



E P S

biotechnology

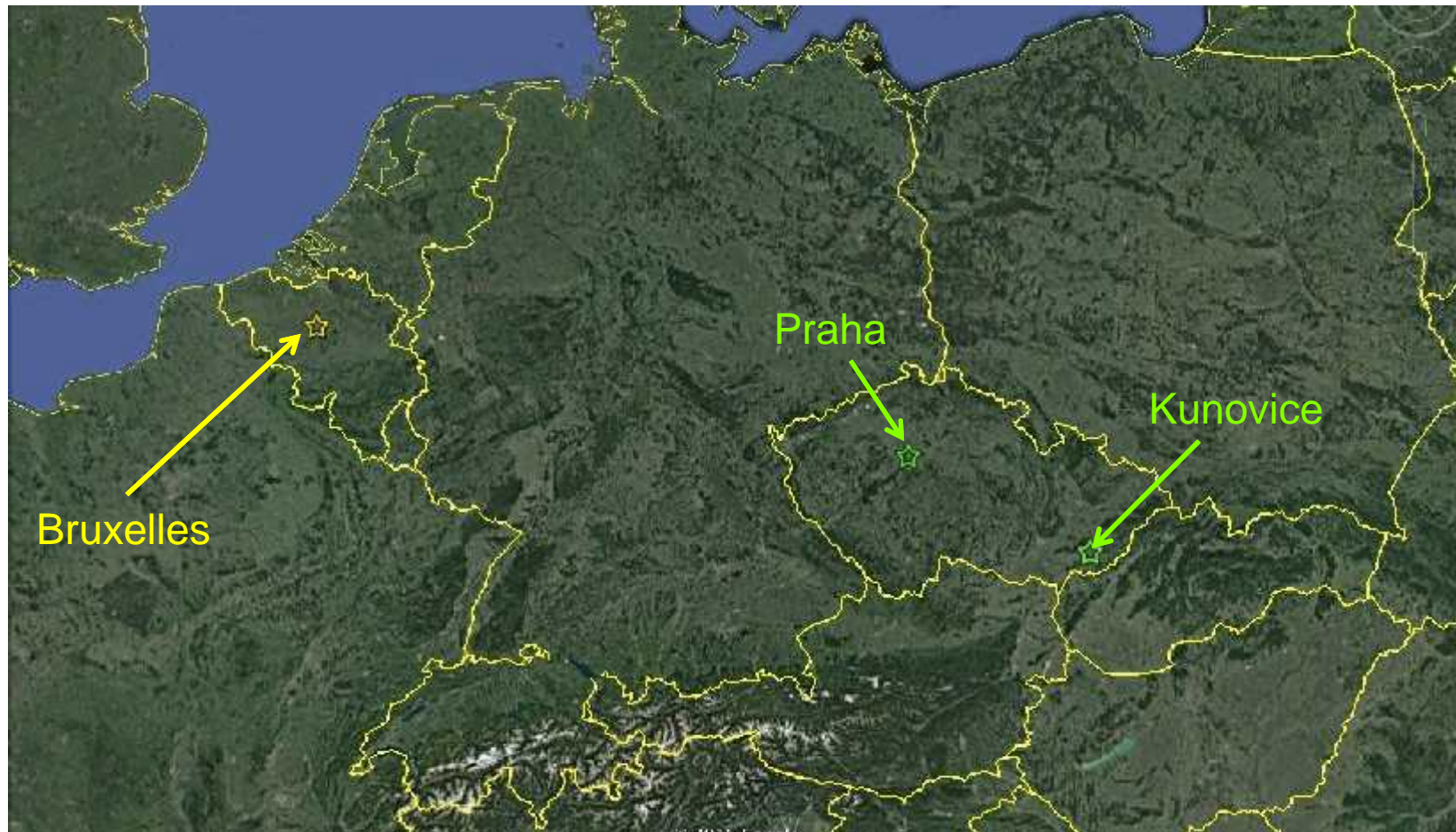
EPS biotechnology, s.r.o.

From thought to technology, from lab to practice.

R&D performing SME

WHERE ARE WE FROM?

Kunovice, Moravia, Czech Republic



Google Earth

WHO ARE WE?



Young collective, eager and enthusiastic for R&D.

- Biotechnology SME founded in 2002
- 40 people, Average age 35 years
- Main activities:
 - Custom research
 - R&D projects & grants
 - Environmental services & consulting
 - Waste management & processing
- Side activities:
 - Education & technology workshops
 - Remediation conferences
 - Public outreach – EPS activity
 - (www.epsactivity.cz)



FACILITIES

Fully poised for R&D



- 2 R&D Technological centers: Prague & Kunovice
 - HPLC-UV (RID), FTIR (wine analyzer), GC-FID, GC-TCD epifluorescence microscopy, spectrophotometry, cultivation equipment
- Biorefinery and composting plant
- Bioremediation fields
- Remediation sites, CZE & SVK



WHAT WE DO?

Biotechnology R&D projects: past & future

www.epsbiotechnology.cz

- **Environmental Remediation**
 - TA04020258, TA04021210, TA04020431
- **Renewable Energy Resources**
 - TA01020798, Coal gas enrichment (future)
- **Wastewater Management**
 - TA01020798
- **Industrial Biotechnology**
 - FR-TI3/668, Terroir-based yeast cultures (future)



Biotechnology R&D activities

Food biotechnology + Wine region = Our interest

Our vision → Cooperate with research organizations to complete the picture of wine research.



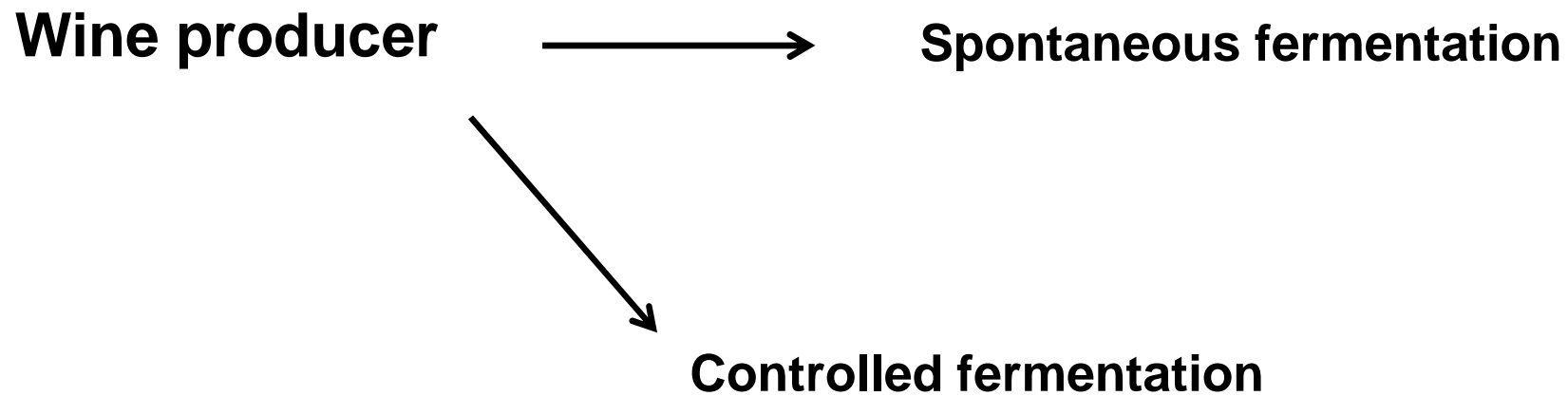
**UNIVERSITY OF
CHEMISTRY AND TECHNOLOGY
PRAGUE**



Tomas Bata University in Zlín

Mendel
University
of Agriculture
and Forestry
in Brno





Uniform yeast – “euro yeast“

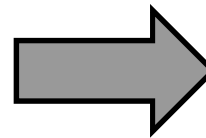


Controlled fermentation



X

Terroir yeast - For concrete variety
- From local wineyard



Preservation of local character
Better sensoric properties

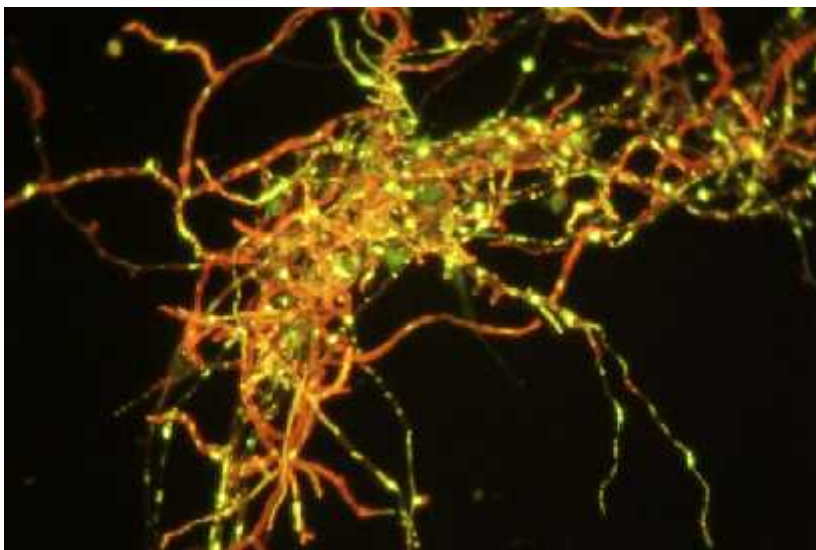


- Selected R&D activities**
- **Bio-remediation of oil based hydrocarbons**
 - **Bio-surfactant and bio-immobilization agents**
 - **Nano – fiber carriers**
 - **Biological desulfurization of biogas**



- Rich experineces with aplication of public acknowldged bio-remediation techniques
- Extensive collection of biodegrading microorganisms
- The use of modern and inovative techniques for sites monitoring
 - e.g. molecular biology, ATP luminometry, thermometry, L&D

- Pollutants:



**Oil-based hydrocarbons,
Chlorinated hydrocarbons
PAH, Phtalates, Phenols,
BTEX, MTBE, pesticides**

Examples of treated sites



Unipetrol Litvínov

**Petrochemical plant
BTEX + PHC
Anaerobic degradation
Bio-stimulation**



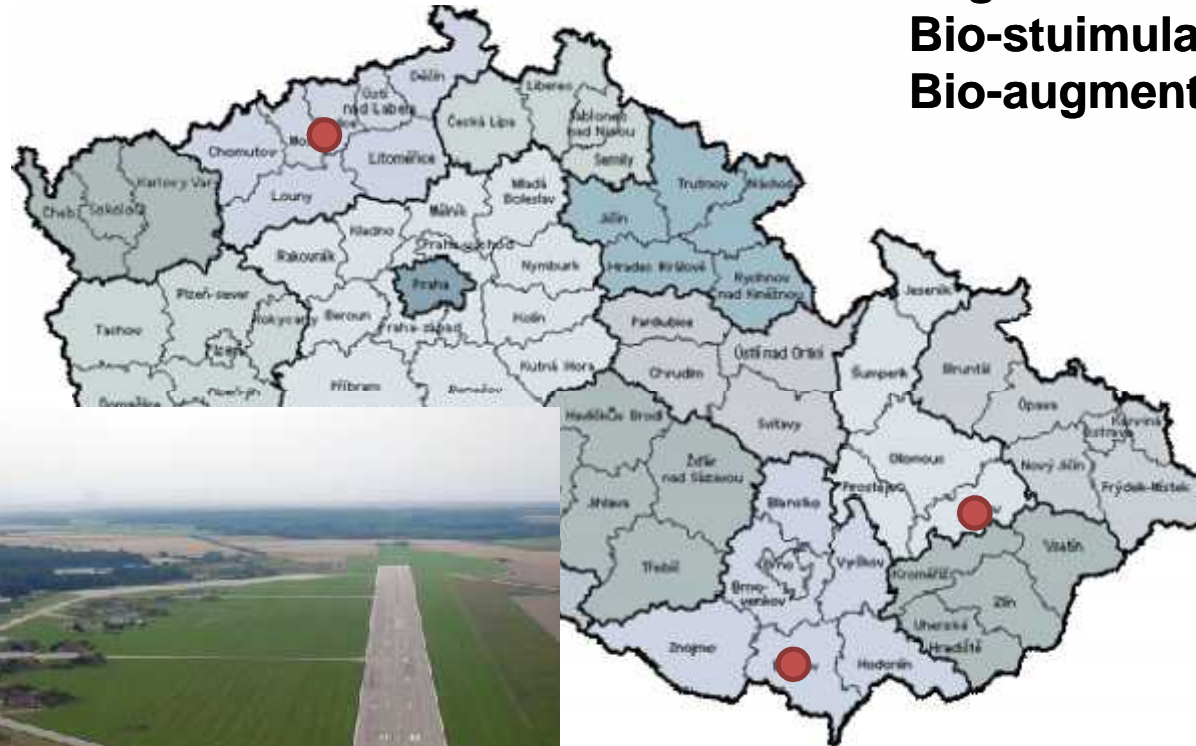
B eclair: D CARGO

**Railway depot
CHC
Bio-stimulation
Bio-augmentation**



P erov Airport Military base

Avgas
Bio-stuimulation
Bio-augmentation



Sokolnice Transformer station



**Oil-based HC
Pumping
Air-stripping
Bio-stimulation**



Ostramo Chemical plant & Sludge lagoons

Mineral oils, C10 – C40
Bio-stimulation
Bio-augmentation



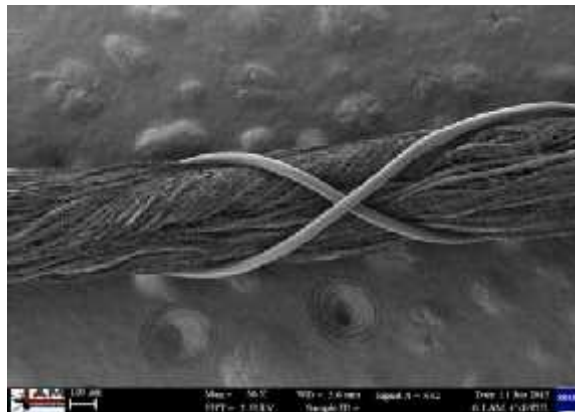
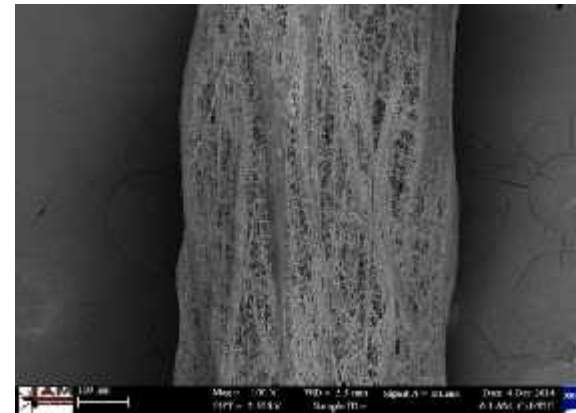
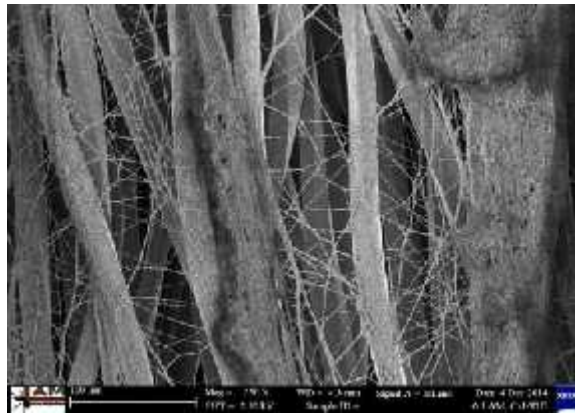
Biodegradation fields



- Project announced by TACR
 - Cooperation with **UPce** and **UTB**
- Finding a new, alternative, low cost technologies to bio-remediated sites with BTEX contamination.
- Simultaneously, the question of finding a biological way which leads to precipitation of heavy metals is studied



- EPS, s.r.o. - member of the NANOPROGRES excellence cluster
- Project announced by TACR
 - Cooperation with **Technical University of Liberec**
- Conservation of fully functional consortia of microorganisms
- Tool for more relevant monitoring of qualitative and quantitative changes in underground biota representation (using molecular techniques e.g.: PCR, qPCR)
- Carrier material for biofilm reactors (e.g. biofilters)



- EPS biogas plant
- Research grant
- Increase the quality of biogas
- One of the major problems => Sulfur compounds => Corrosion of engine parts
- Decrease sulfur amount by the useage of a biofilter with sulfur bacteria biofilm



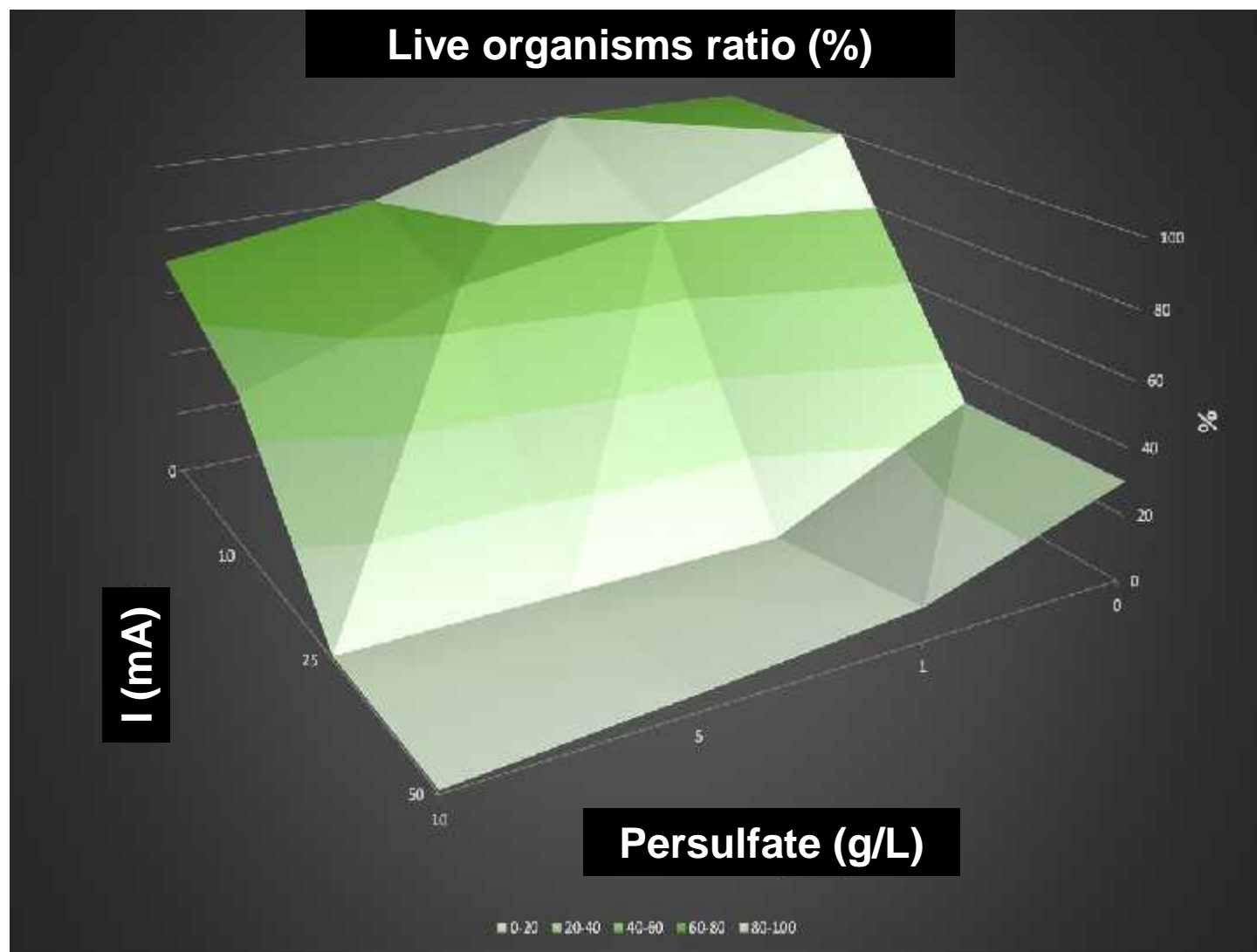
Abiotic R&D in EPS biotechnology

Techniques intensified by electric current

- Synergistic effect of direct electric current with reducing and oxidizing agents and/or biota
- Redox reactions characterized by the exchange of electrons
- Supply of electrons through the direct current is several orders less expensive than supply electrons by redox processes
- Electromigration utilization
- Strongly effective for hydrogeologically complicated fields with low soil permeability

Combination with bio? YES





R&D is followed directly by pilot/semi-industrial scale tests



Full scale application follows...

FROM LAB TO FIELD AND BACK AGAIN

*A case study of a large bioremediation project in the
Czech Republic*



- Site in the Northern Bohemia, Czech Republic
- Founded by Nazi in 1940, later operated by the Czechoslovak Army, from 1968 till 1991 by the Soviet Army
- The biggest western-most army airport of Warsaw Treaty (so-called Eastern Block) during the Cold War, intensively operated in eighties during the Cold War escalation



Soil cores - field scale

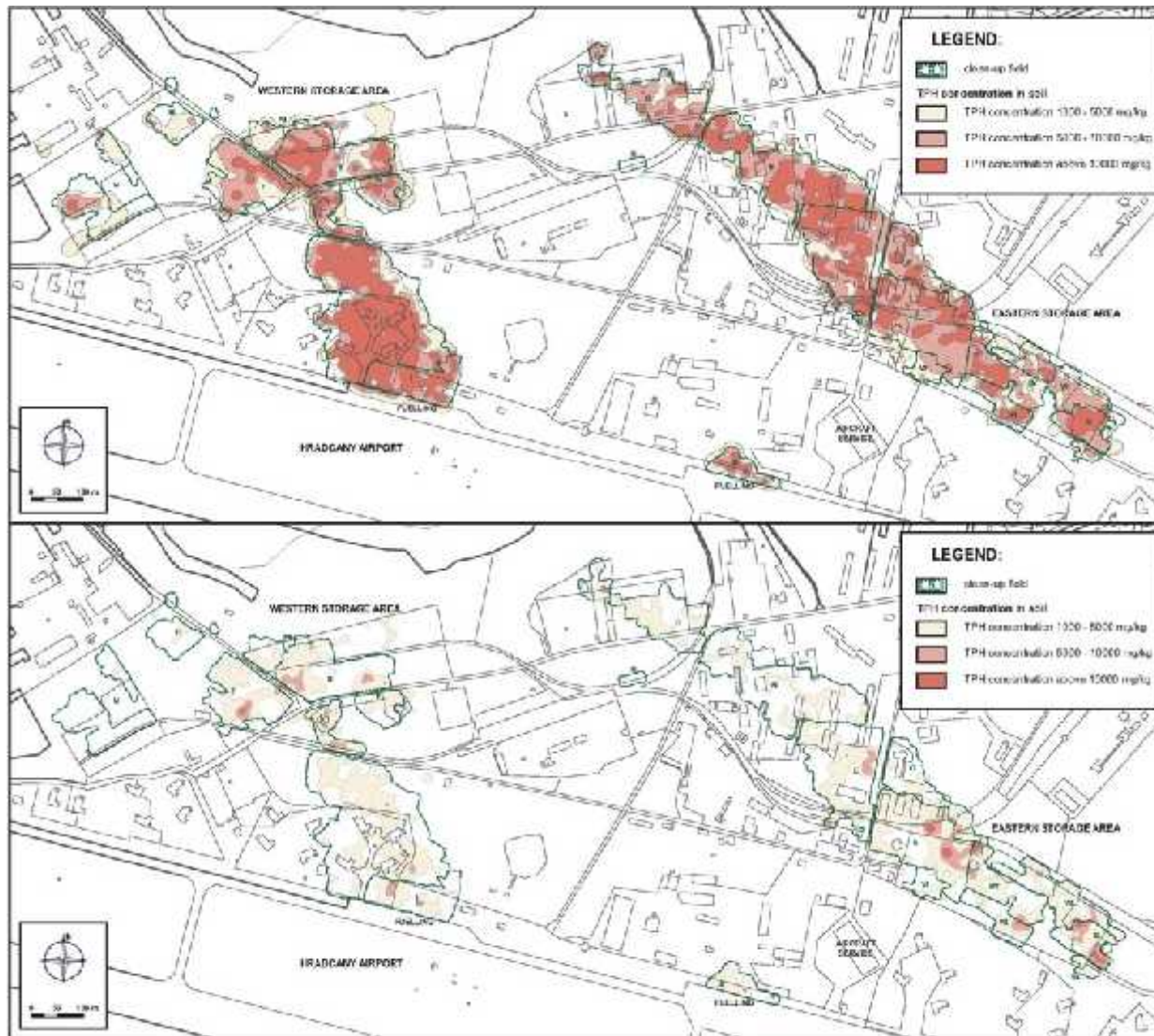


Column test - lab scale



RESULTS OF THE PROJECT

Soil TPH content



Initial TPH soil mass estimation was 7,150 metric tons ($\pm 30\%$) in 1997, closure TPH soil mass estimation was 1,665 metric tons ($\pm 30\%$), based on difference of these mass estimates, the amount removed from the site was 5,485 metric tons ($\pm 30\%$).

RESULTS OF THE PROJECT

Groundwater contamination

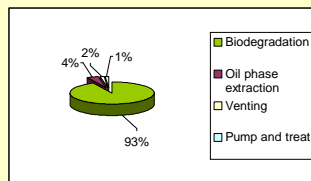
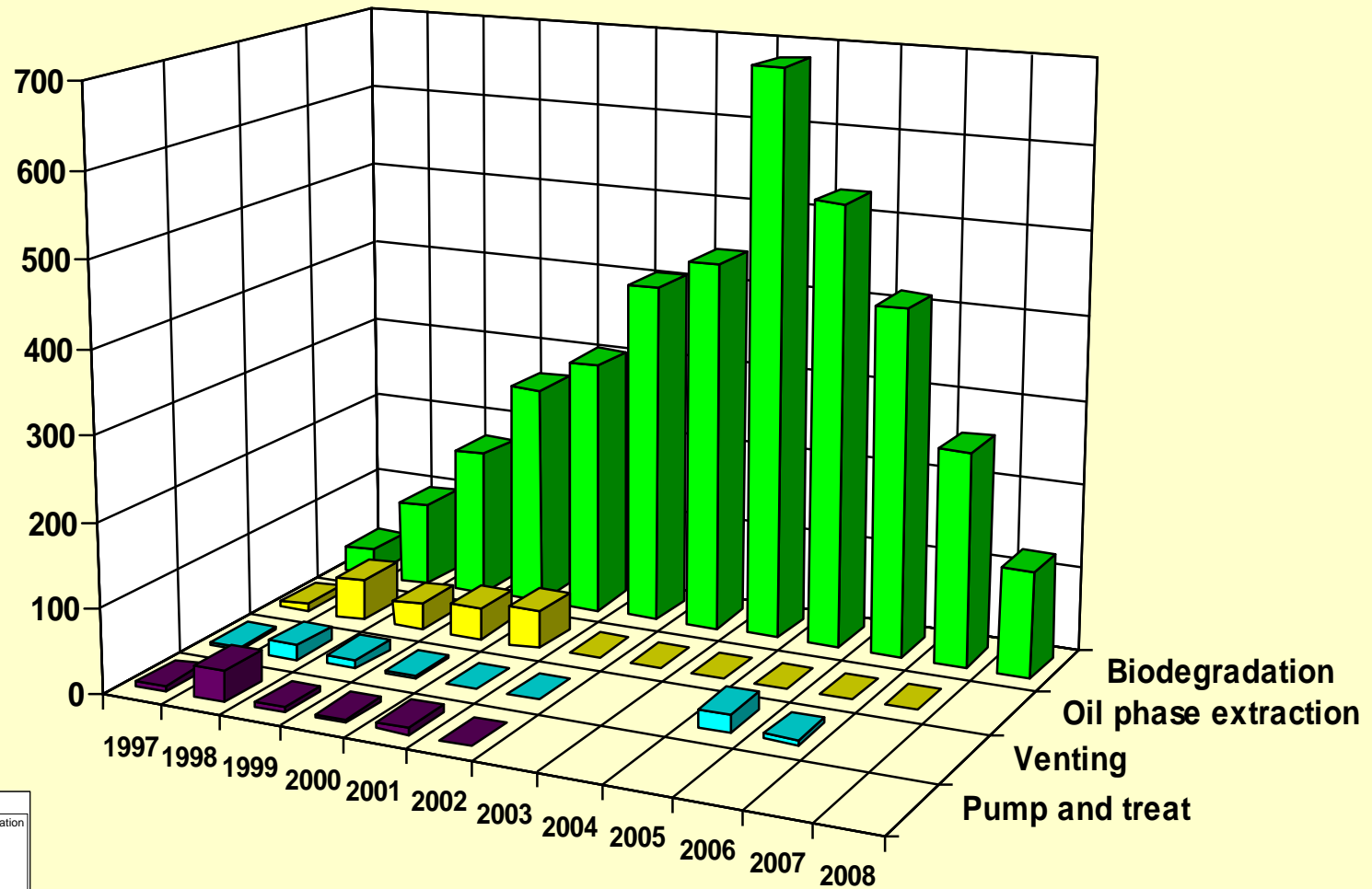


LNAPL extent decreased from 13.8 ha in 1997 to 0.01 ha in the end 2007 (LNAPL presence detected in four wells, total number of monitoring points is 1,893)

The site closure monitoring (2009-2011) verified reaching of the risk-based clean-up goals.

COMPARING THE EFFICIENCY OF CLEAN-UP METHODS

TPH removal – metric tons per year



- Machá kova J., Wittlingerova Z., Vlk K., Zima J., Linka A.: Comparison of Two Methods for Assessment of In Situ Jet-Fuel Remediation Efficiency, DOI 10.1007/s11270-007-9507-9, Water Air Soil Pollut, 2008, vol 187, pages 181-194/ Springer
- J.. Masak , J. Machackova , M. Siglova , A. Cejkova , V. Jirku (2003) : Capacity of the Bioremediation Technology for Clean-Up of Soil and Groundwater Contaminated with Petroleum Hydrocarbons , Journal of Environmental Science and Health, Volume 38, Number 10 / Taylor & Francis
- Jirina Machackova, Zdena Wittlingerova, Kvetoslav Vlk & Jaroslav Zima (2012): Major factors affecting insitu biodegradation rates of jet-fuel during large-scale biosparging project in sedimentary bedrock, Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 47:8, 1152-1165
- [Appl Microbiol Biotechnol](#). 2009 Mar;82(3):565-77. doi: 10.1007/s00253-009-1868-0. Epub 2009 Jan 27. **Enhancement of the microbial community biomass and diversity during air sparging bioremediation of a soil highly contaminated with kerosene and BTEX.** [Kabelitz N¹](#), [Machackova J](#), [Imfeld G](#), [Brennerova M](#), [Pieper DH](#), [Heipieper HJ](#), [Junca H](#).
- [FEMS Microbiol Ecol](#). 2011 Oct;78(1):137-49. doi: 10.1111/j.1574-6941.2011.01169.x. Epub 2011 Aug 1. **Functional adaptation of microbial communities from jet fuel-contaminated soil under bioremediation treatment: simulation of pollutant rebound.** [Korotkevych O¹](#), [Josefiova J](#), [Praveckova M](#), [Cajthaml T](#), [Stavelova M](#), [Brennerova MV](#)
- [Clean Soil and Safe Water](#) Part of the series [NATO Science for Peace and Security Series C: Environmental Security](#) pp 281-290 Date: 03 October 2011 **The Role of the Regulator in the Water Management in the Czech Republic – Case Study of a Large Remediation Project**, Kvetoslav Vlk_ Jaroslav Zima Zdena Wittlingerova Jirina Machackova.

7th International Conference on Protection and Restoration of the Environment, Chania, Crete 2006

Battelle
The Business of Innovation

Biosymposiums and Chlorcons –
Baltimore 2005, Monterey 2008,
Miami 2016, USA

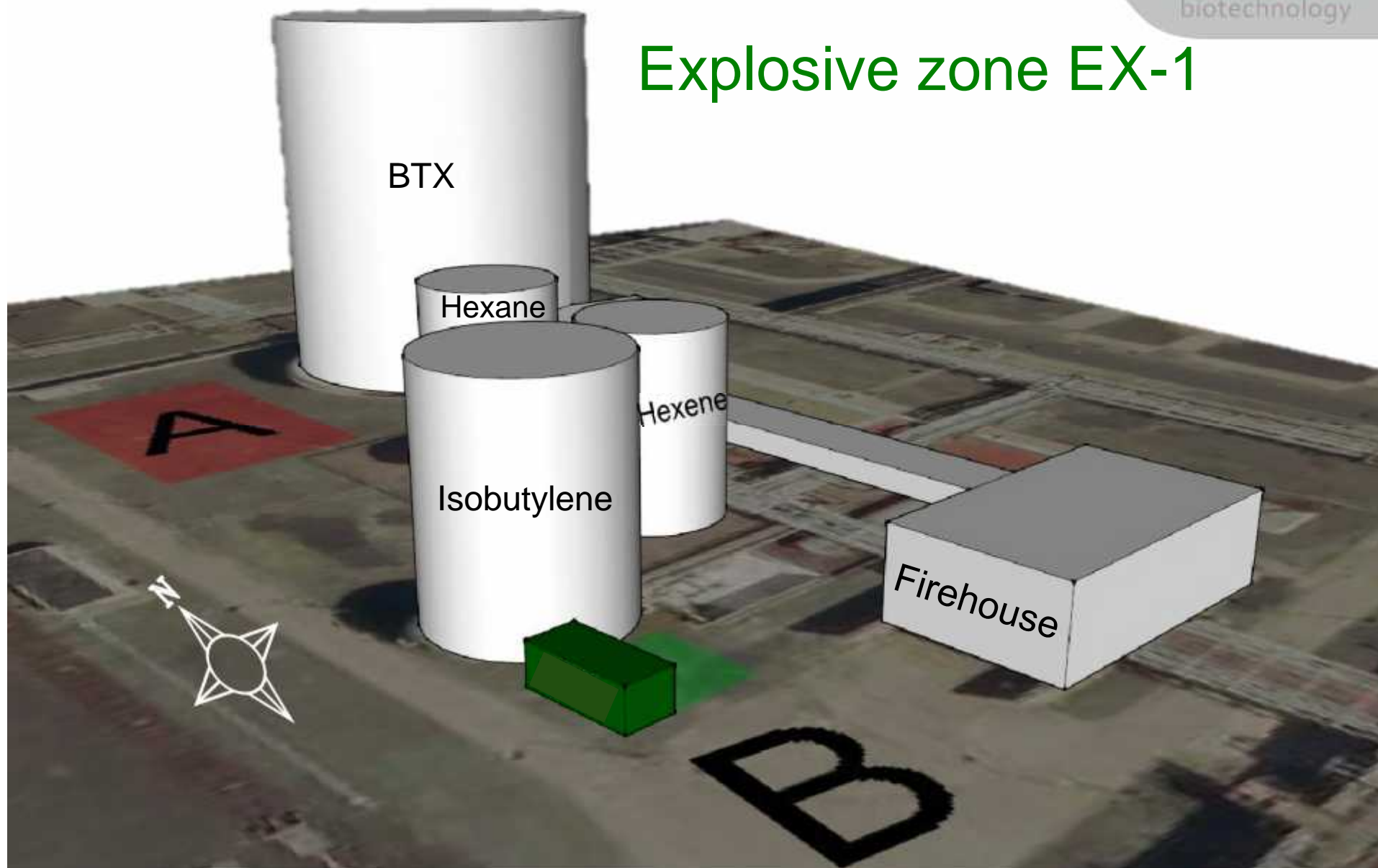
EXPLOSIVE ZONE REMEDIATION CONTROL

Active petrochemical production

Strict safety measures!



Explosive zone EX-1





- Cheap, very strong oxidant (ROS)
- **UNSTABLE**
 - Fast disintegration, **exothermic decay (!!)**
 - Produces large amounts of gas: O₂ & VOCs
- **STABILIZATION**
 - critical know-how
 - Addition of stabilizer (phosphates, chelates, organic acids = pH drops !)



Primary pilot test objectives

1. To verify the technology,
2. to reduce contamination levels,
3. to comply with rigorous safety regulations (EX-1),
4. to optimize on site process and reaction control.

Studied risk factors

1. Exothermic reaction course,
2. reagent corrosiveness,
3. generation of VOCs as daughter products.

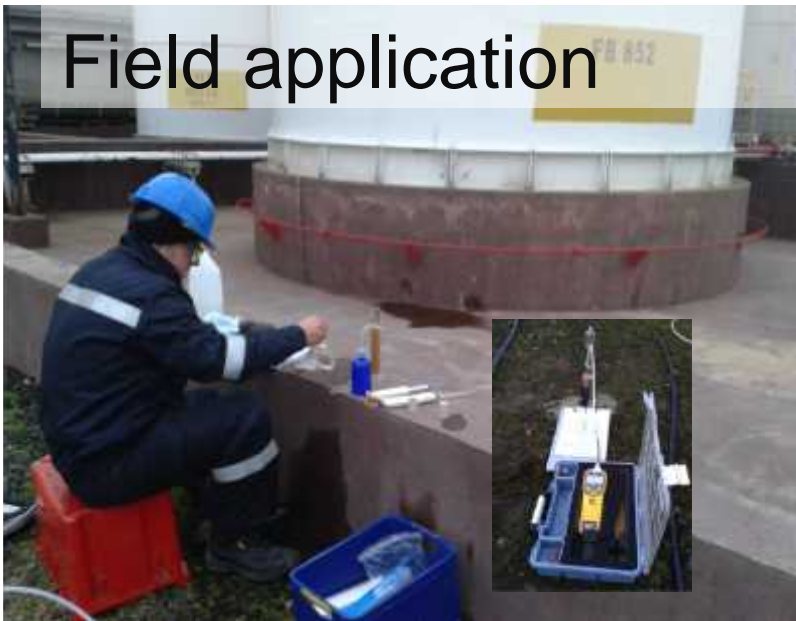
- Ethylene production => pollutants:
 - BTEX, Naphthalene, Non-polar organics (NOCs)

HV-8857: pollutant evolution

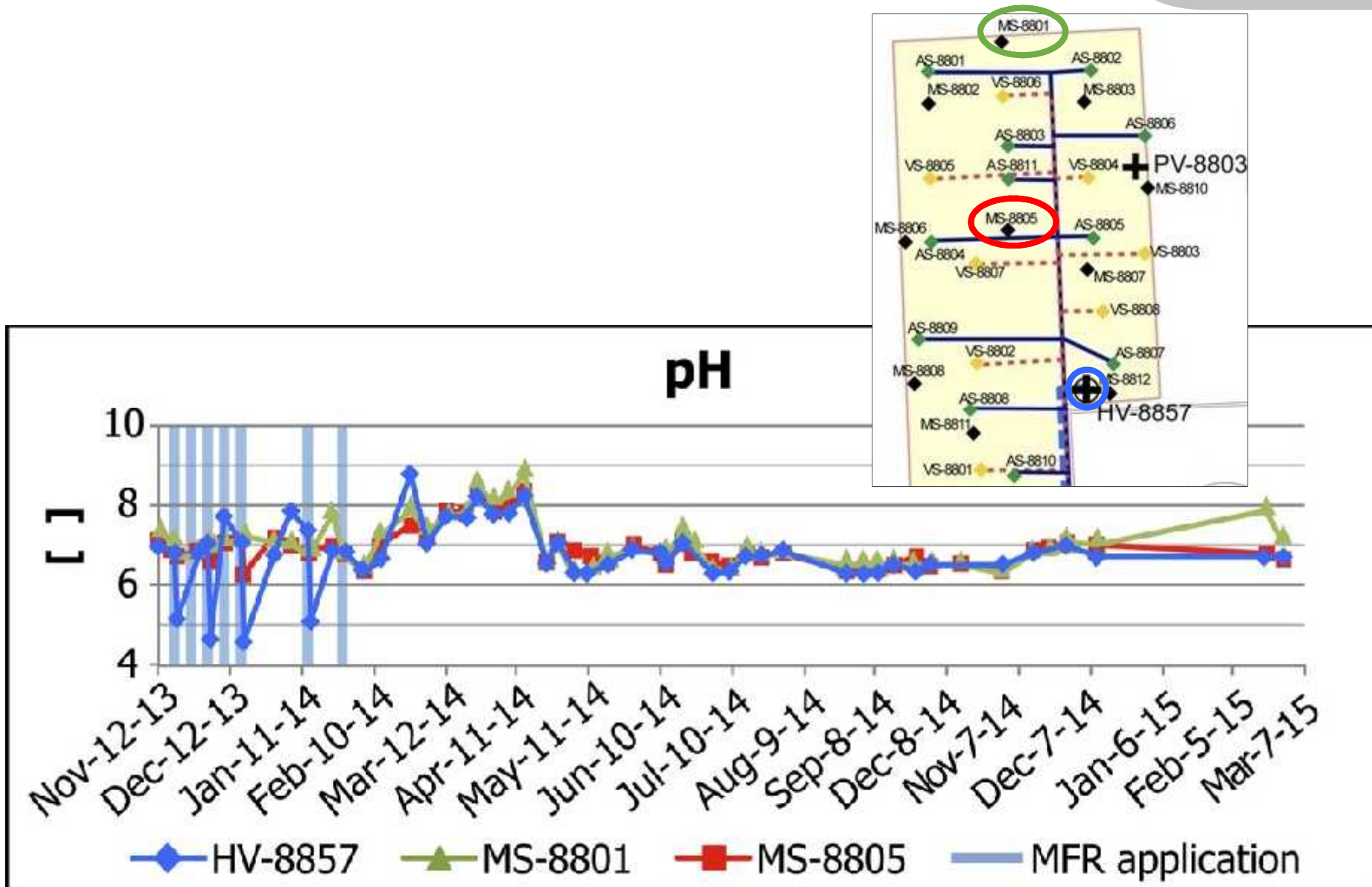
Contaminant	Benzene [$\mu\text{g/L}$]	Naphthalene [$\mu\text{g/L}$]	NOCs [mg/L]
RC	400	1700	no FPLH
TC	2 500	2 500	20
2004	-	-	FPLH
2006	125 000	< 0.5	> 200
2013*	10 300	162	13.2

*(pre-pilot)

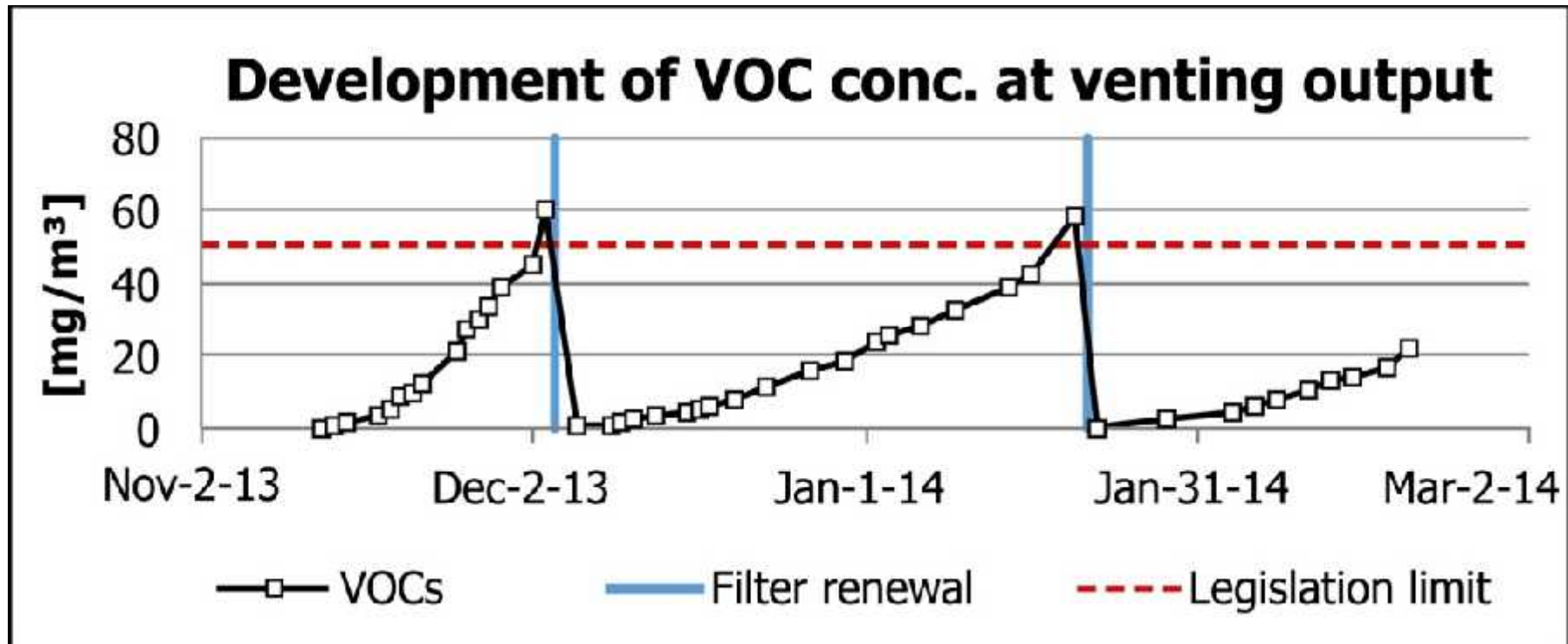
Field application



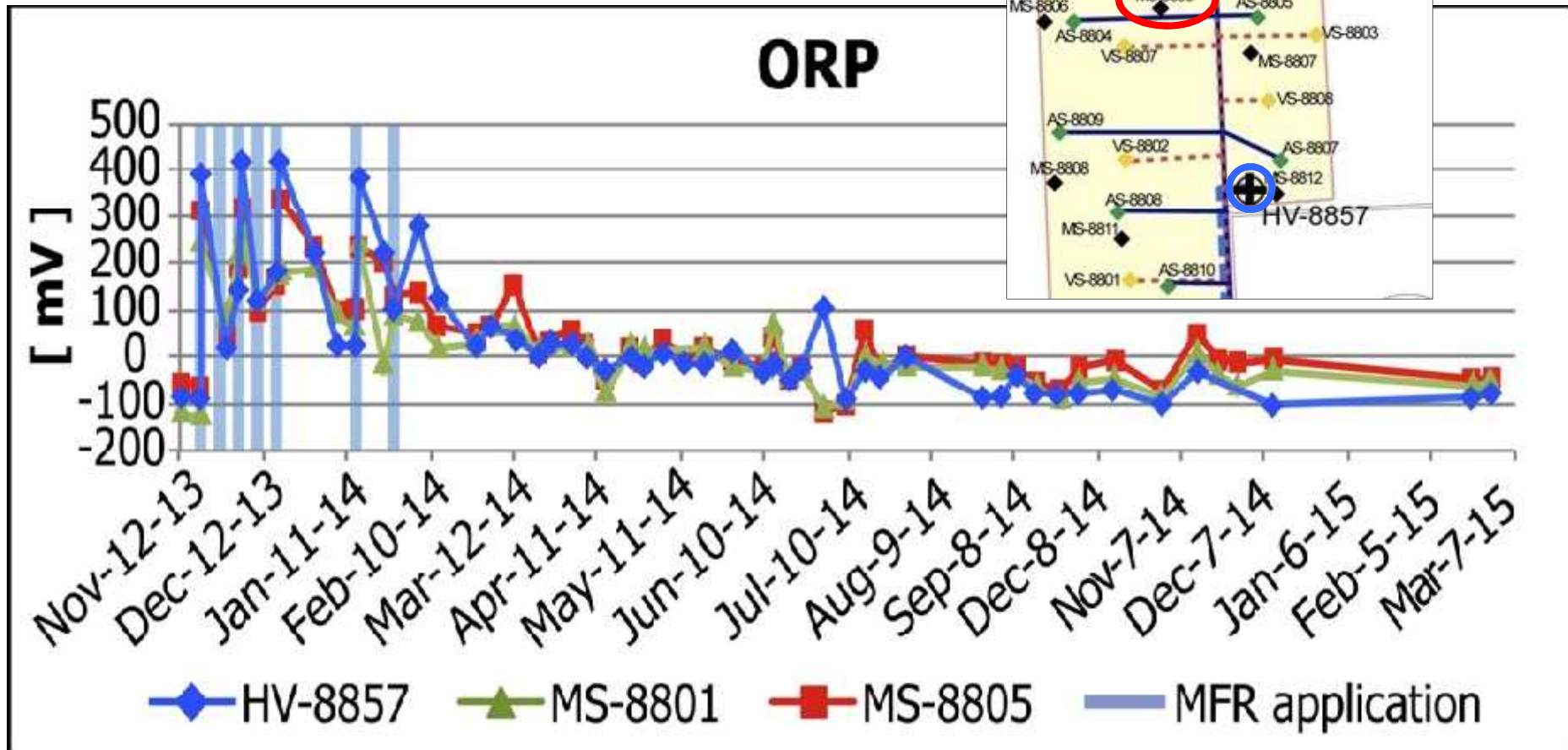
Safety: pH monitoring



Venting filter outflow



Parallel with the DO parameter



Contaminant destruction: HV-8857

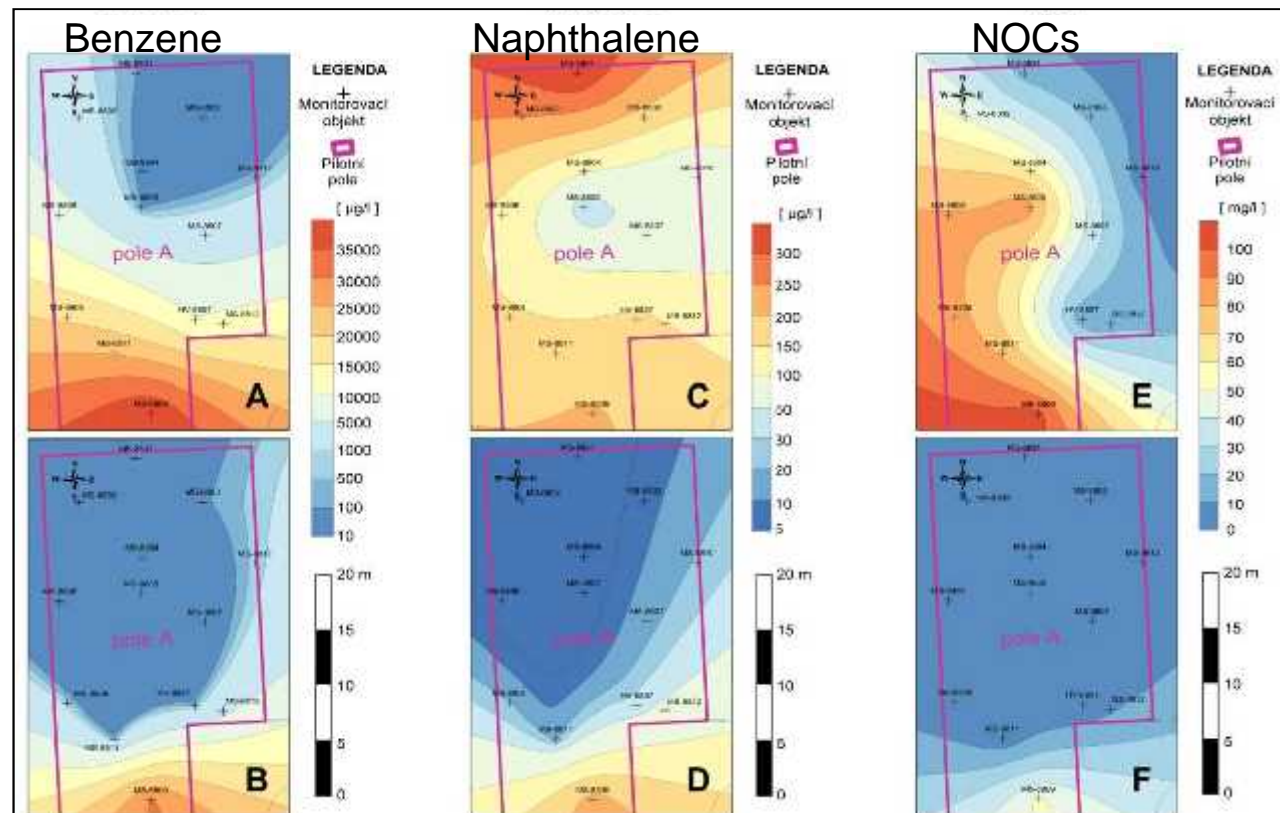
Contaminant	Benzene [µg/L]	Naphthalene [µg/L]	NOCs [mg/L]
RC	400	1700	no FPLH
TC	2 500	2 500	20
2013*	10 300	162	13.2
2014*	404	36.8	2.87

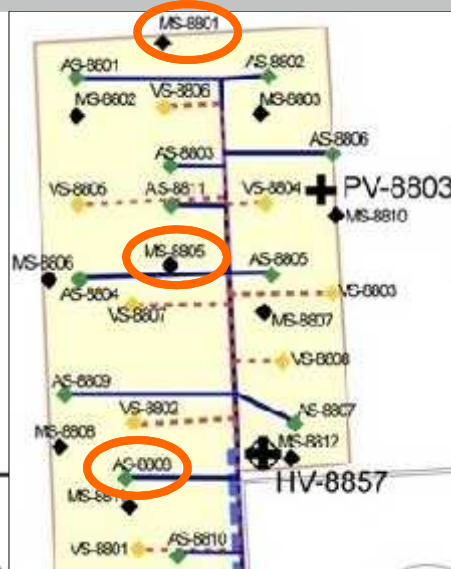
**Before pilot test*

– A, C, E

**After pilot test*

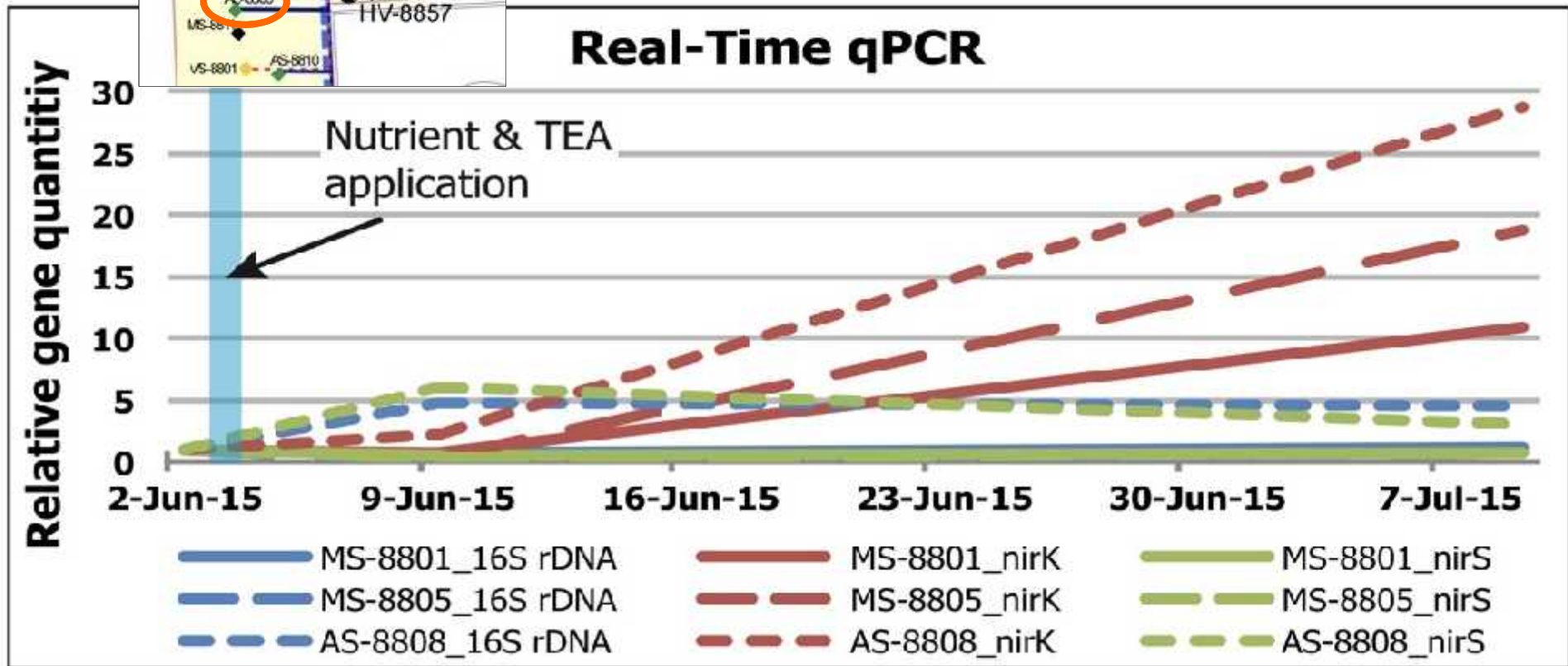
– B, D, F





Enhanced natural attenuation:

- Nutrients: NH_4^+ & PO_4^{3-}
- Terminal Electron Acceptor: NO_3^-



Primary pilot test objectives

- ✓ 1. Technology verification,
- ✓ 2. reduction of contamination levels,
- ✓ 3. rigorous safety regulations (zone EX-1), and
- ✓ 4. Control tools optimization (real time monitoring).

Studied risk factors

- 1. Exothermic rxn': Temp. increase less than 5°C
- 2. Corrosiveness: pH > 4,5 & g.w. level below the level of utility networks
- 3. VOCs generation: concentration decrease along time

Enhanced attenuation potential => denitrification