

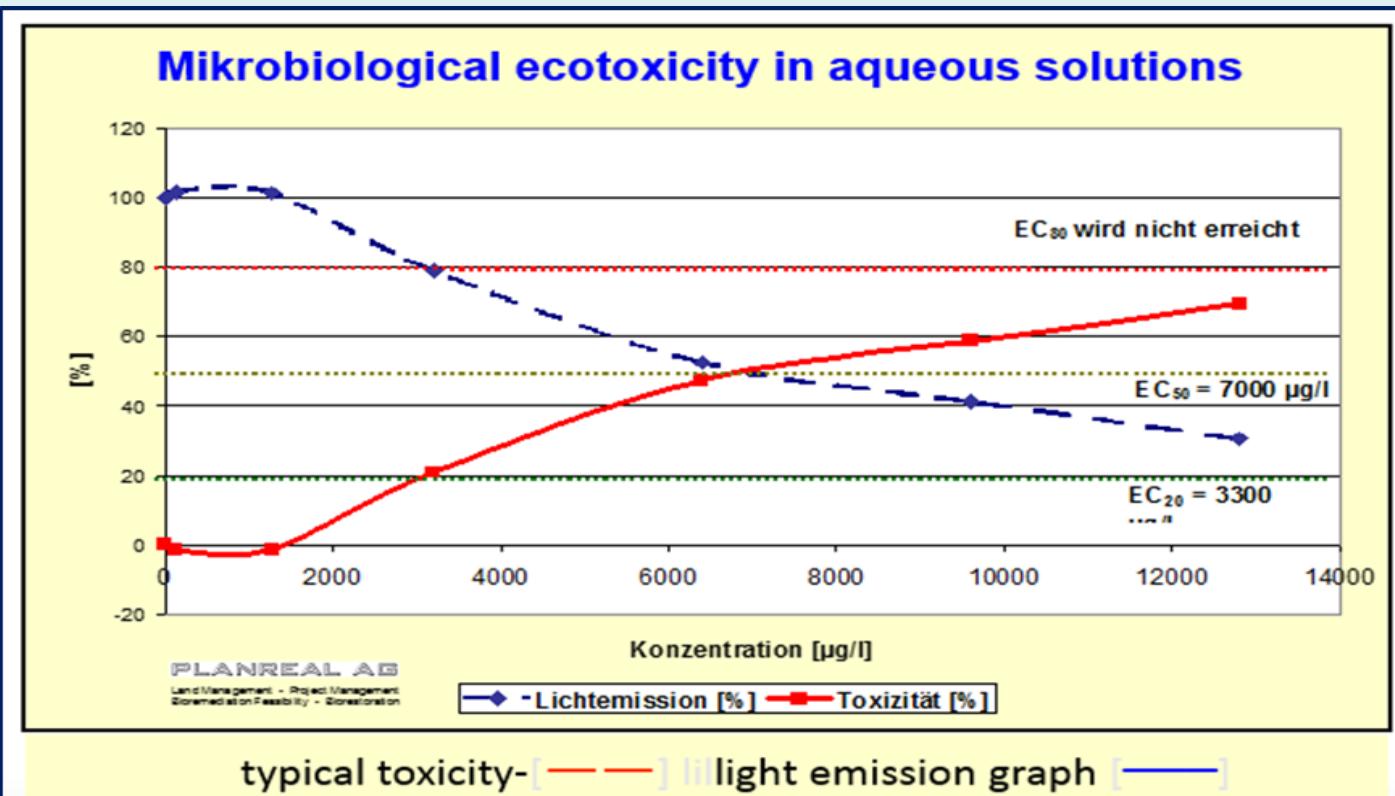
**Toxiometer tests** (Measurement of the metabolic activity of selected soil bacteria strains by using luminous soil bacteria)

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



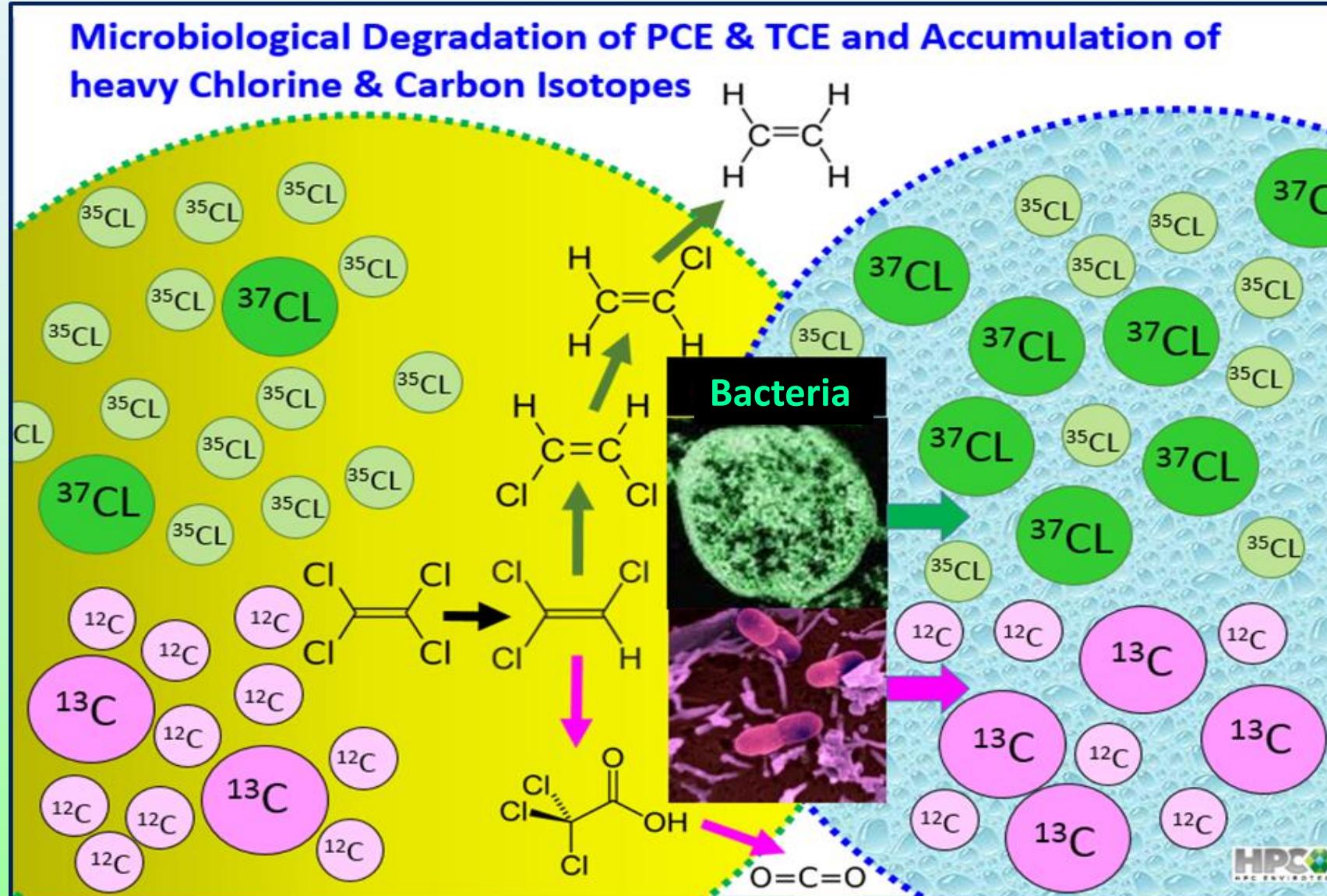
## Microbiological Excotoxicity:

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



Mapping of **constraint zones** of microbiological TCE & PCE degradation → Treatment of Cr<sup>6+</sup> first!

## Isotopic Fractioning to Identify in-situ Microbial Biodegradation



## DNBA-Treatment:

- **SENS: Substratum – Emulsifier – Nutriments - Stabilizer** for anaerobic Aquifer Conditions:

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

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

>Bio-available degradable Surfactants / Alginates from Brittany / Roscoff or Emulsion Stabilization,

>Special Nutriments & Oligo-elements, to dynamize the Bio-degradation (constituted Site specifically):

→ For reductive, anaerobic Dehalogenation of the HVOC and

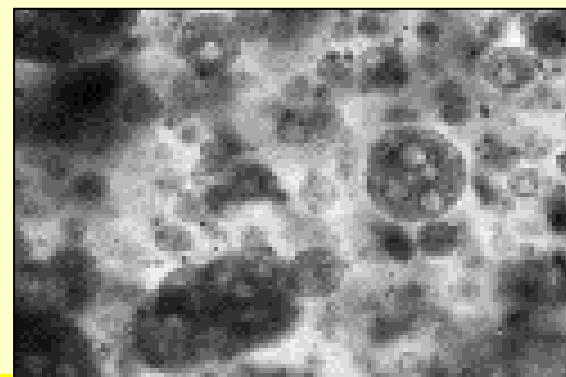
→ For reductive Immobilization of CrVI to CrIII.

- **SSMC: Site Specific Microbiological Consortium:**

*Dehalococcoides restrictus, Dehalococcoides multivorans, Dehalococcoides ethenogenes:*

→ Anaerobic Degradation of PCE, TCE to cis-DCE (SENS + SSMC-KB1®),

→ Aerobic Degradation of cis-DCE to Ethene / CO<sub>2</sub> SSMC-162).



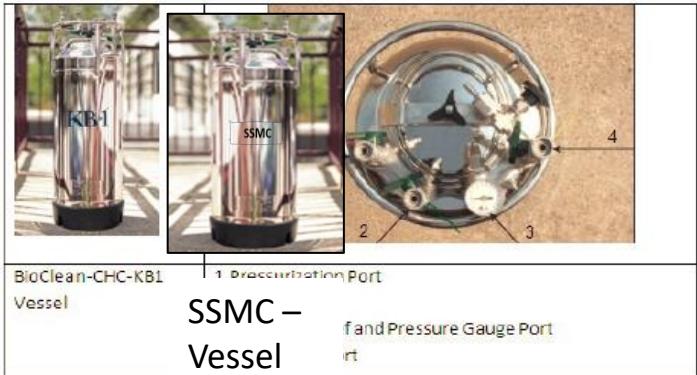
## Project Example: DNBA: Chlorinated Solvents + Cr6: Microbiological In-situ-Treatment via DNBA

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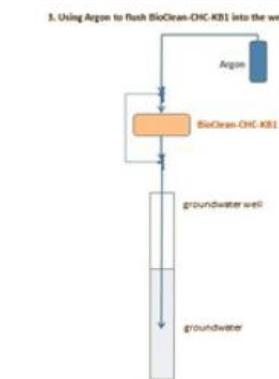
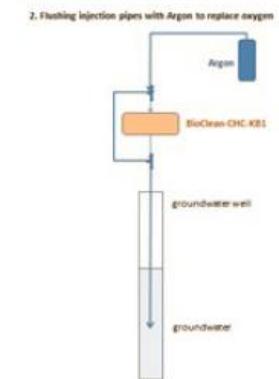
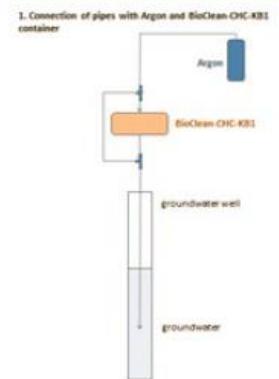
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### Injection Phase 2 → SSMC-CHC-KB-1

SSMC is delivered in stainless steel container for injection under Nitrogen or Argon



#### Injection of SSMC at the site "B"



Step 1: Connecting clean pipes with N<sub>2</sub> or Ar container & SSMC and Injection pipe into the Groundwater

Step 2: Flushing Injection pipe with N<sub>2</sub> or Ar Flow

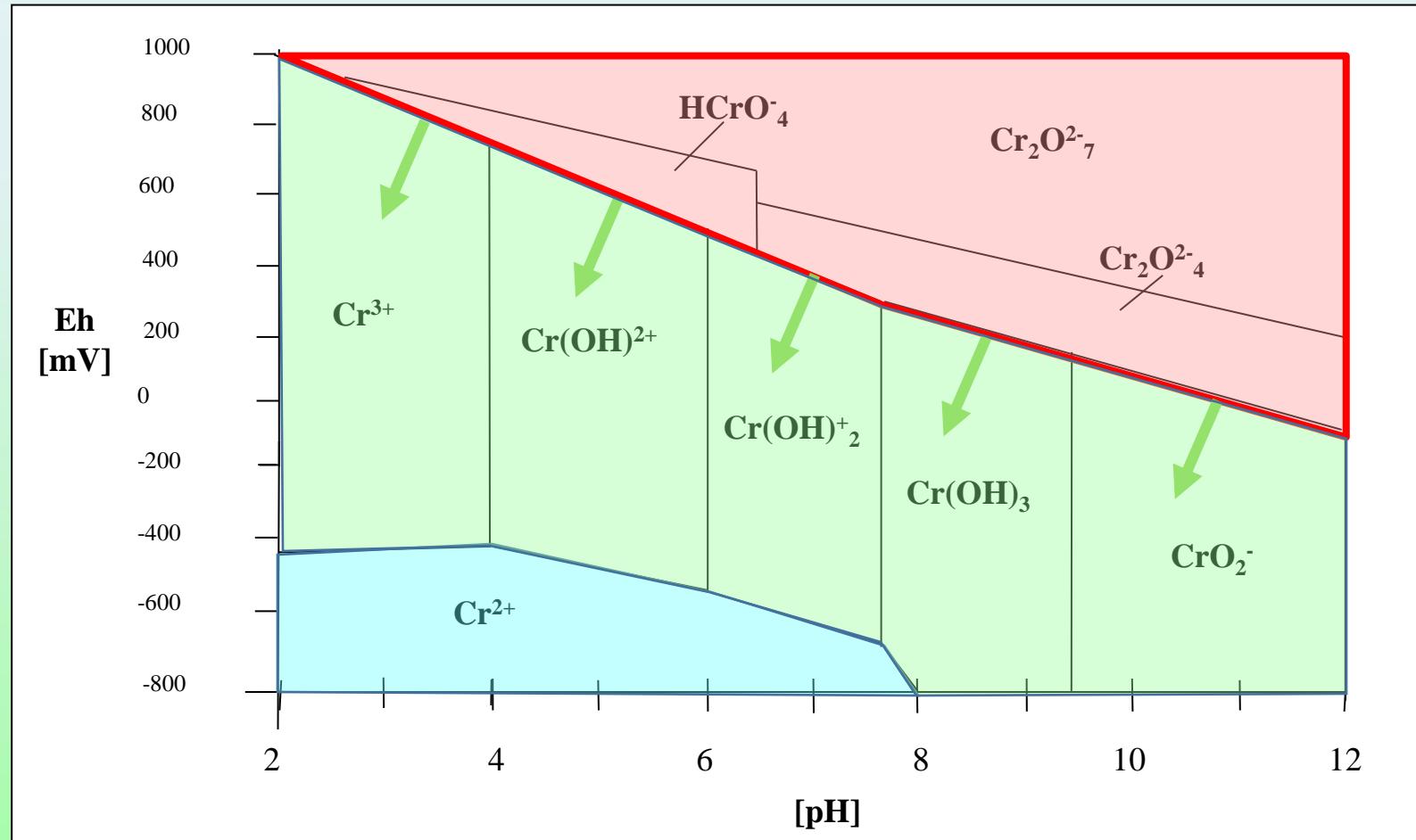
N<sub>2</sub> or Ar flushing SSMC out of the container directly into the Groundwater

 A Partner of  
Inogen Environmental Alliance

### Production and injection of Bacteria Consortium



## Goal: Reduction of CrVI to CrIII and Immobilisation

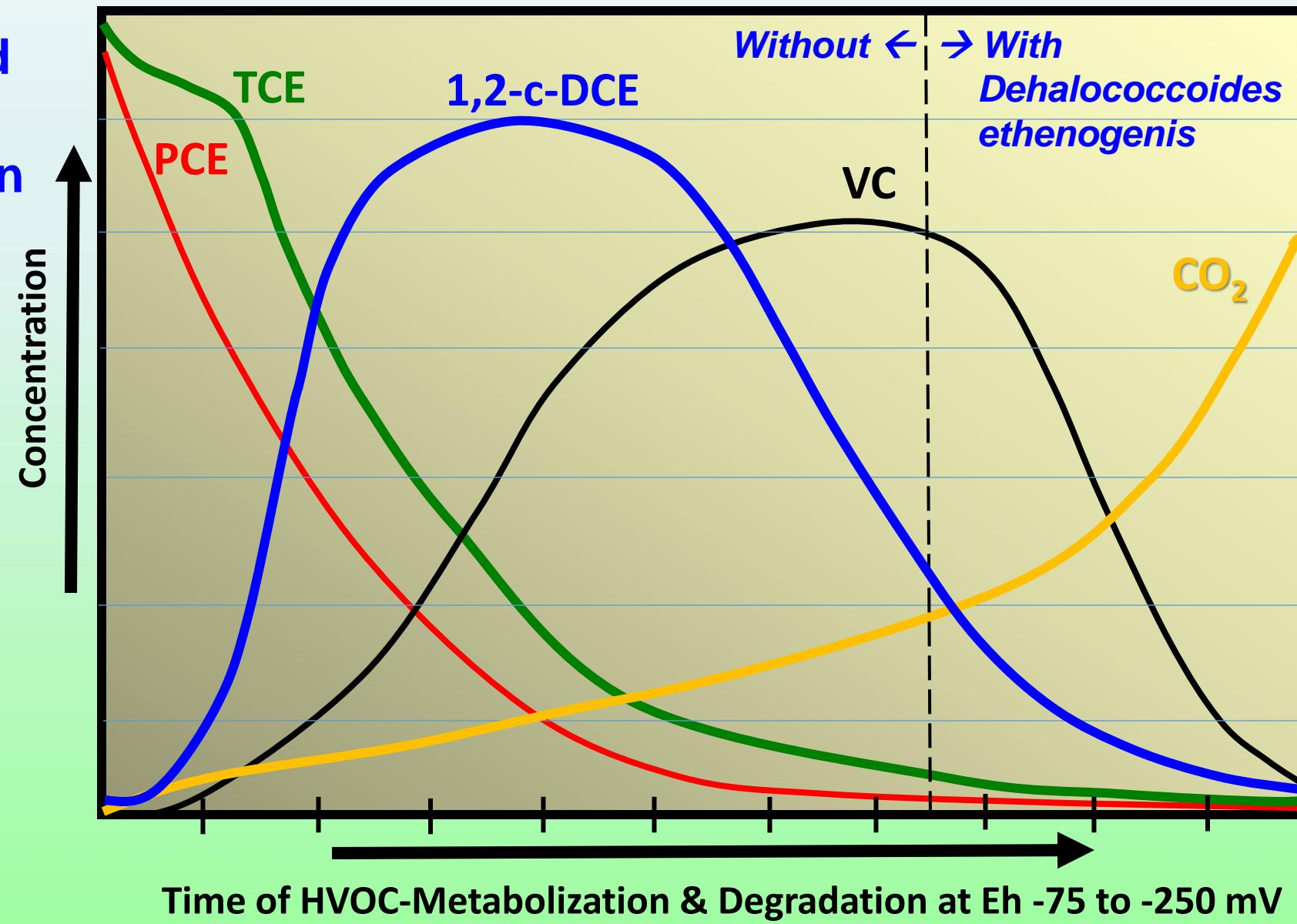


Project Example: DNBA: Chlorinated Solvents + Cr6:  
Microbiological In-situ-Treatment via DNBA



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Chlorinated  
Solvent  
Degradation

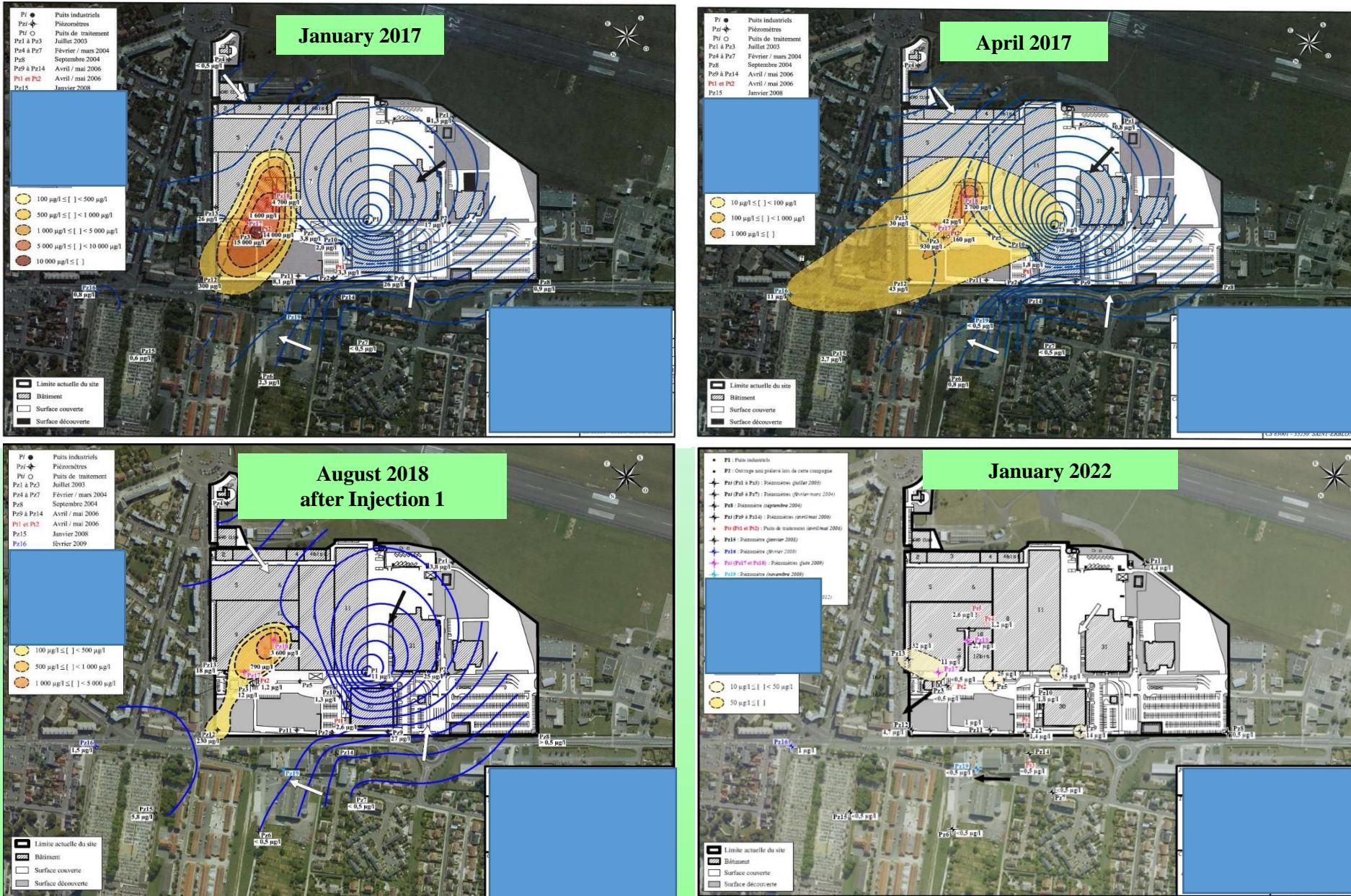


# Project Example: DNBA: Chlorinated Solvents + Cr6: Microbiological In-situ-Treatment via DNBA

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TCE

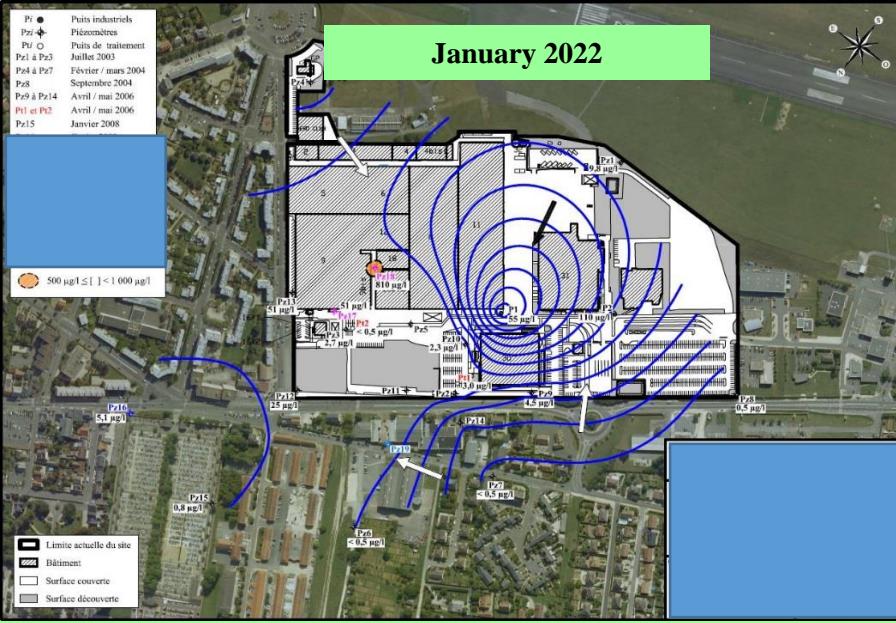
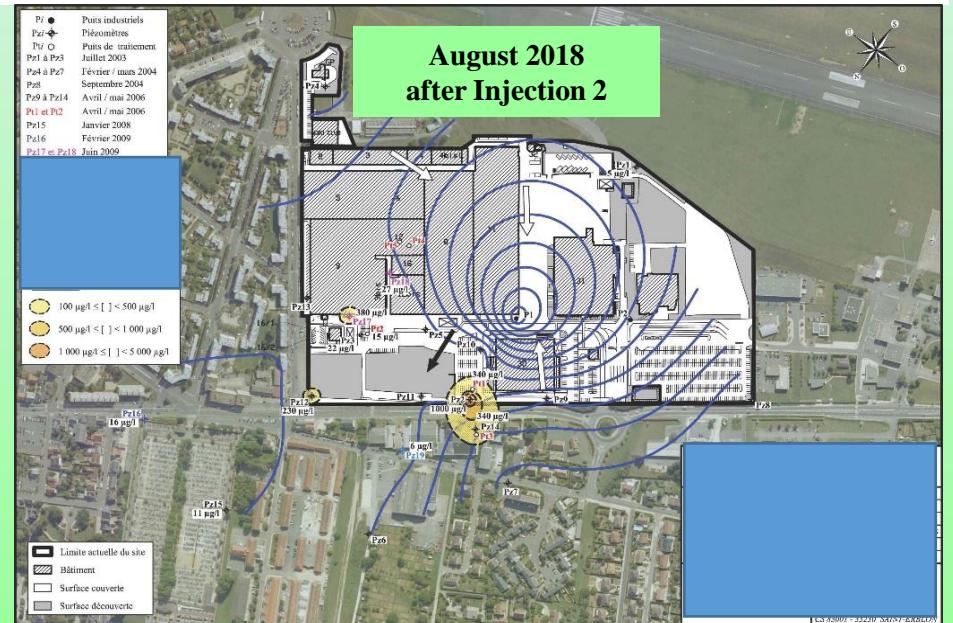
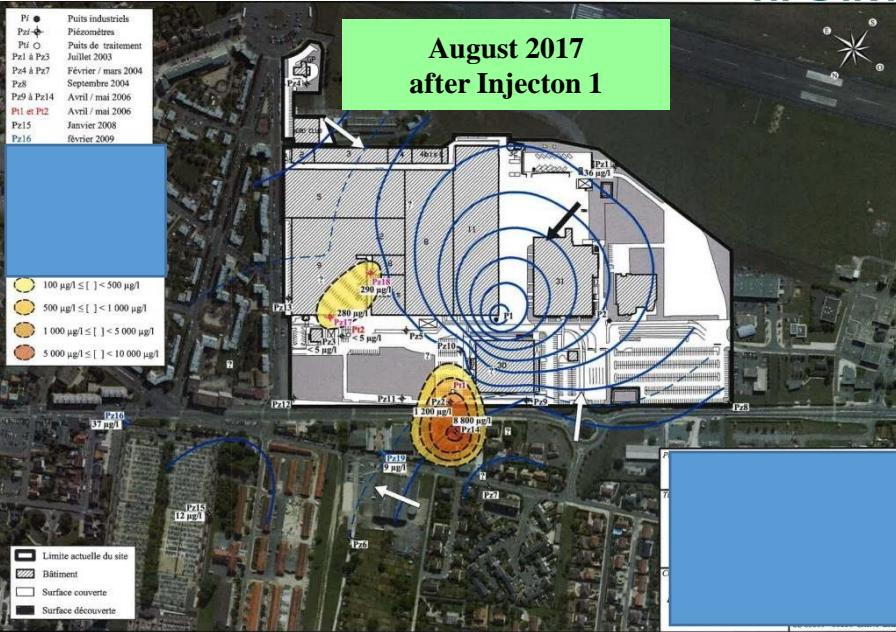
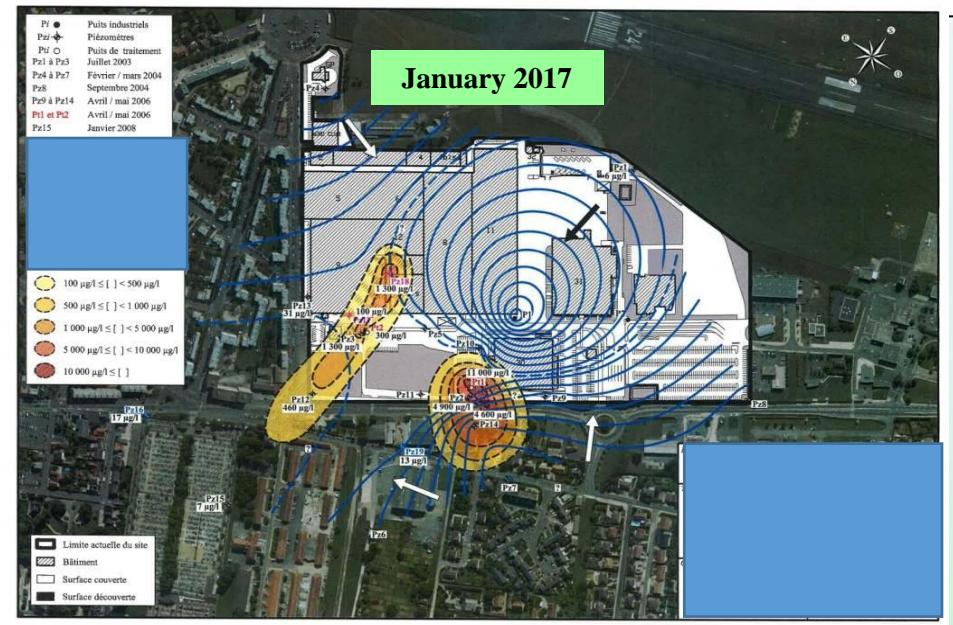


# Project Example: DNBA: Chlorinated Solvents + Cr6: Microbiological In-situ-Treatment via DNBA

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**Cr6**



Project Example: DNBA: Chlorinated Solvents + Cr6:  
Microbiological In-situ-Treatment via DNBA

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## Full DNBA-Treatment



## *Conclusion: DNBA Aerospaciale Site*

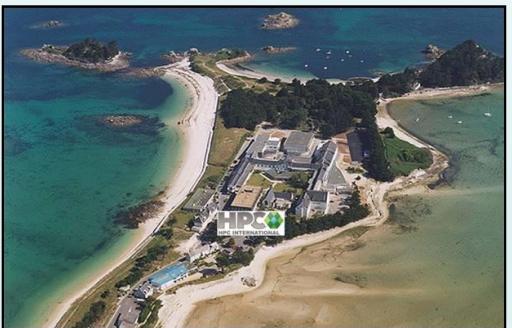


The technical-economic Feasibility Study and detailed DNBA Investigations was the Basement for the Successfully microbiological DNBA-Remediation by NBA Dynamization.

→ Cost Savings: in Comparison to P&T: Pump & Treat:  
Minimum: 7,8 Million €uros

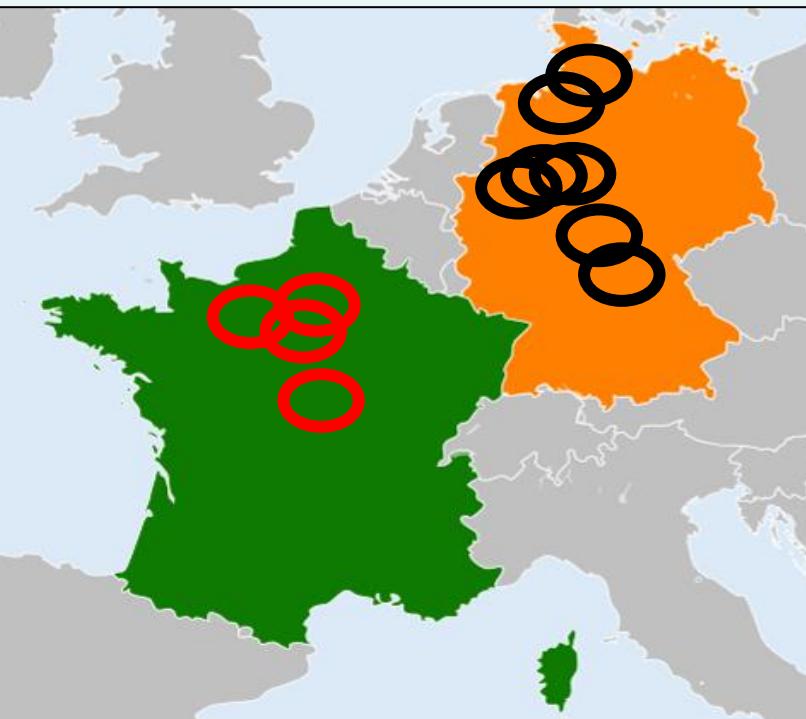
→ Time Savings: Remediation Time of 2 - 3 Years in  
Comparison to P&T: 22 – 35 Years.





## In-situ DNBA: Dynamized Natural Bio-Attenuation

Project Examples: Chemical &  
Electronic Industries:  
Microbiological In-situ-Treatment  
Bio-Technologies & Genetic Bacteria Selection

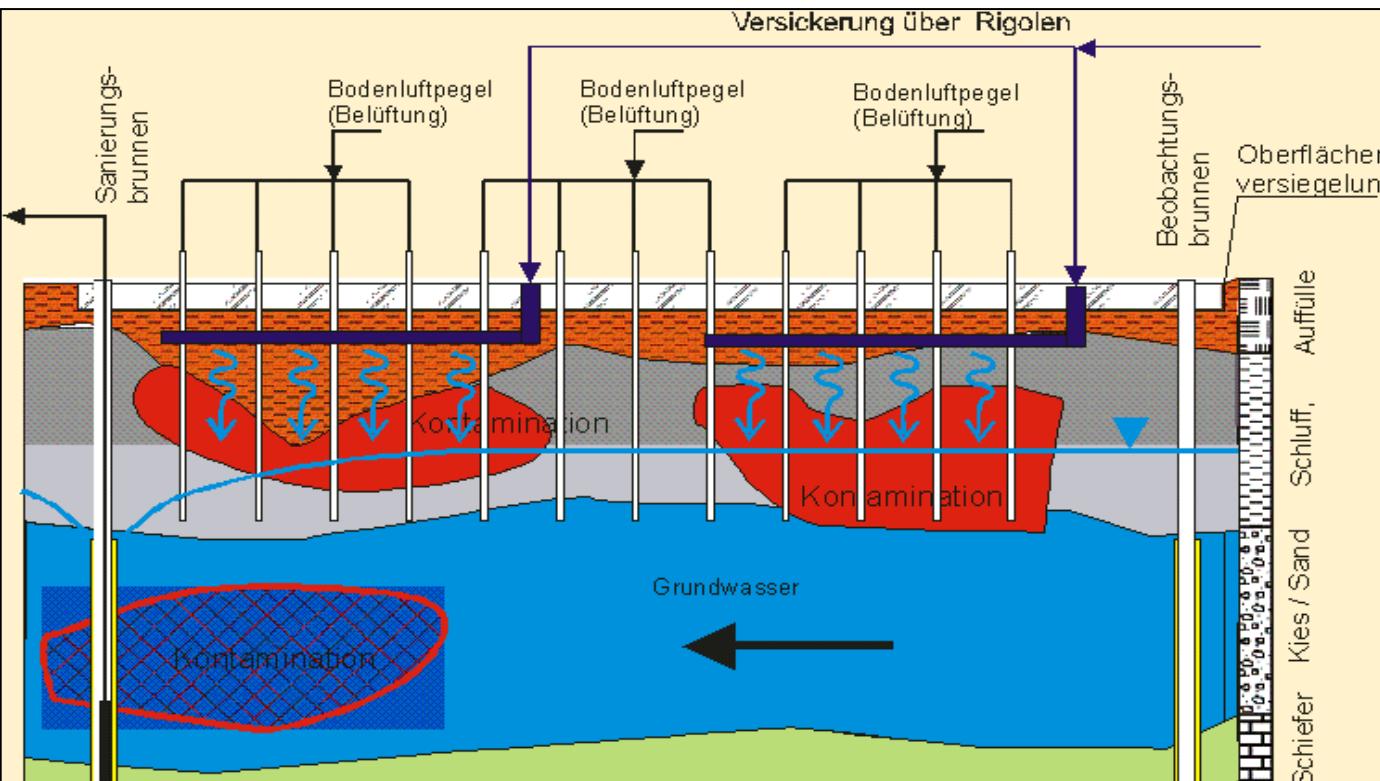
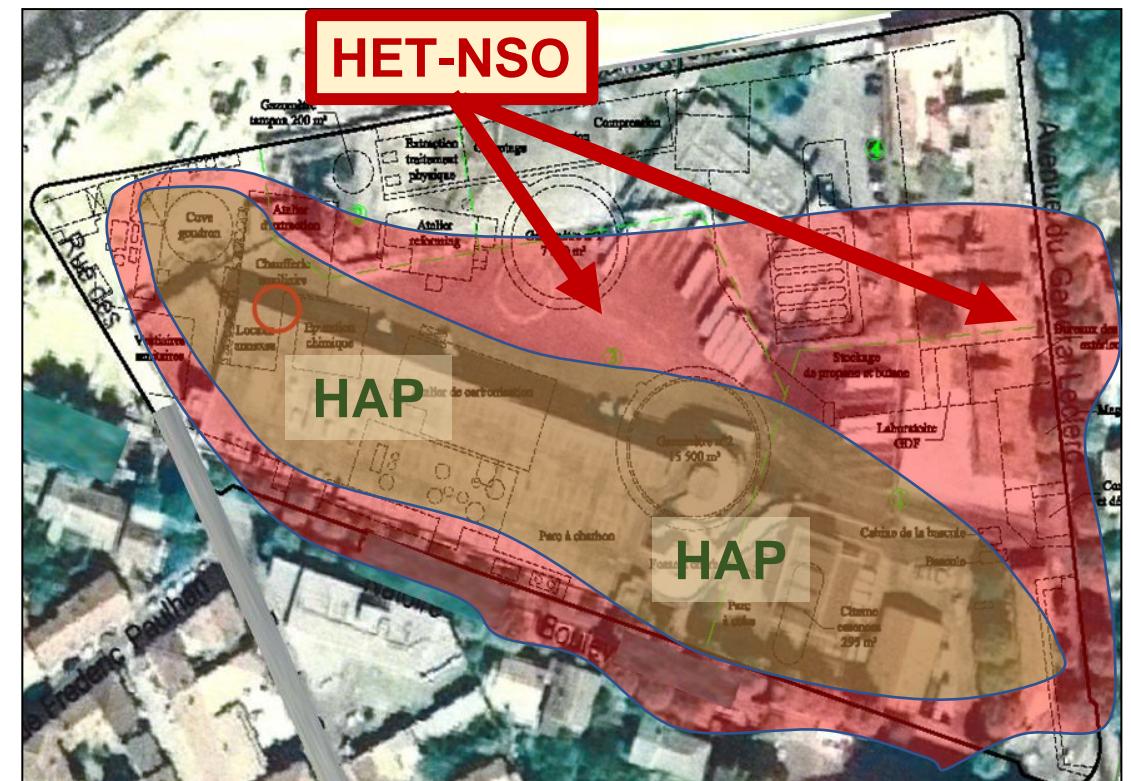
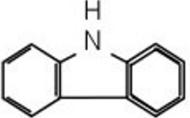


## Traitements in-situ des HET-NSO & AA: BAND (DNBA) microbiologique aérobie

→ Bio-Atténuation Naturelle Dynamisée : Example via Percolation 25°C + Aération & B-Consortium :  
 Site de l'ancienne Usine à Gaz d'EON

→ A préalable: Etude de Faisabilité: > Sélection d'un Consortium Bactériologique par PCRq, Ecotoxicologie microbiol  
 > Fractionnement d'isotopes  $^{12}\text{C}/^{13}\text{C}$ ,  $^{32}\text{S}/^{33}\text{S}$ ,  $^{16}\text{O}/^{17}\text{O}$  &  $^{14}\text{N}/^{15}\text{N}$ ,  
 > Tests de Challenge, de Respiration et de Minéralisation

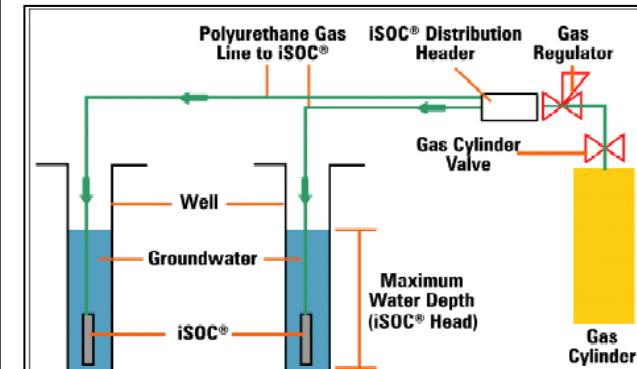
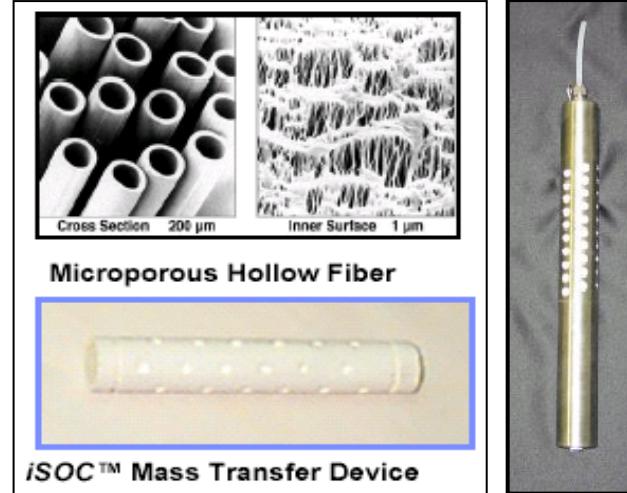
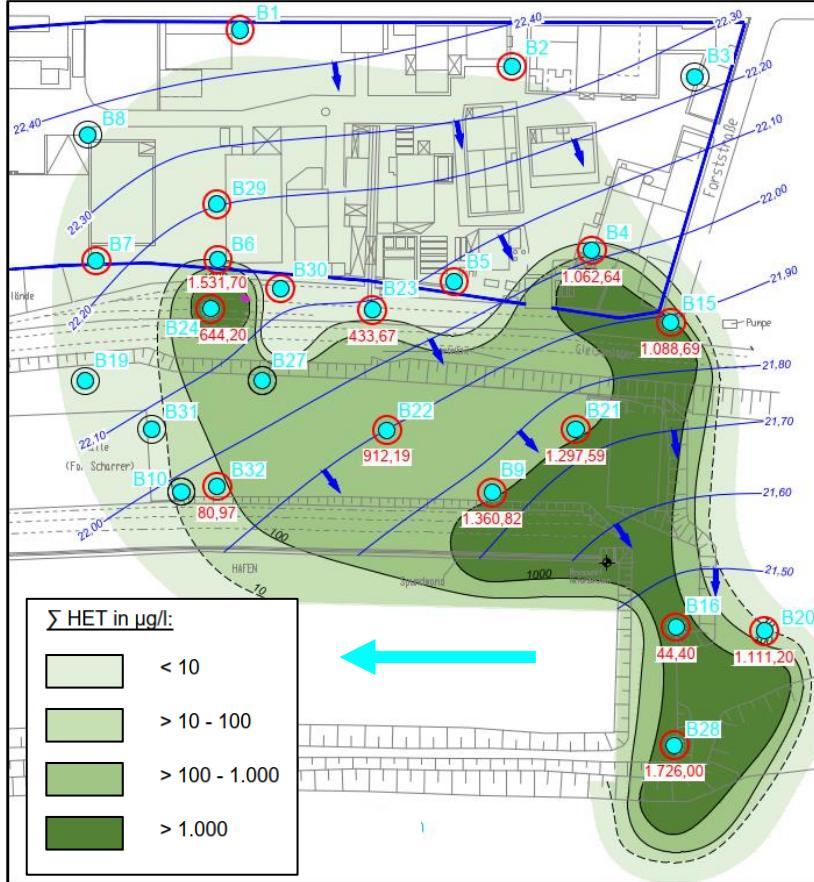
Carbazole



## Traitements in-situ des HET-NSO & AA: BAND (DNBA) microbiologique aérobie

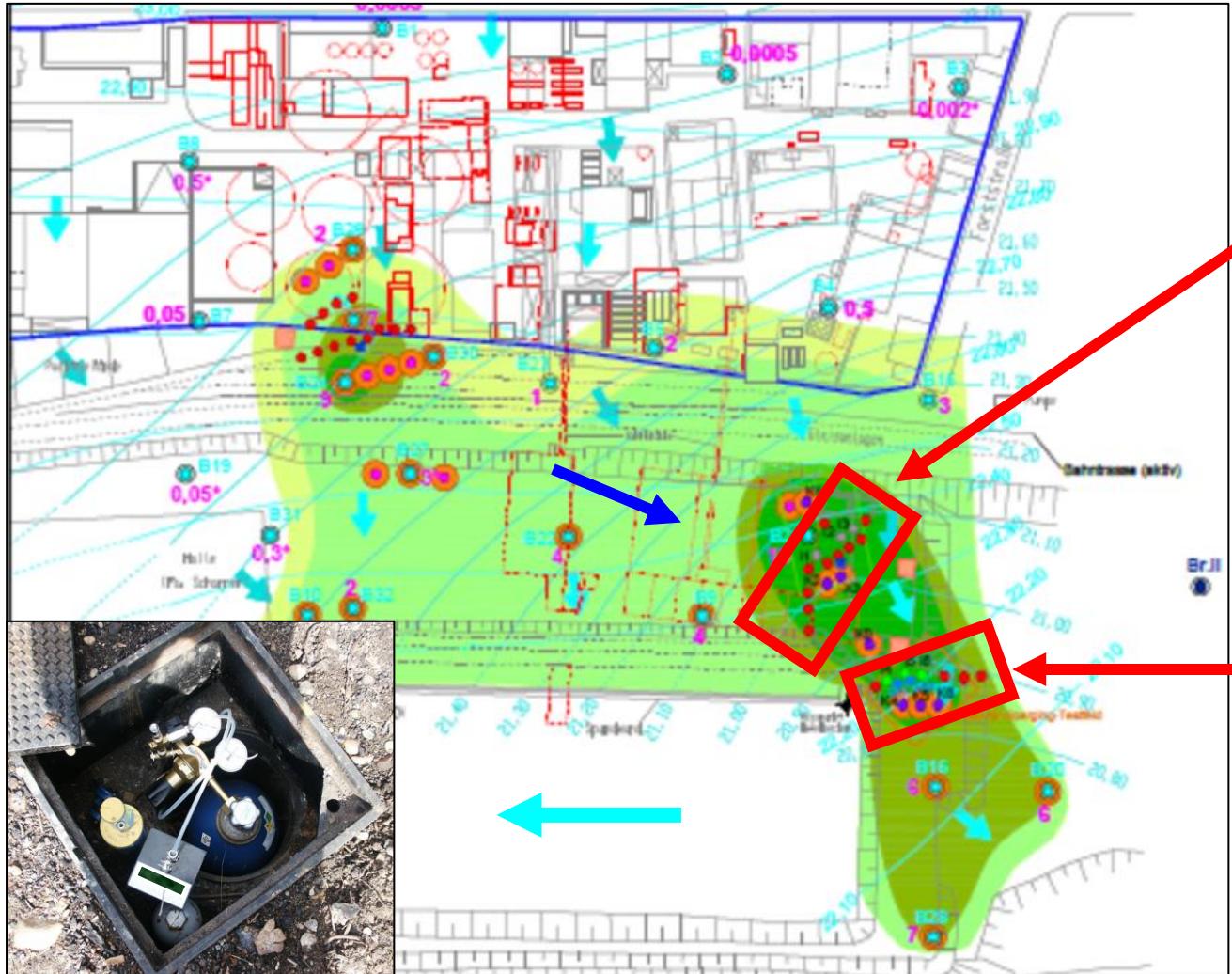
→ Bio-Atténuation Naturelle Dynamisée : Example via BAS & B-Consortium : Site de l'ancienne Usine Carbochimique C

→ A préalable: Etude de Faisabilité: > Sélection d'un Consortium Bactériologique par PCRq, Ecotoxicologie microb.,  
 > Fractionnement d'isotopes  $^{12}\text{C}/^{13}\text{C}$  &  $^{32}\text{S}/^{33}\text{S}$ , &  $^{16}\text{O}/^{17}\text{O}$  et  $^{14}\text{N}/^{15}\text{N}$ ,  
 > Tests de Challenge, de Respiration et de Minéralisation, Tests BAS & ISOC.



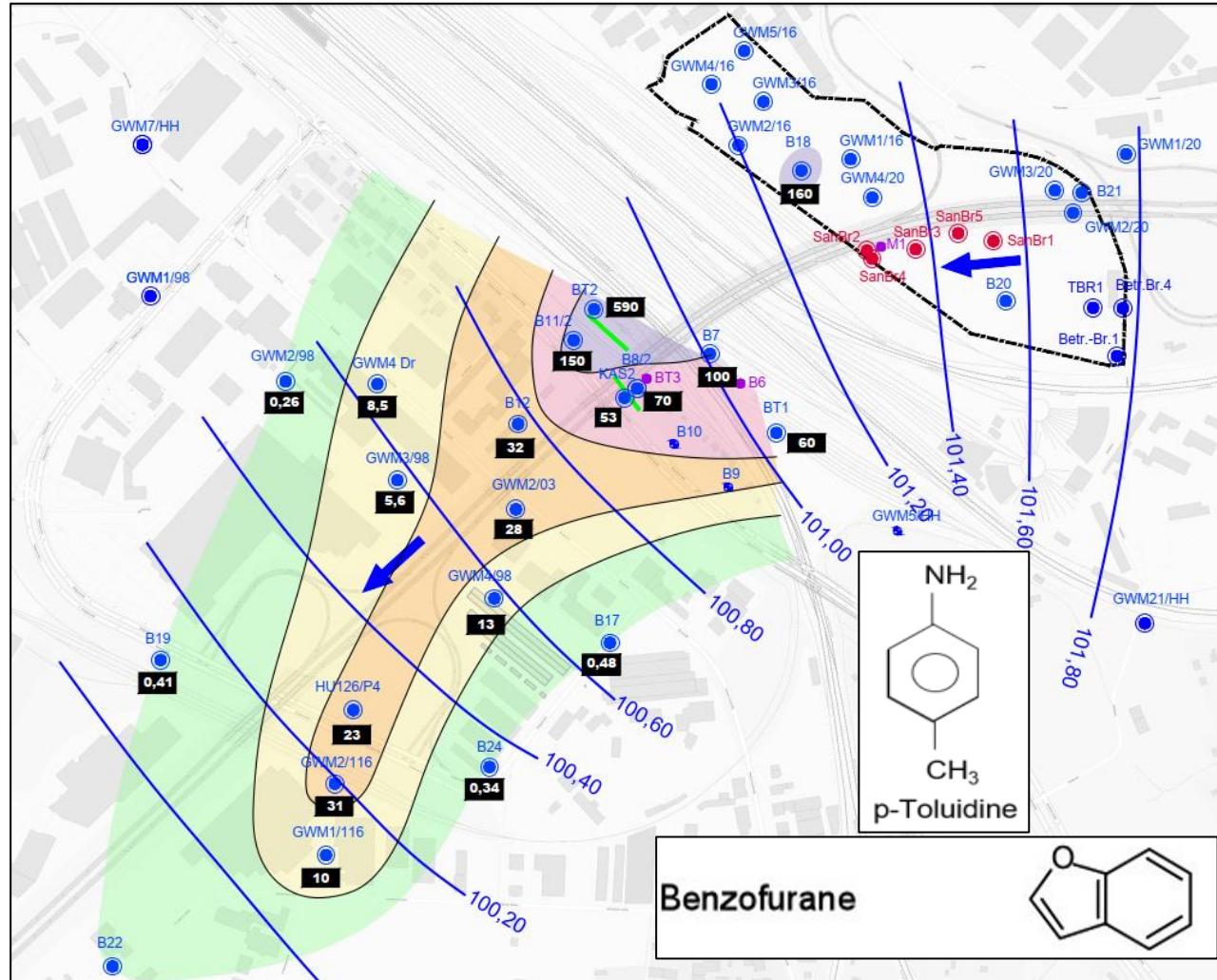
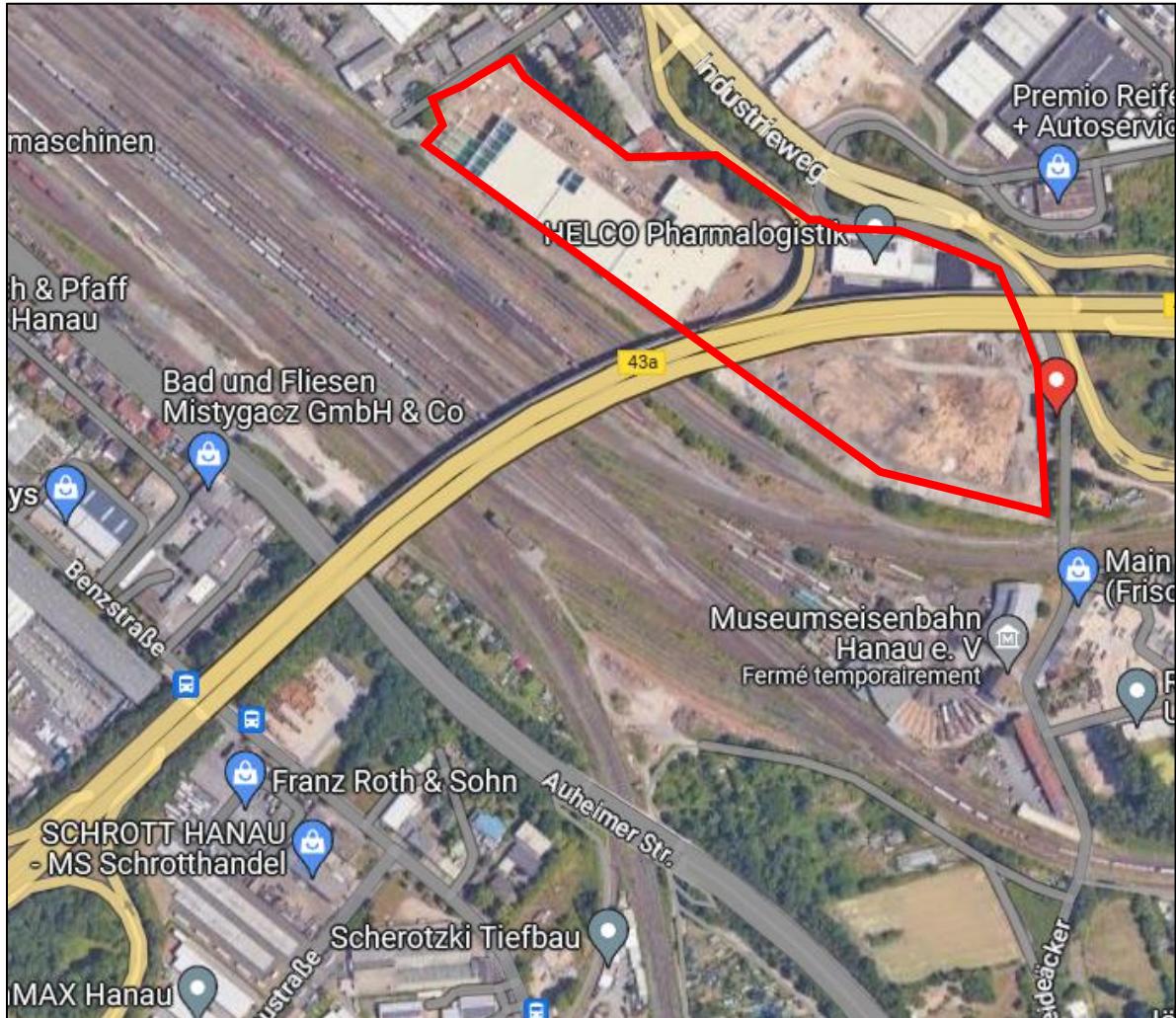
## Traitements in-situ des HET-NSO & HAP: BAND (DNBA) microbiologique aérobie

→ Bio-Atténuation Naturelle Dynamisée : Example via BAS & B-Consortium : Site de l'ancienne Usine Carbochimique C



## Traitements in-situ des HET-NSO & AA: BAND (DNBA) microbiologique aérobie

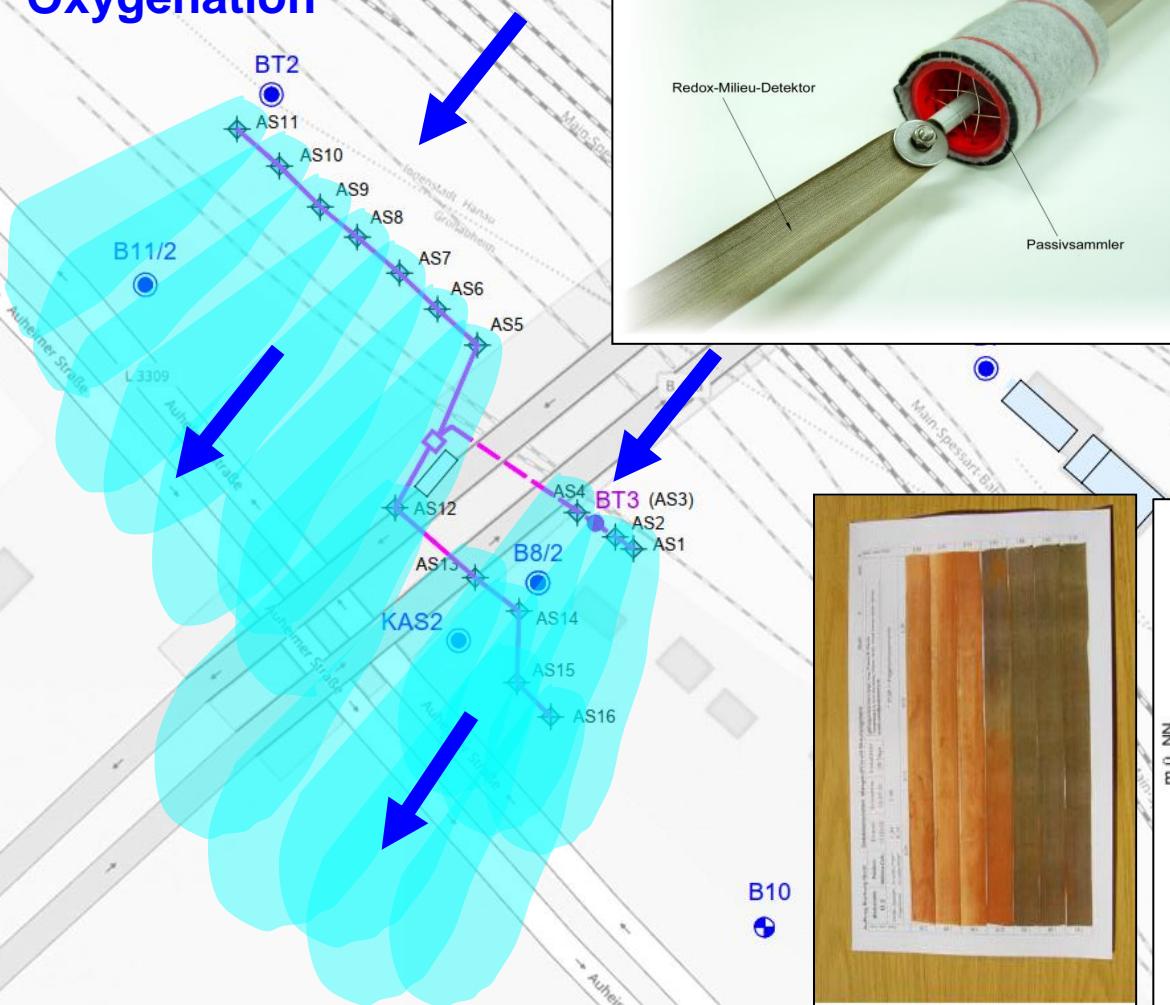
→ Bio-Atténuation Naturelle Dynamisée : Example via In-situ BAS: Site de l'ancienne imprégnation de bois: Hanau



## Traitements in-situ des AA & HET-NSO: BAND (DNBA) microbiologique aérobie

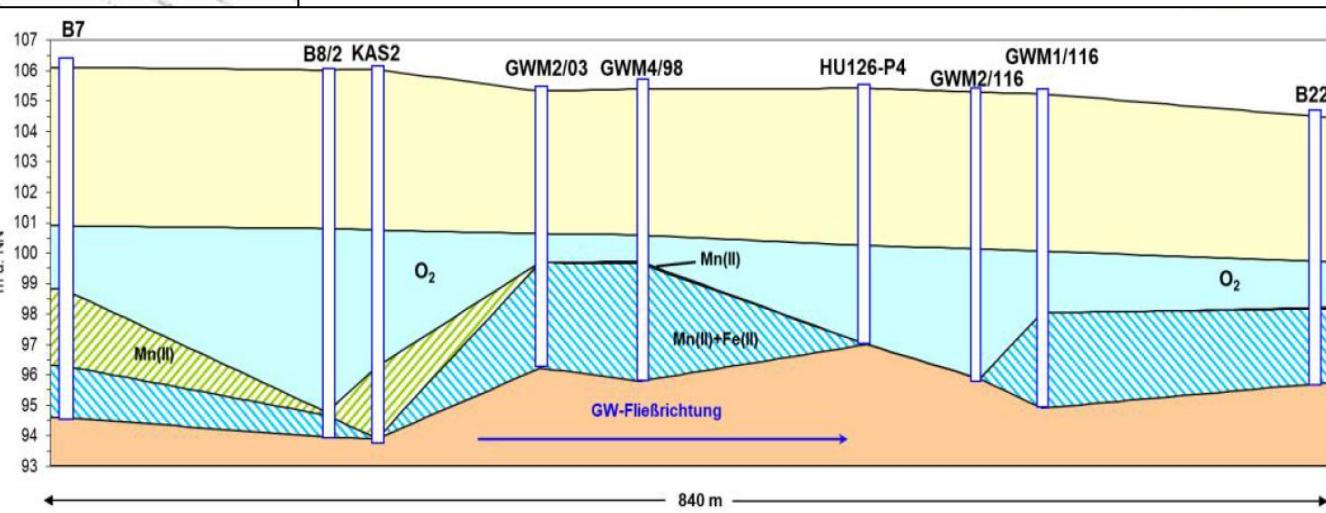
→ Bio-Atténuation Naturelle Dynamisée : Example via In-situ BAS: Site de l'ancienne imprégnation de bois: Site H.

### Oxygénéation



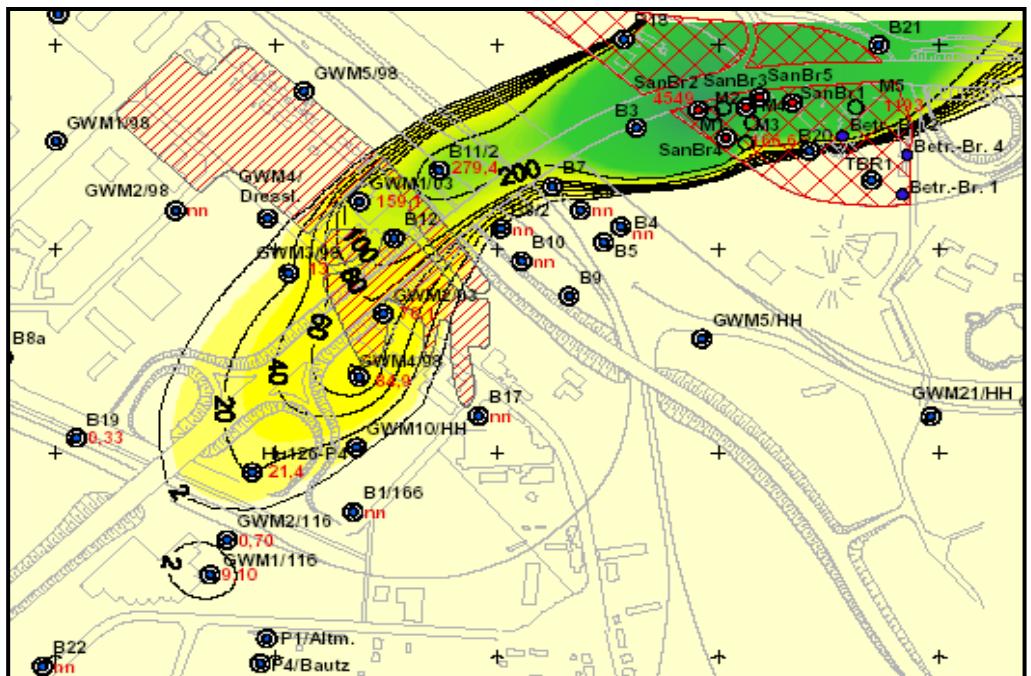
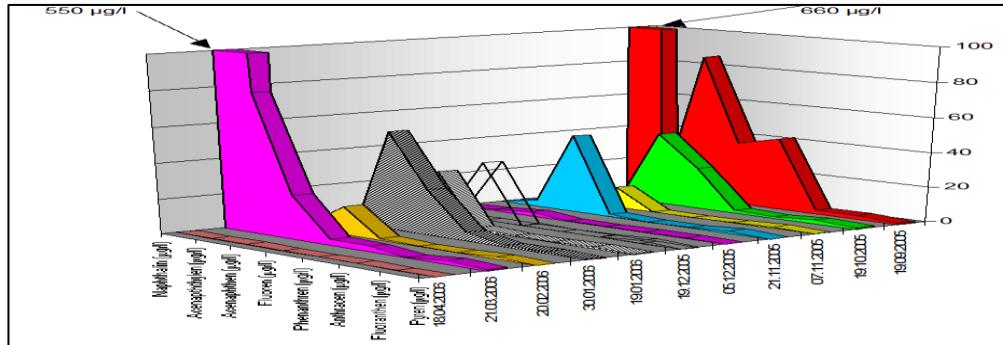
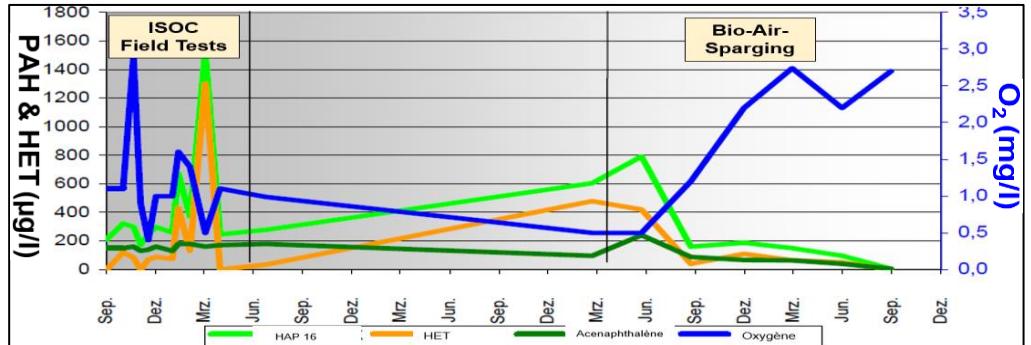
Electron-Acceptor use	Course of Reaction
Use of Oxygen	$C_{10}H_8 + 12 O_2 \rightarrow 10 CO_2 + 4 H_2O$
Use of Nitrate	$C_{10}H_8 + 9,6 NO_3^- + 9,6 H^+ \rightarrow 10 CO_2 + 4,8 N_2 + 8,8 H_2O$
Use of Nitrate	$C_{10}H_8 + 9,6 NO_3^- + 38,4 H^+ \rightarrow 10 CO_2 + 9,6 NH_3^+ + 8,8 H_2O$
Use of Manganese	$C_{10}H_8 + 48 H^+ + 24 MnO_2 \rightarrow 24 Mn^{2+} + 10 CO_2 + 28 H_2O$
Use of Iron	$C_{10}H_8 + 20 H_2O + 48 Fe^{3+} \rightarrow 48 Fe^{2+} + 10 CO_2 + 48 H^+$
Use of Sulfate	$C_{10}H_8 + 9 H^+ + 6 SO_4^{2-} \rightarrow 3 H_2S + 3 HS^- + 10 CO_2 + 4 H_2O$
Methanogenic Cond.	$C_{10}H_8 + 8 H_2O \rightarrow 6 CH_4 + 4 CO_2$

### Redox Zones



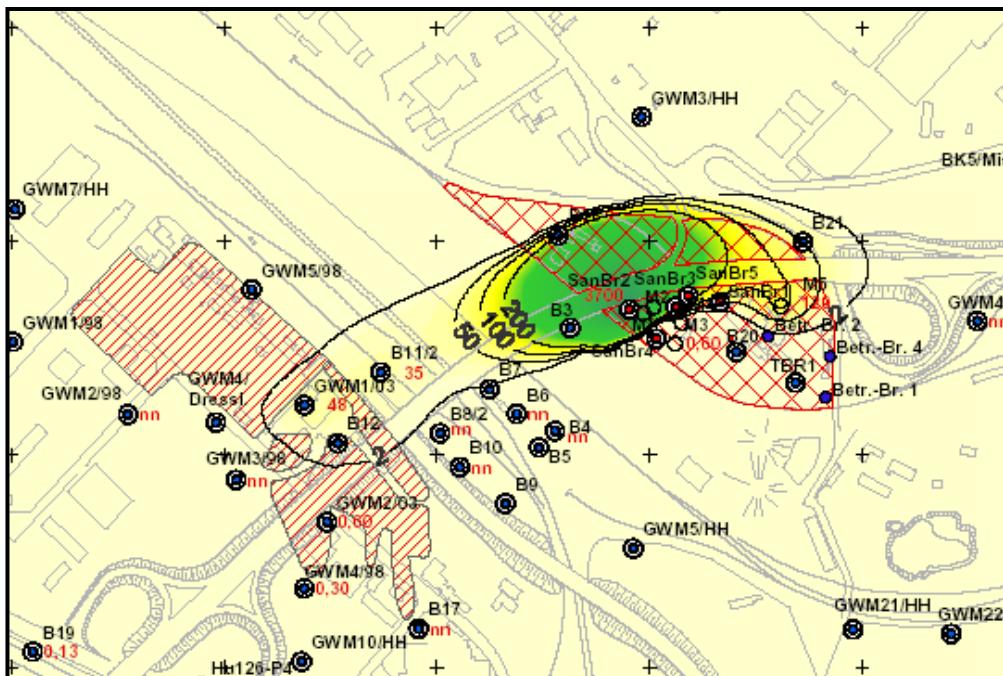
## Traitements in-situ des AA, HET-NSO: BAND (DNBA) microbiologique aérobie

→ Bio-Atténuation Naturelle Dynamisée : Example via In-situ BAS : Site de l'ancienne imprégnation de bois: Site H.



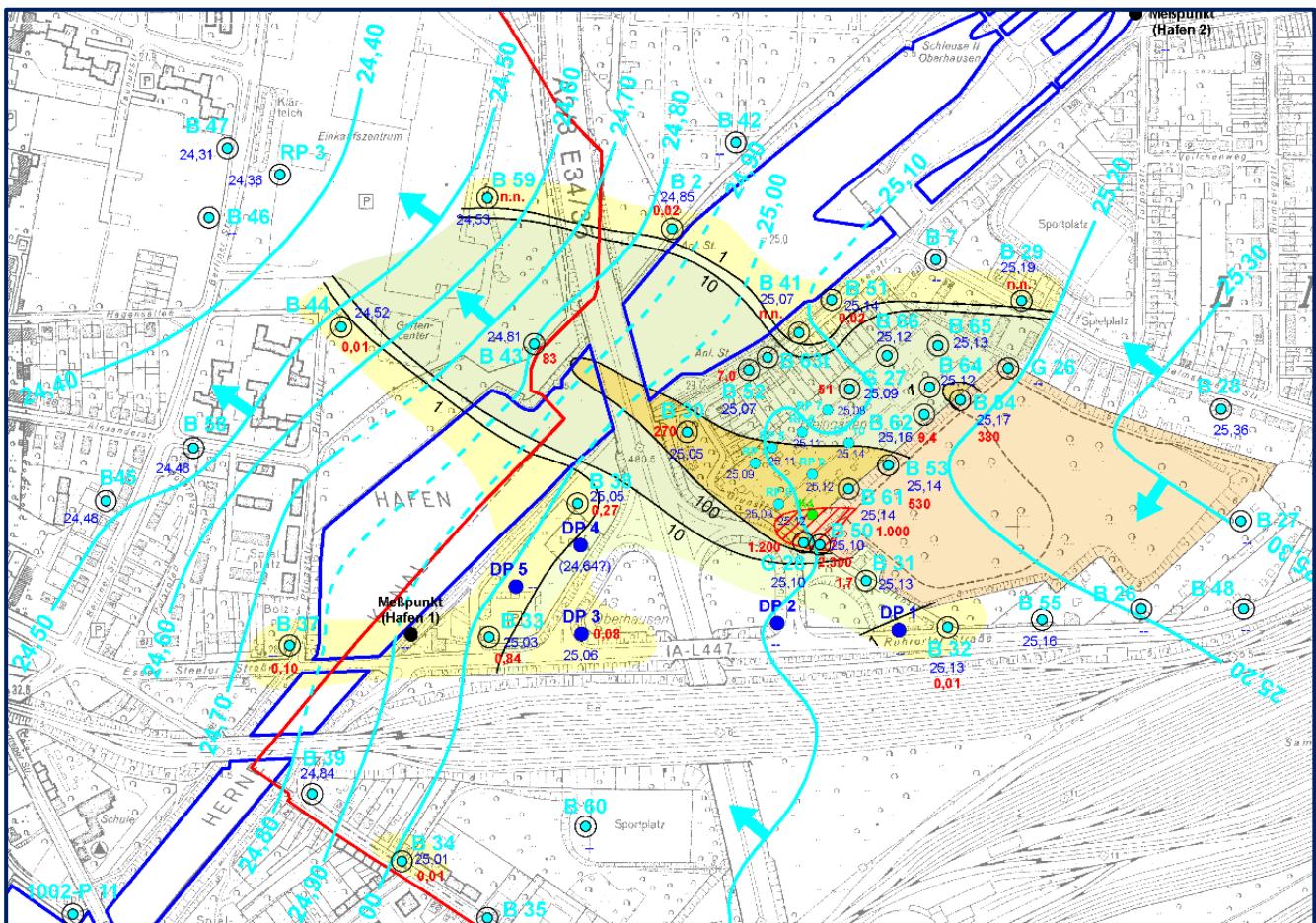
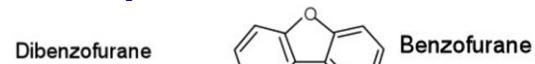
8 months  


Carbazole  
 (µg/l)



## Traitements in-situ des BTEX & HET: BAND (DNBA) microbiologique aérobie

- Bio-Atténuation Naturelle Dynamisée : Example via In-situ Oxyactivator: Site de l'ancienne décharge Oberhausen-L.
- A préalable: Etude de Faisabilité: > Sélection d'un Consortium Bactériologique par qPCR, Ecotoxicologie microbiol., > Fractionnement d'isotopes  $^{12}\text{C}/^{13}\text{C}$ ,  $^{32}\text{S}/^{33}\text{S}$ , Tests de Challenge, Respiration.



Production of Site specific Bacteria Consortium

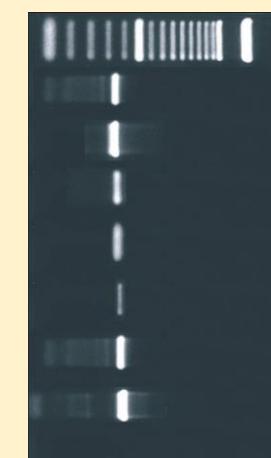


Microbiological Passiv Sampling for PCRq-Bacteria Selection

Floater ← GW-Table

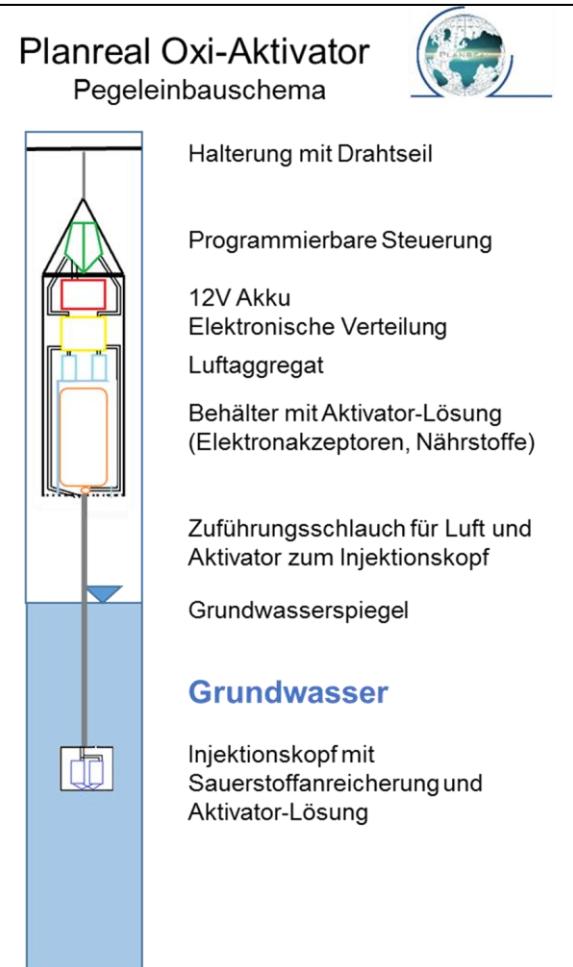
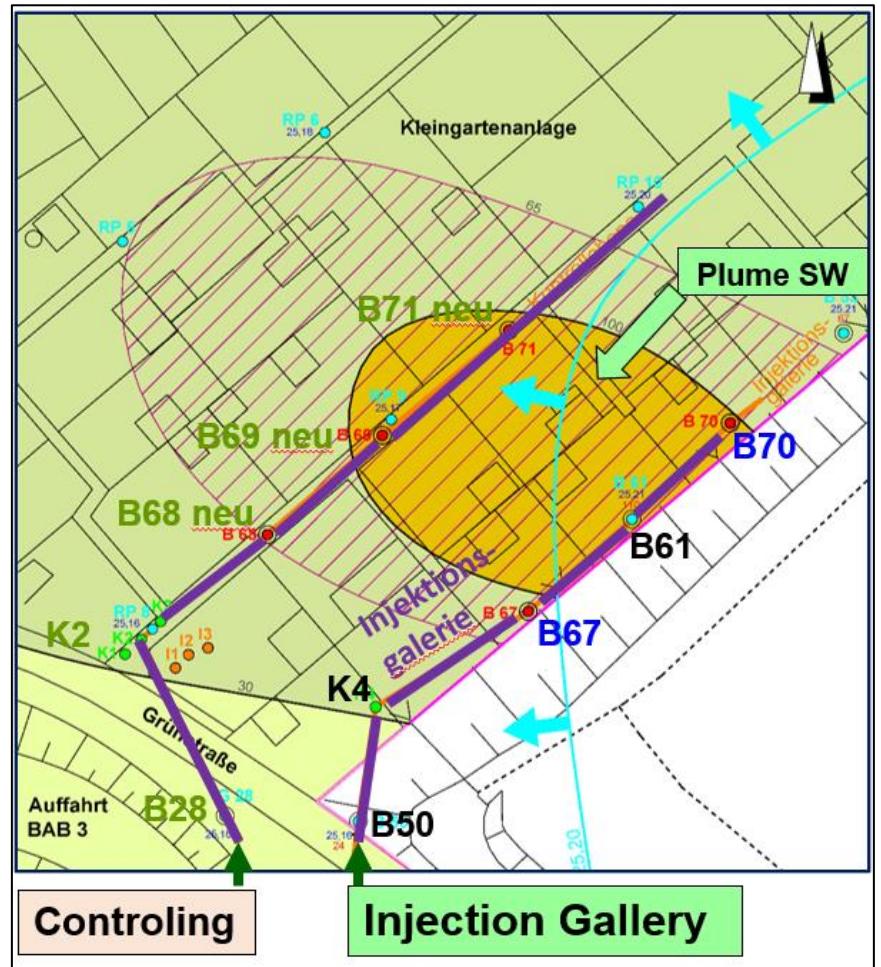
Attachment

MB-Passivsampler



## Traitements in-situ des BTEX & HET: BAND (DNBA) microbiologique aérobie

→ Bio-Atténuation Naturelle Dynamisée : Example via In-situ Oxyactivator: Site de l'ancienne décharge Oberhausen-L.  
 Infiltration d'oxygène, de nitrate et des nutriments. Mesures in-situ Eh, pH, O<sub>2</sub>

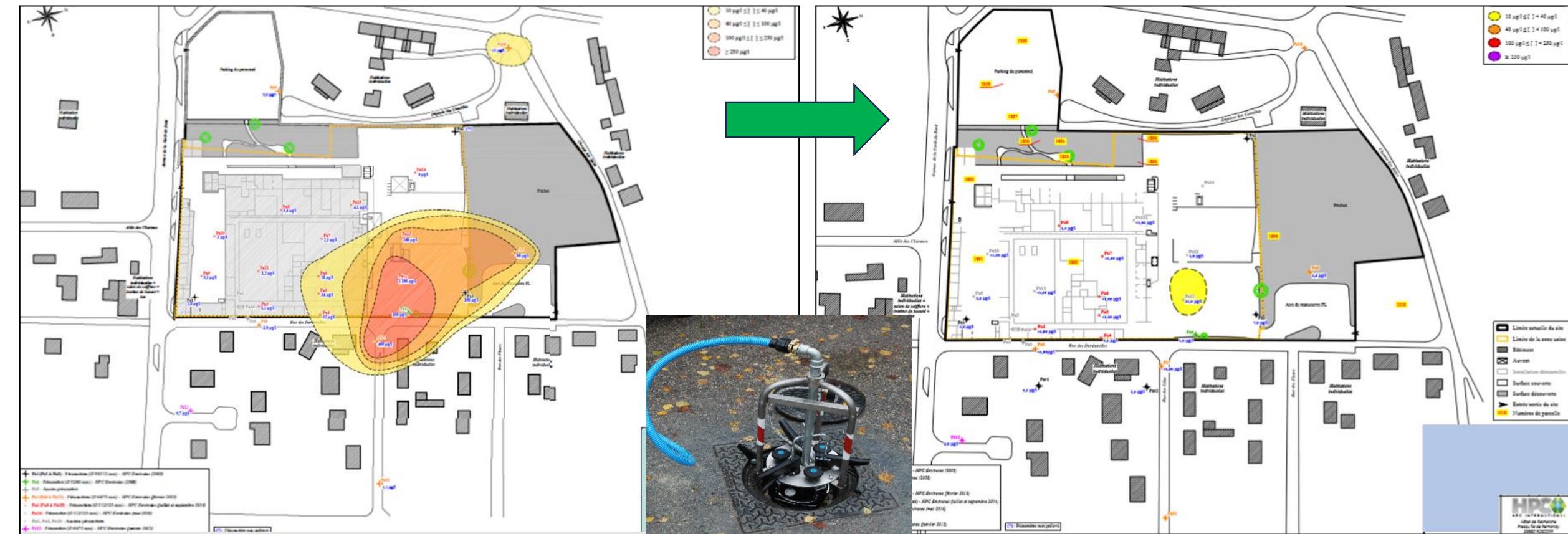


**Oxy-activators (O<sub>2</sub> & NO<sub>3</sub><sup>-</sup>)**



## Traitements in-situ des PCE, TCE, DCE, CV: BAND microbiologique anaérobie

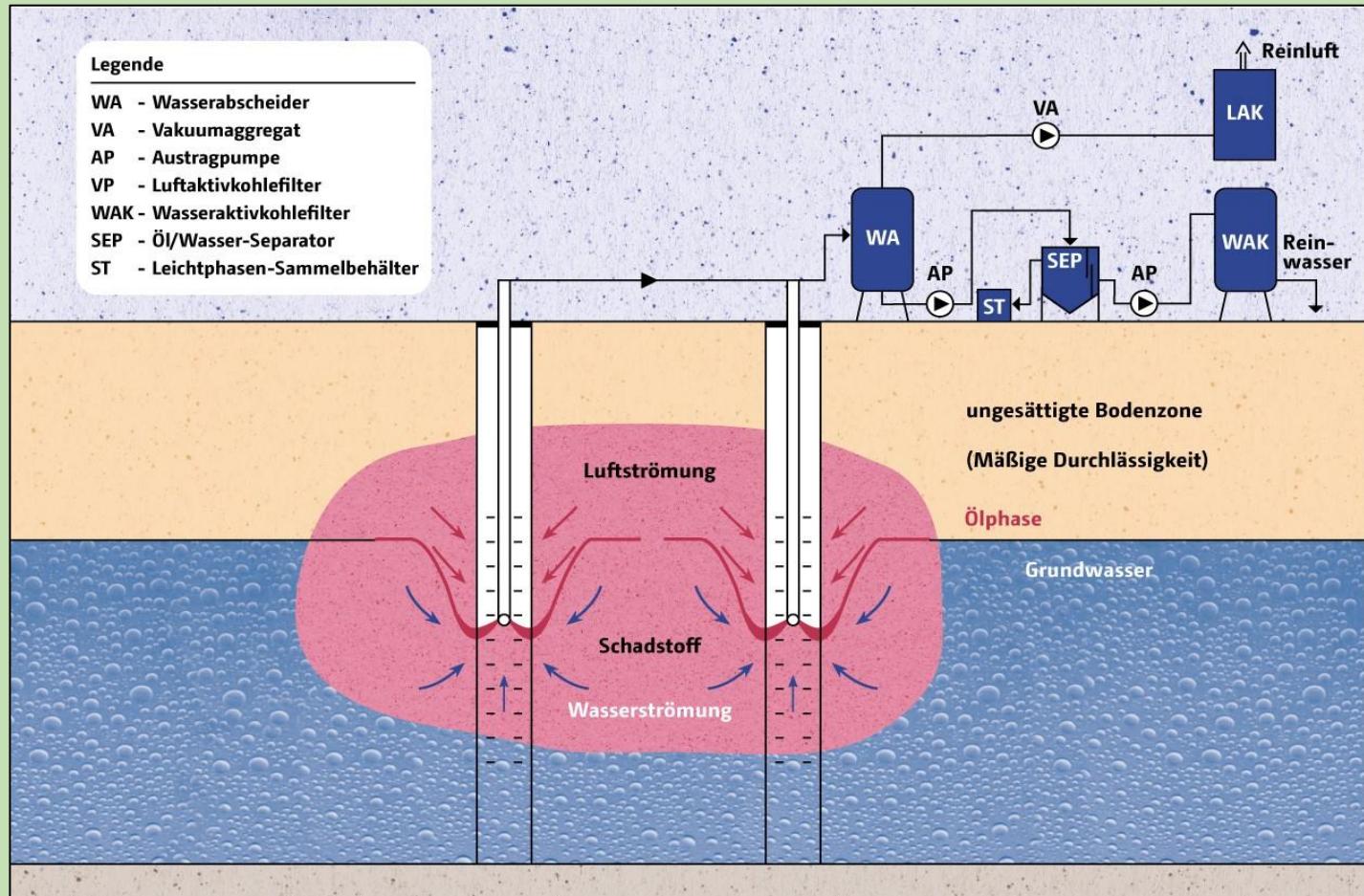
→ Bio-Atténuation Naturelle Dynamisée : Site en Normandie : Example via Injections n-situ sous Eh de -75 à -250 mV  
 Infiltration des Substrates - Nutriment et Consortium Bactériologique spécifique sous Azote Mesures in-situ Eh, pH, O<sub>2</sub>



Après 8 mois : PCE: 6 800 µg/l → 26 µg/l max.

## Traitements in-situ des HET-NSO, BTEX, HAP & AA: Alternatives couteuses: P&T !

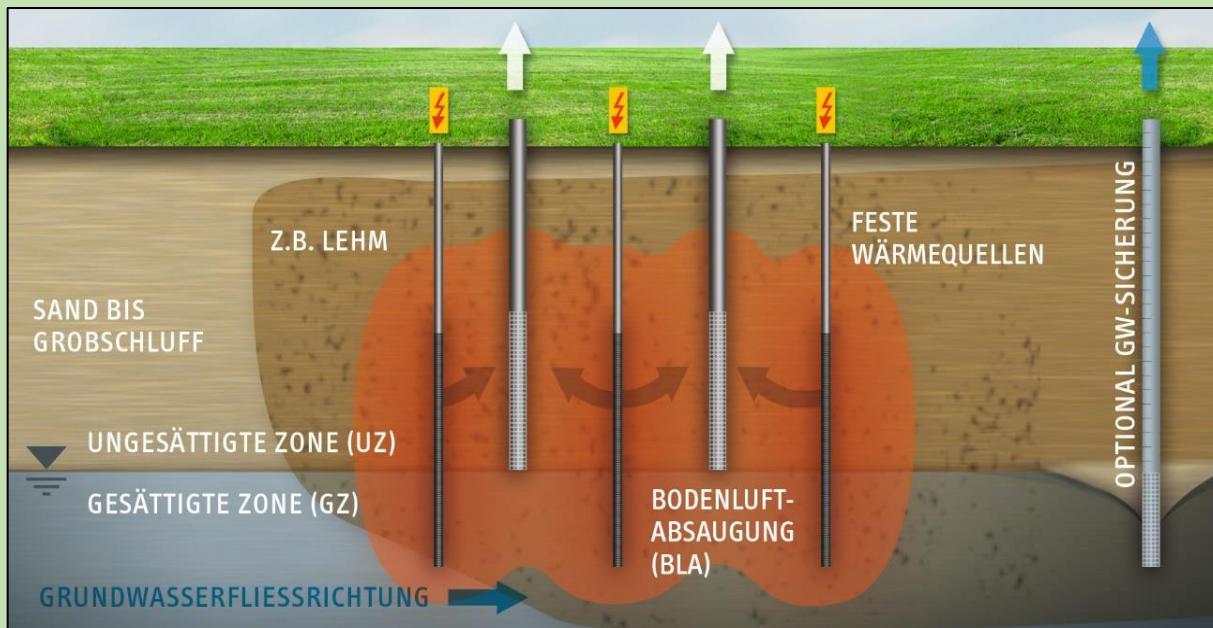
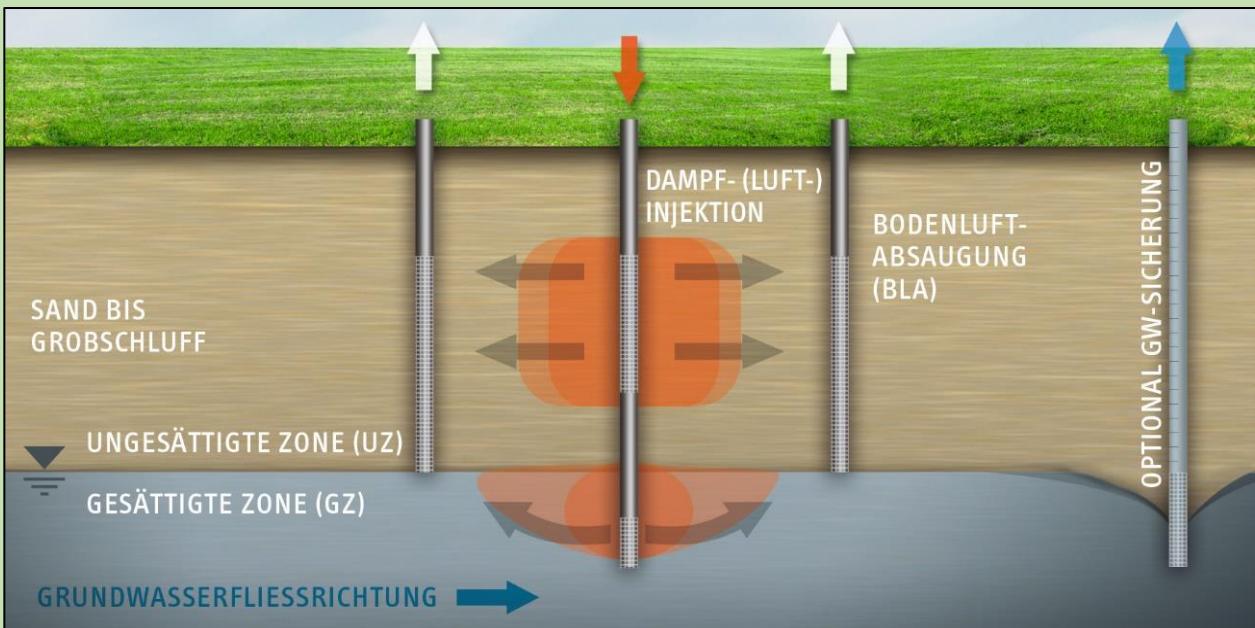
→ MPE: Multiphase Extraction (Cornelsen)



## Traitements in-situ des HET-NSO & AA: Alternatives thermiques (220 °C !)

→ TUBA: Injection de Vapeur sous pression (Reconsite)  
 Volatilisation & remobilisation des polluants et MPE

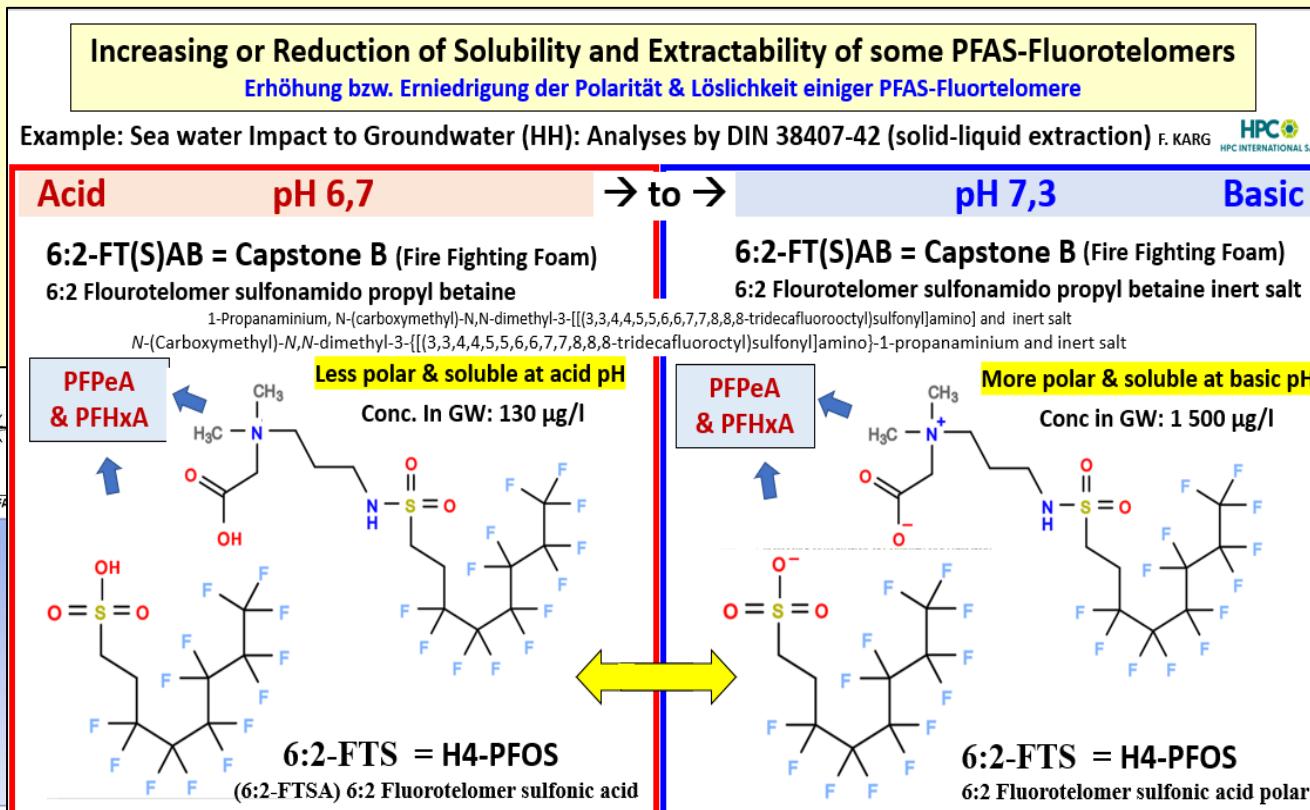
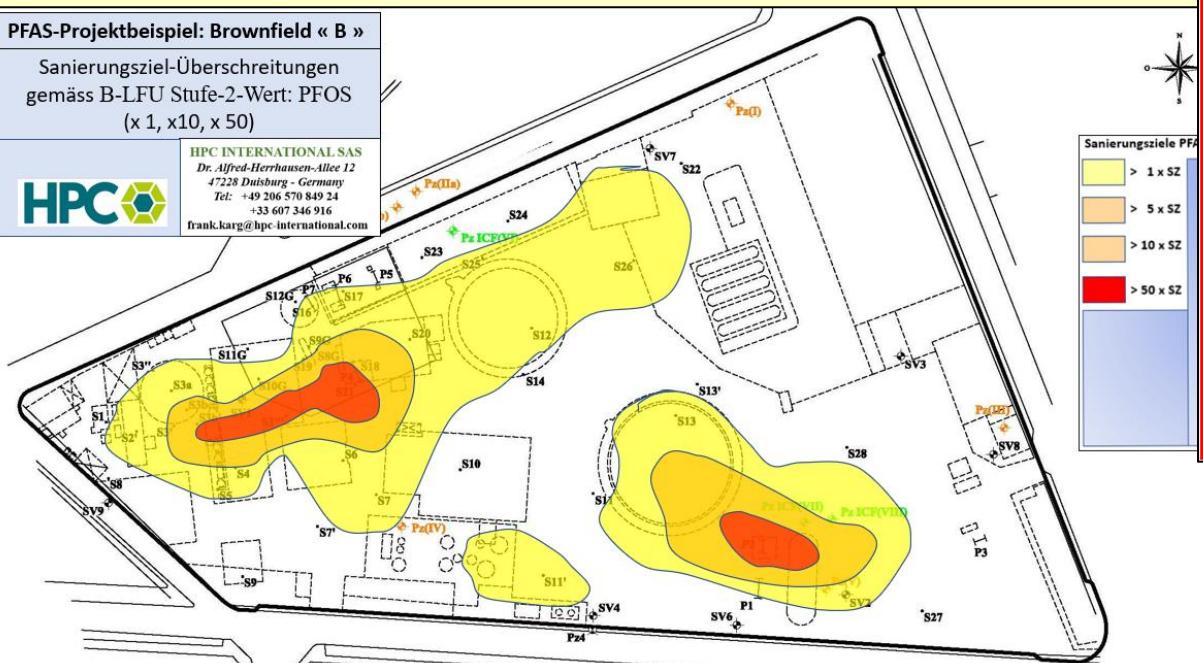
→ THERIS: Traitement thermique électrique (Reconsite)  
 Volatilisation & remobilisation des polluants et MPE



## Traitements in-situ des PFAS: Immobilisation in-situ

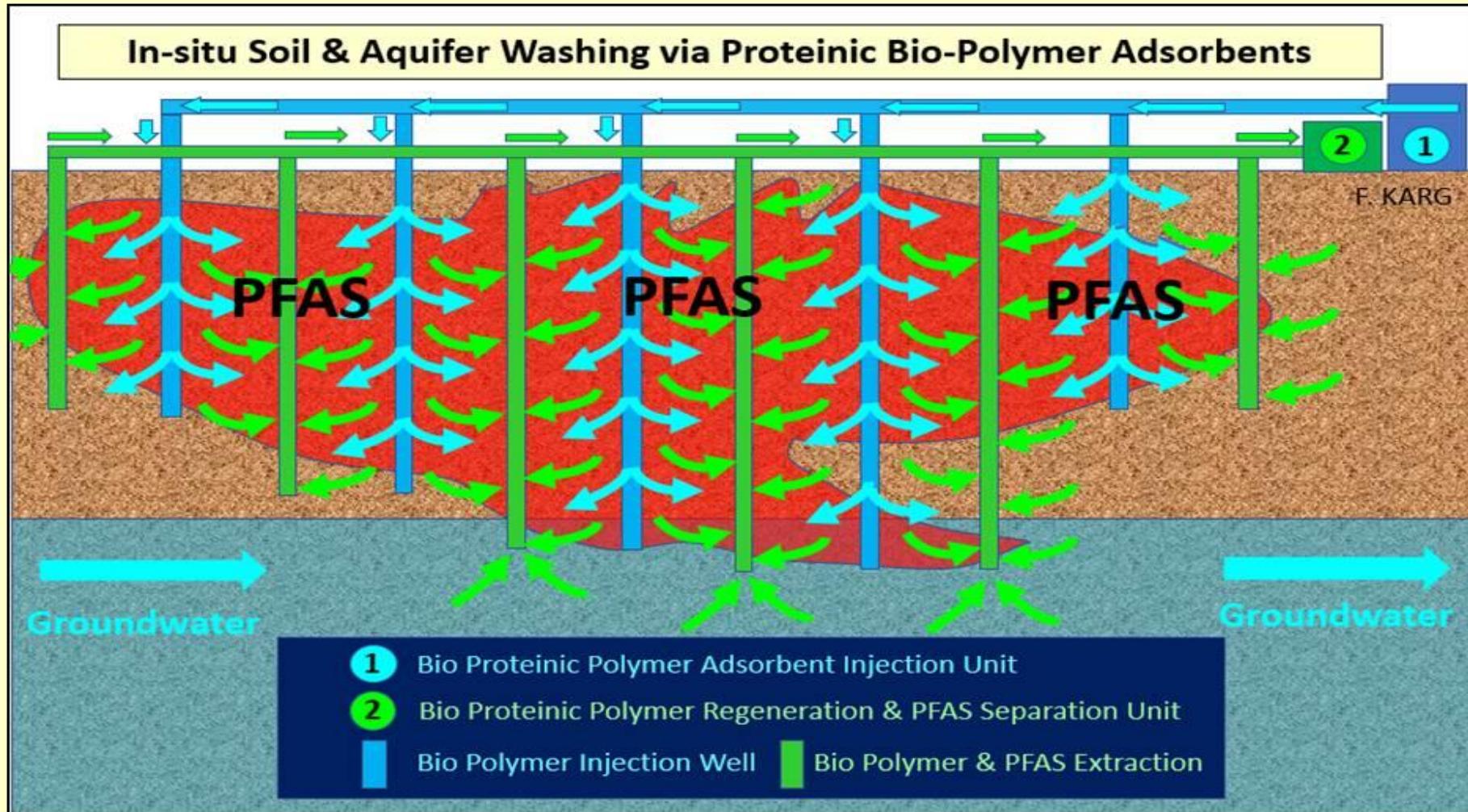
→ Application du Charbon Colloïdal ou des Adsorbants spécifiques

**PFOS, etc. dans les sols  
et eaux souterraines  
Site pétrolier HH**



## Traitements in-situ des PFAS: in-situ Washing (Belgique: site confidentiel)

→ Application des produits de lavage du type Bio-polymères protéiniques et régénération des réactifs



# Micropolluants toxiques et les biotechnologies des traitements de dépollution in-situ microbiologiques et biochimiques applicables : Exemples des BAND (Bio-Atténuation Naturelle Dynamisée) / L'Approche au meilleur bilan Coût-avantages

Merci !

Questions ? Remarques ?

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