

EOS Remediation



Monitoring Gene Potential to
Evaluate the Effectiveness of EOx™
at a Gasoline Impacted Site

By: Gary M. Birk, P.E.

YOUR NATURAL SOLUTIONS

Patented Methods for *In Situ* Bioremediation

Acknowledgements

- Holly Parsons, L.P.G. Project Manager, American Environmental Corporation, Indianapolis, IN
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Reference: Lisa Alvarez-Cohen, Civil and Environmental Engineering
University of California, Berkeley, Earth Science Division, LBNL

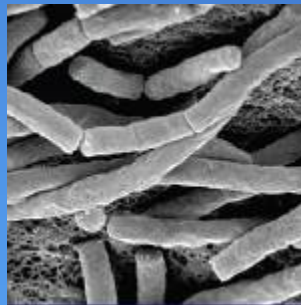
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In Situ Bioremediation

In Situ
↓
In Place

Bio
↓
Microbial

Remediation
↓
Method to Fix

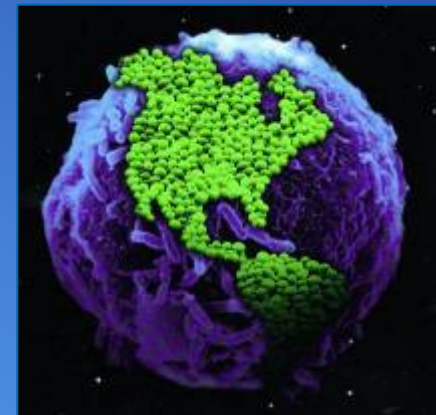


Microorganisms are used to Clean up Pollution
in the Environment

Microbial World: The unseen majority

- ~20,000 strains/species of bacteria in 1g topsoil
- Bacteria in 5g topsoil = ~total human population of Earth
- Total bacterial population of Earth = $\sim 5 \times 10^{30}$ cells
- **>90% of bacteria are in the subsurface below 30 feet**

Ref : Whitman et al. (1998) Proc. Nat Acad. Sci. USA 95: 6578-6583



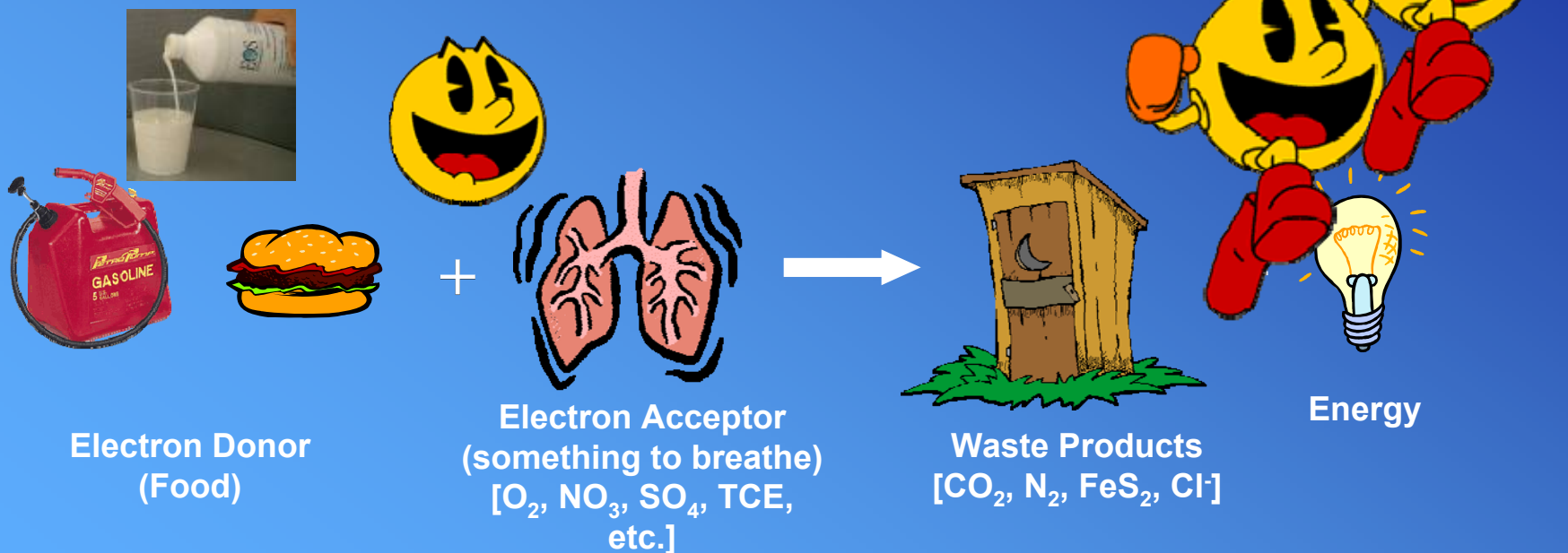
How Does It Work?

- How does *in situ* bioremediation clean up pollution?
 - Soil microbes are remarkably versatile!
 - They eat (electron donor) and breathe (electron acceptor) many types of organic and inorganic chemicals to generate energy to live.
 - (Gasoline, oil, chromium, iron, solvents, perchlorate, nitrate, ammonia, MTBE, etc)
- They live in complex communities capable of degrading contaminants by “bucket brigade”.



How Does It Work?

➤ Growth-Promoting Biological Reduction



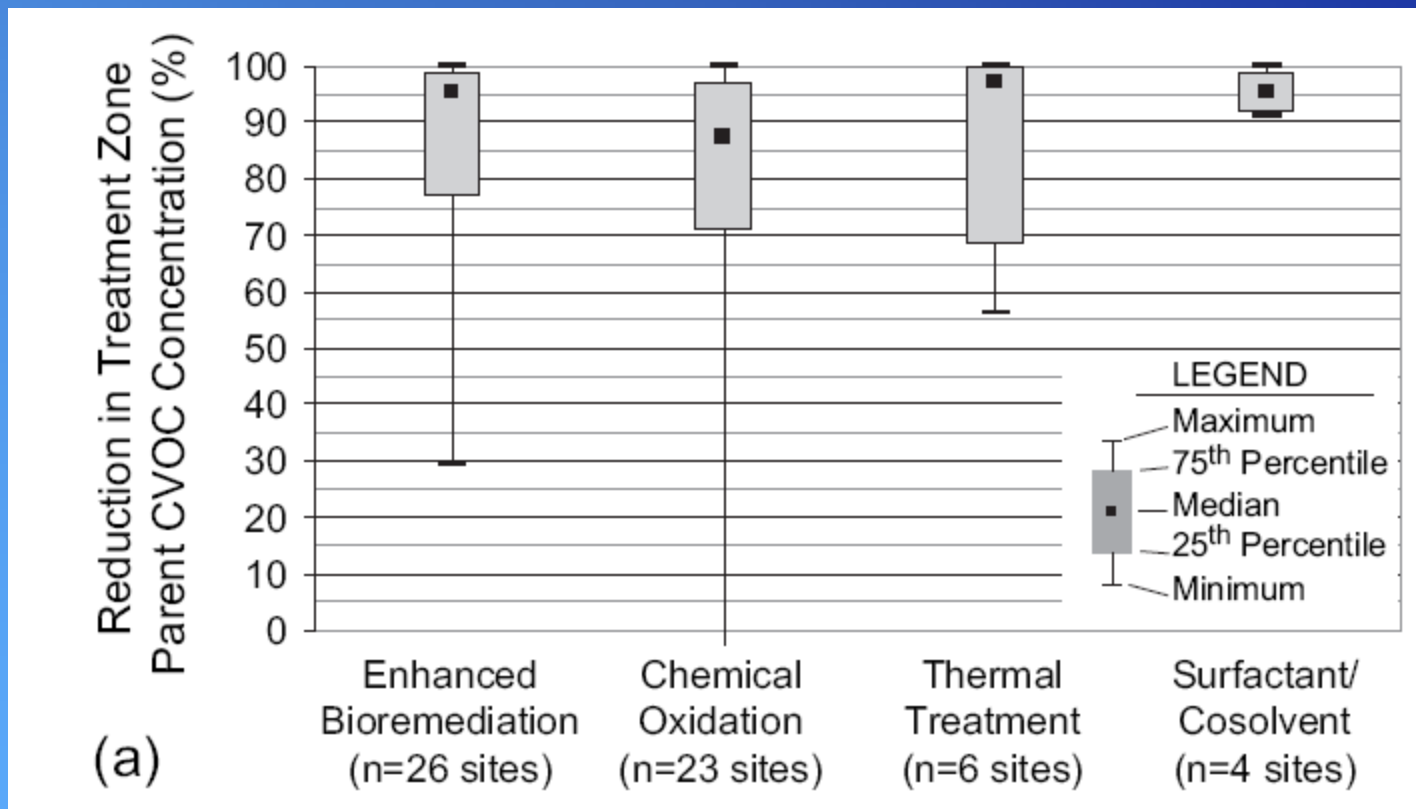
(Drawing Modified from AFCEE and Wiedemeier)

Applying *In Situ* Bioremediation

- Natural Attenuation – biotransformation occurs naturally: indigenous microbes present, substrates & nutrients present (can be MNA)
- Biostimulation - indigenous microbes present, substrates &/or nutrients must be added
- Bioaugmentation – indigenous microbes not present, organisms are added

Why Bioremediation?

Remedial Performance and Cost



\$29/yd³

\$125/yd³

\$88/yd³

\$385/yd³



From:

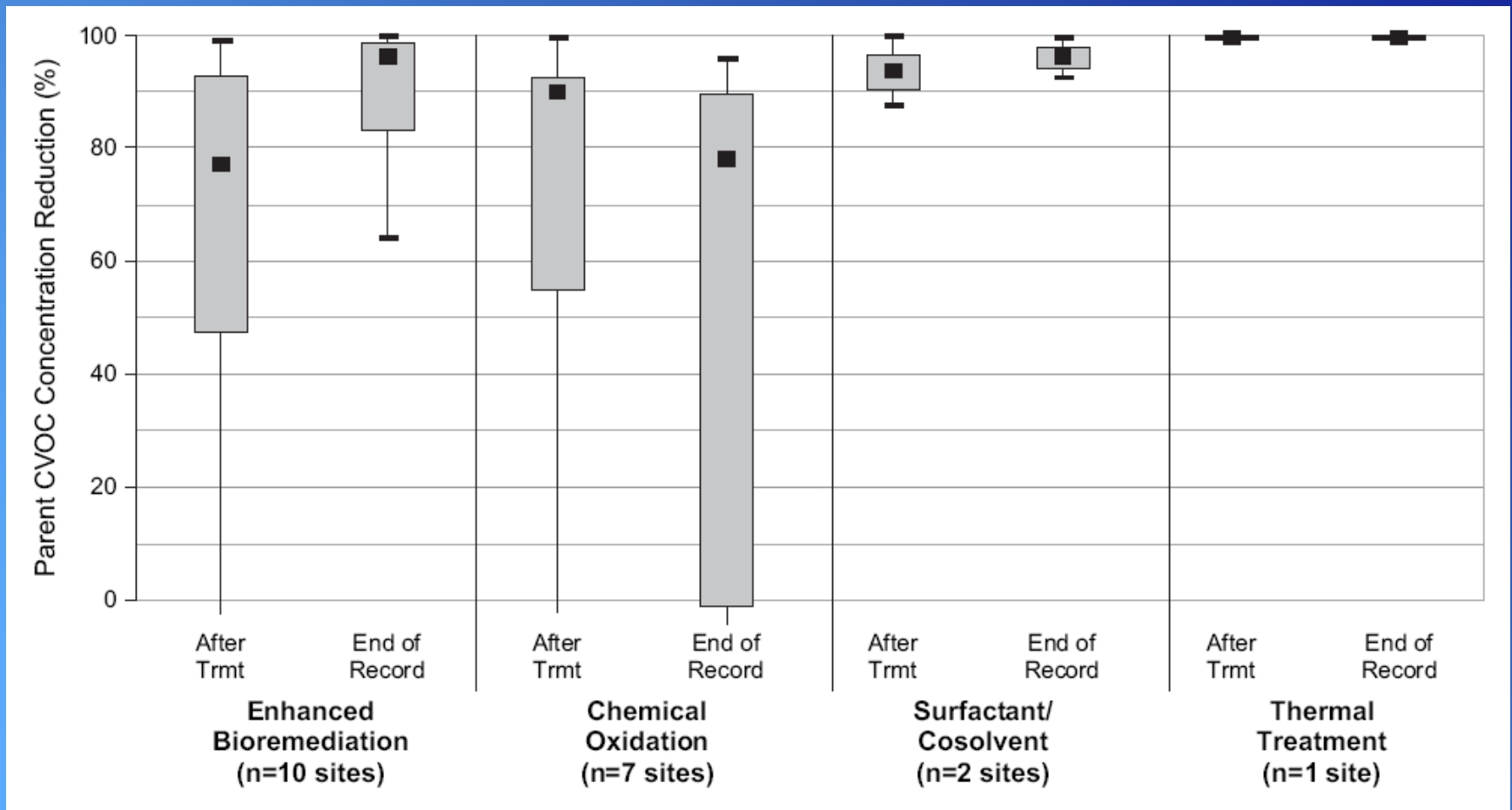
McDade et al, *Remediation Journal* 15, 9-18, 2005.

McGuire et al, *Ground Water Monitoring and Remediation* 26, 73-84, 2006.

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Contaminant Rebound



From: McGuire et al, Ground Water Monitoring and Remediation 26, 73–84, 2006.



An Oxygen Releasing Compound for Enhanced Aerobic Bioremediation

- Time-release metal peroxide (CaO_2)
- Accelerates bioremediation of aerobically degradable contaminants
- Reaction





An Oxygen Releasing Compound for Enhanced Aerobic Bioremediation

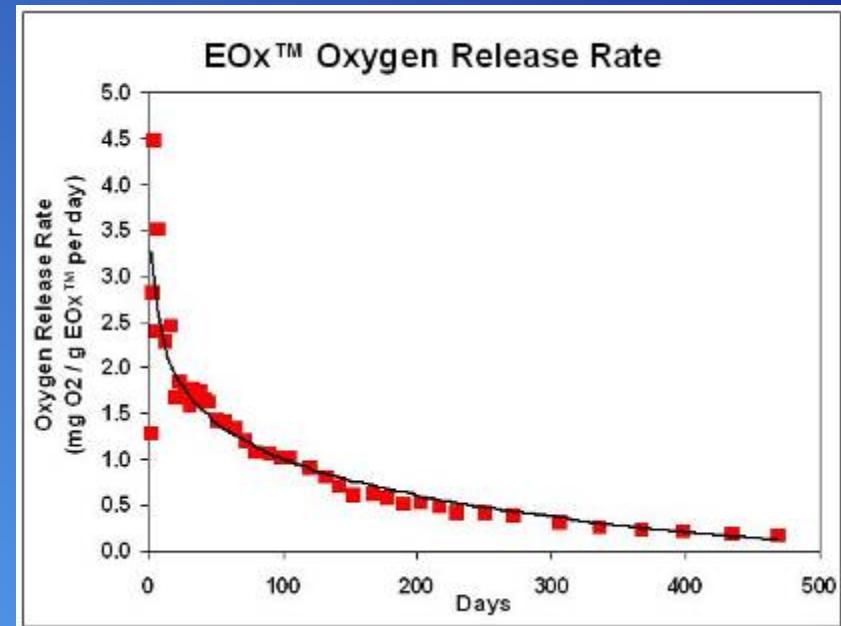
Product Properties	<i>EOx</i> TM Standard Specification
Composition	CaO ₂ , CaO, Ca(OH) ₂
Calcium Peroxide, % by weight	Min.75
Available Oxygen, % by weight	Min.16.6
Particle Size Distribution	Through 200 mesh Min.99 Through 325 mesh Min.50
Bulk Density, g/L	500-650
Moisture, %	Max. 2.0
pH Approx.	10.5 - 12
Appearance	White or yellowish powder
Packaging	Fiber Drum, 110 lbs net contents





An Oxygen Releasing Compound for Enhanced Aerobic Bioremediation

- Controlled release of oxygen
 - Accelerates biodegradation by promoting activity of indigenous aerobic microorganisms
 - Results in a higher level of sustained bioactivity and increased contaminant removal





An Oxygen Releasing Compound for Enhanced Aerobic Bioremediation

- **EOx™ Applications:**
 - Direct application of *EOx™* powder or water slurry in excavations
 - *EOx™* and water slurry injection for source treatment or permeable reactive barriers (PRBs)

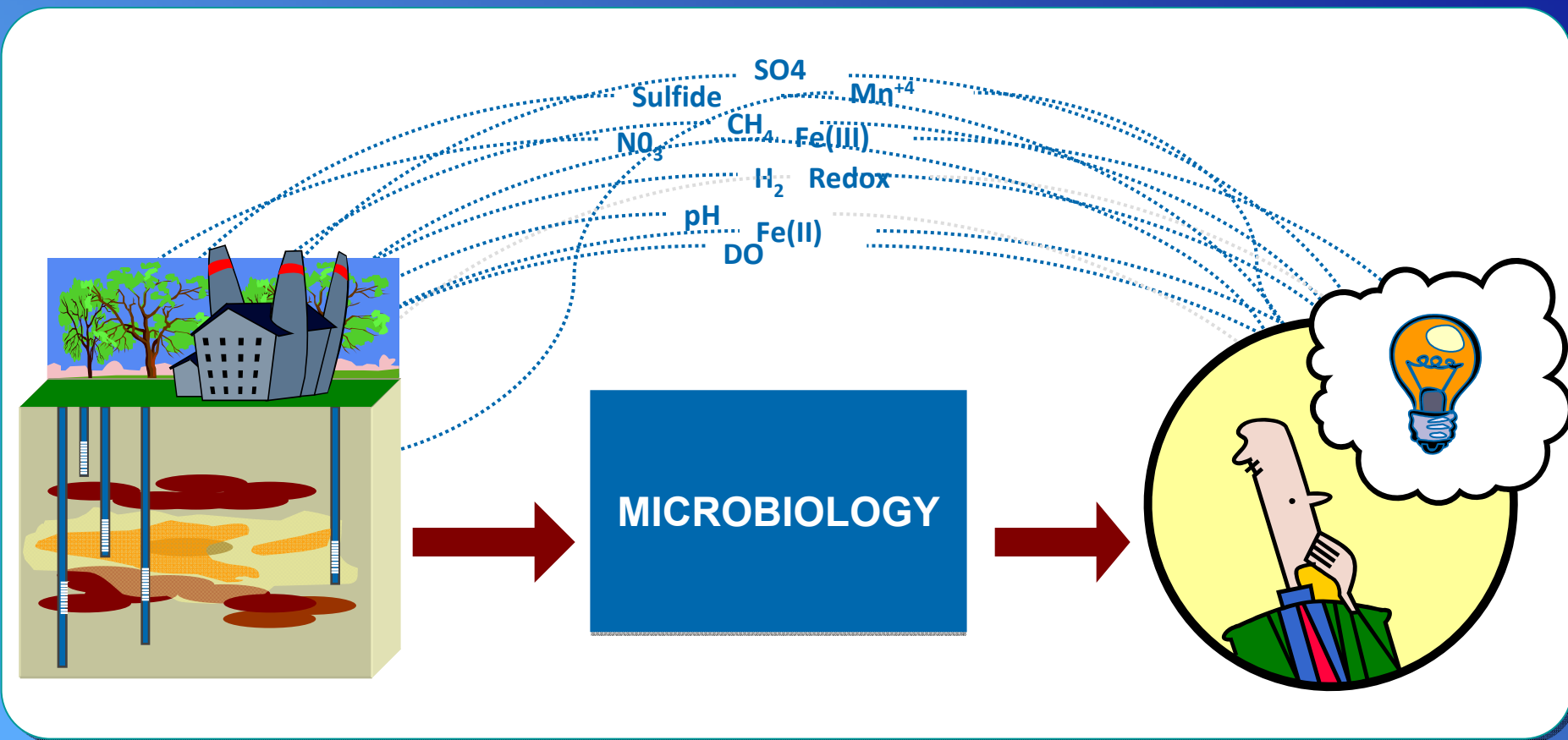


So why analyze for microbes at your site?

- Only a small segment of the population is capable of doing the desired process
 - *Dehalococcoides spp.* are the only known organisms that can completely degrade PCE to ethene under anaerobic conditions
 - Even if the compound is readily degradable, we don't know if it is being utilized under current site conditions.

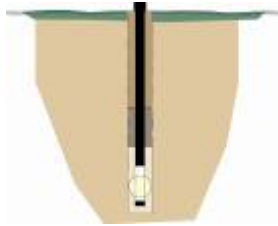


Site Assessment



How does CENSUS work?

Sample Collection



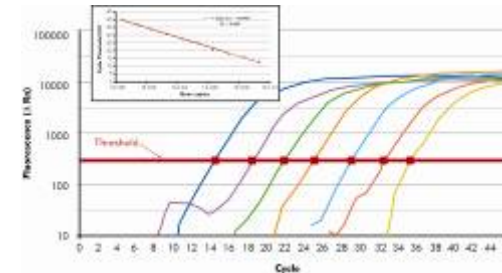
Groundwater, soil, or Bio-Trap samplers collected and shipped overnight on ice (4°C)

DNA Extraction



DNA extracted from sample upon arrival

Amplification



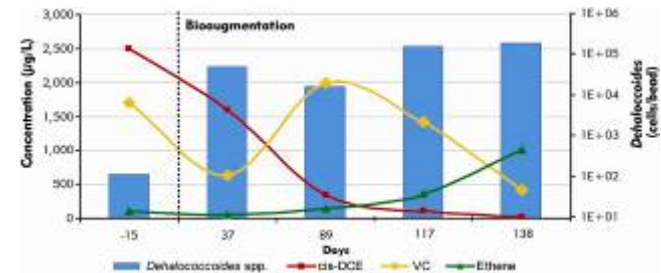
Quantitative Real-Time PCR (polmerase chain reaction) is used to detect & quantify targets of interest (i.e. Dehalococcoides spp.)

Results



Results are emailed to project contact (7 to 10 day turn around time)

Assessment



Results are integrated with other site parameters to evaluate site management decisions

CENSUS Applications Include



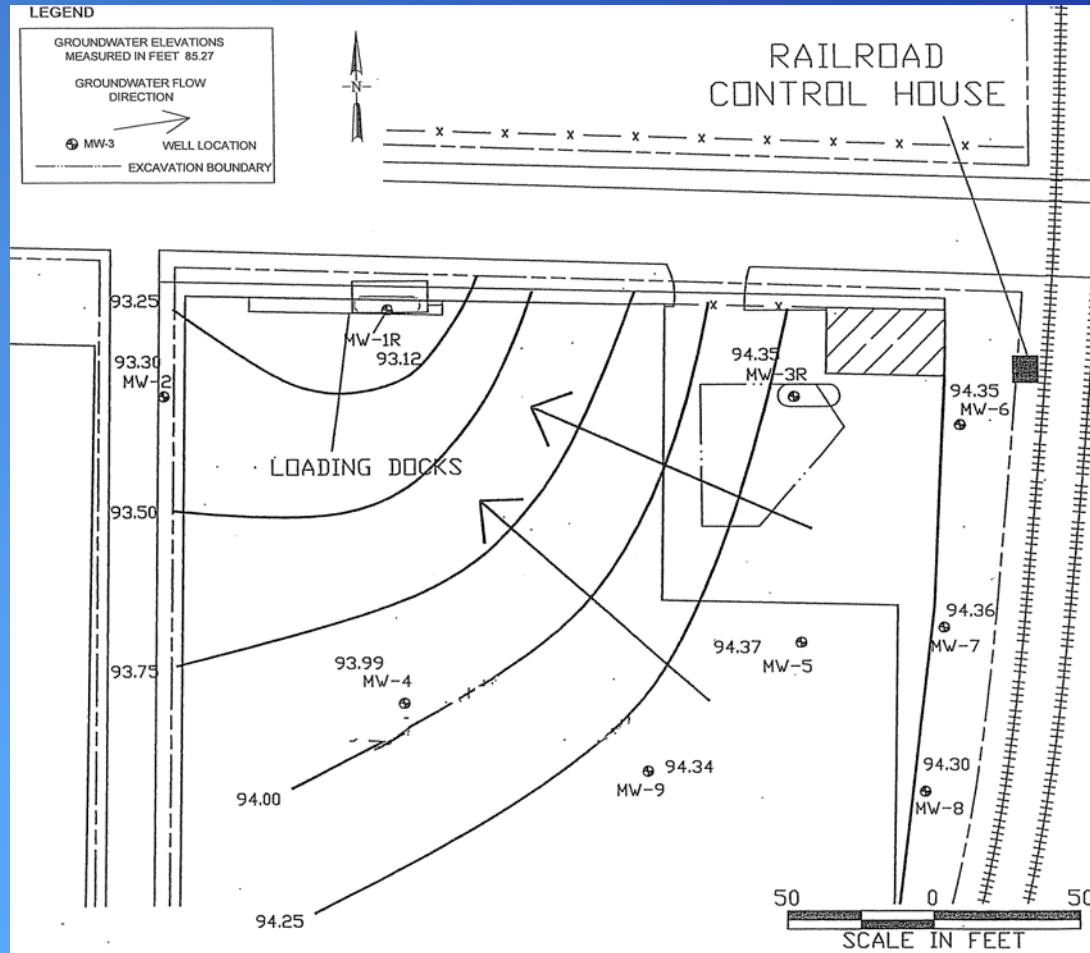
- Directly evaluate the feasibility of monitored natural attenuation (MNA)
- Evaluate the efficacy of enhanced bioremediation approaches
- Assess the need for bioaugmentation

EOX™



COC	CENSUS® Assay	Description
BTEX	<ul style="list-style-type: none"> • Phenol Hydroxylase (qPHE) • Toluene Dioxygenase (qTOD) 	<ul style="list-style-type: none"> • Metabolism of BTEX compounds • Initiates aerobic biodegradation of B, T, and E
MTBE	<ul style="list-style-type: none"> • MTBE utilizing PM1 (qPM1) 	<ul style="list-style-type: none"> • <i>Methylibium petroleiphilum</i> PM1, one of the few bacteria isolated that is capable of growth on MTBE
PAHs	<ul style="list-style-type: none"> • Naphthalene Dioxygenase (qNAH) 	<ul style="list-style-type: none"> • Initiates aerobic biodegradation of naphthalene and some additional PAHs
Pentachlorophenol (PCP)	<ul style="list-style-type: none"> • PCP Monooxygenase (qPCP) 	<ul style="list-style-type: none"> • Initiates aerobic biodegradation of pentachlorophenol

Industrial Facility Indianapolis, IN



Microbial Results

- Approximately 4 months after the EOx™ application
- MW-3R (impacted well in EOx™ treated zone)
- MW-5 (impacted well, upgradient of EOx™ application)

CENSUS® Assay	MW-5	MW-3R	Units
Phenol Hydroxylase (PHE)	1.39E+02	2.79E+02	cells/ml
Toluene Dioxygenase (TOD)	1.10E+07	3.06E+07	cells/ml



Analytical Data Courtesy of Holly Parsons, L.P.G., American Environmental Corporation

Analytical Results

MW-5	Naphthalene	TPH GRO	TPH ERO	TPH DRO
RISC RDCL	8.3	1100	100	260
	ppb	ppb	ppb	ppb
11/6/2008	103	8100	5900	NS
4/24/2009	20.8	4600	2700	NS
9/2/2009	127	3700	NS	2400
9/15/2010	EOx™ Application			
11/24/2008	<5.0	<200	NS	160
2/2/2010	<1.0	<200	NS	140
5/13/2010	<5.0	<200	NS	250
MW-3R	MTBE	TPH GRO	TPH ERO	TPH DRO
RISC RDCL	40	1100	100	260
	ppb	ppb	ppb	ppb
11/6/2008	63.3	<200	230	NS
4/24/2009	6.2	<200	200	NS
9/2/2009	23.9	<200	NS	140
9/15/2010	EOx™ Application			
11/24/2008	11.5	<200	NS	460
2/2/2010	11.5	<200	NS	100
5/13/2010	<4.0	<200	NS	120

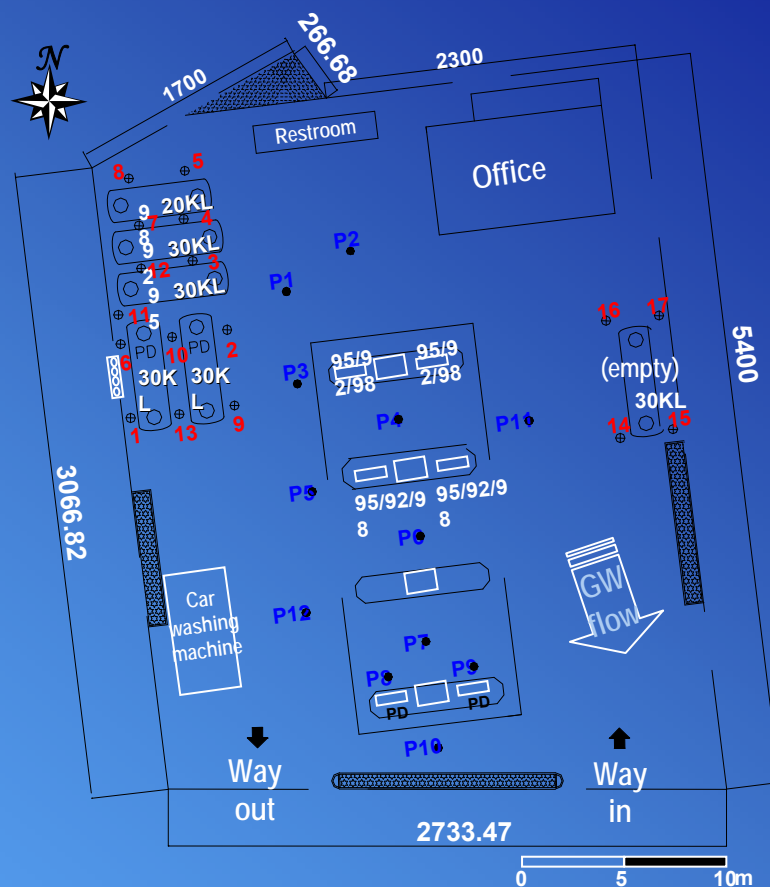


Conclusions

- Dissolved oxygen results still remain evaluated 11 months after treatment
- IDEM dropped groundwater sampling requirements for the site
- Site is currently seeking closure with an Environmental Restrictive Covenant

Site Condition

CPC Service Station
Located in Kaohsiung, Taiwan



◆ Hydrogeology

- Water level: 1.5~2m bgs
- GW flow to SSE
- Silty Sand ($K \sim \times 10^4$)

- ⊕ Pipe leak detector in oil storage tank area
- Pipe leak detector in piping and service area



Target Contaminant - TPH

- Soil sample at **S1**
- Contamination

TPH: 2,303 mg/kg

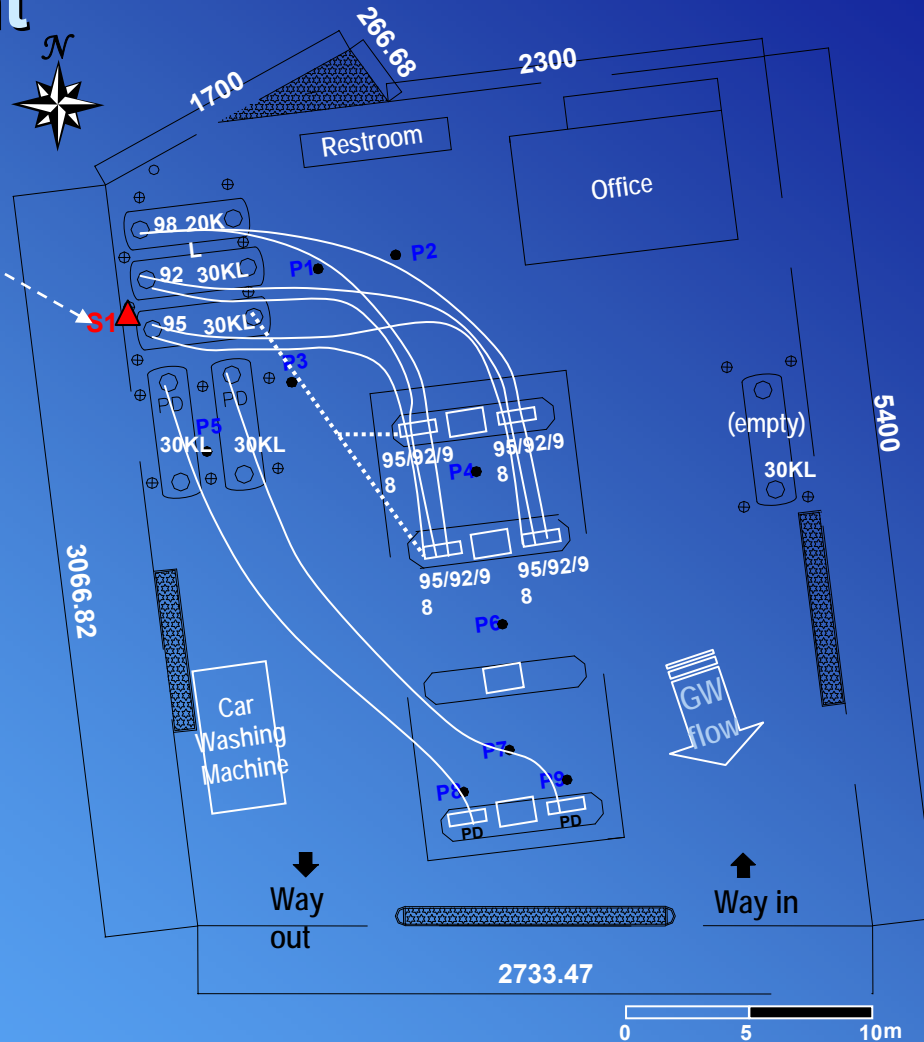
TPH-g: 283
mg/kg

TPH-d: 2,020
mg/kg

Depth: 1.5~3m bgs

- Standard
(Taiwan Soil Pollution Control
Standard)

•TPH: 1,000 mg/kg

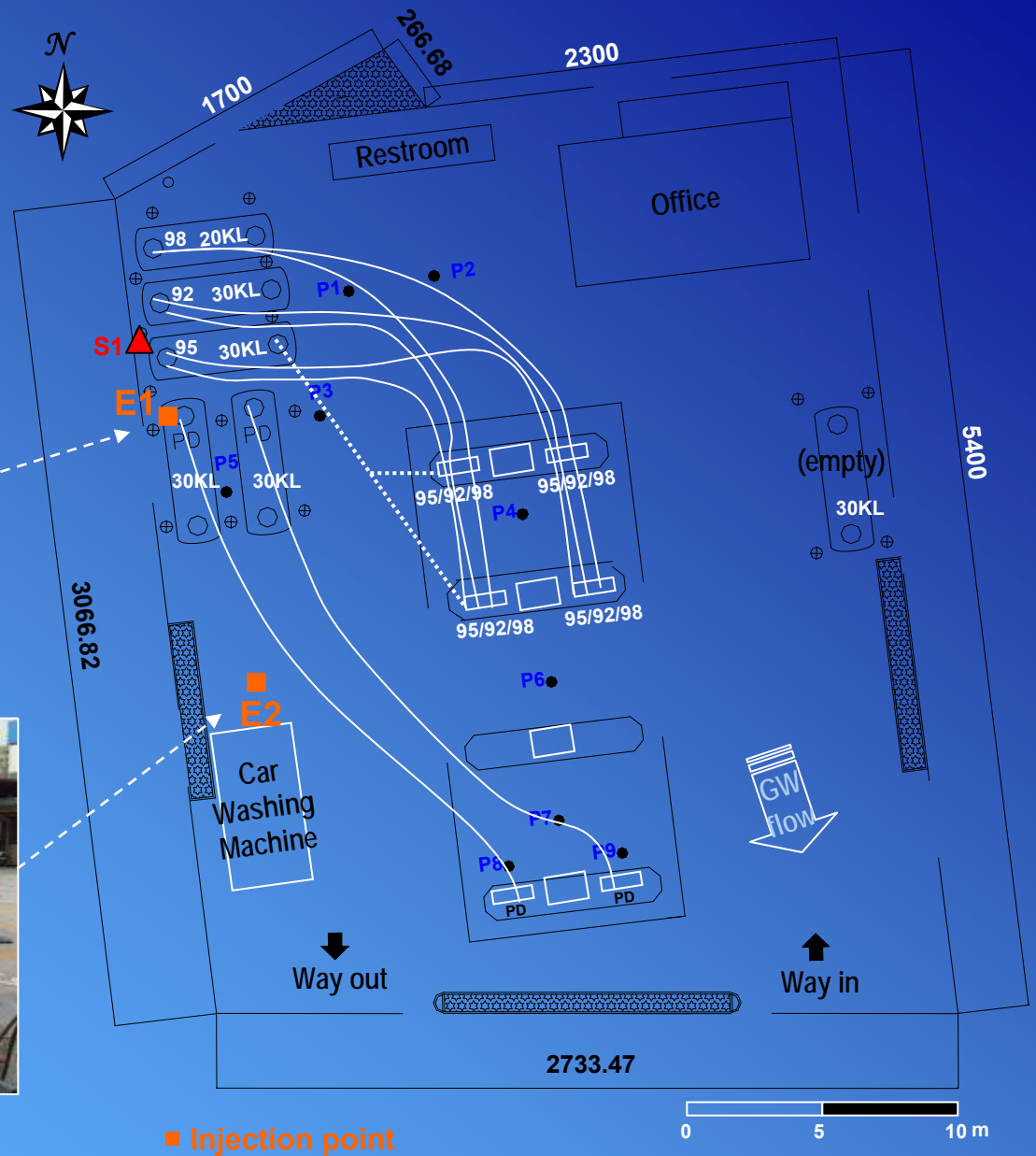


Remediation Activities

- EOX™ Slurry Preparation
 - EOX™ powder : Water = 1:10 (by wt)
- Geoprobe Direct Push
 - Injection depth 1.5~3.0m bgs
- 1st Round Injection on 4/15/2009 (3.a)
 - 2 points: 50kg-EOX™ each point.
- 2nd Round Injection on 9/16/2009 (3.b)
 - 2 points: 50kg-EOX™ each point.

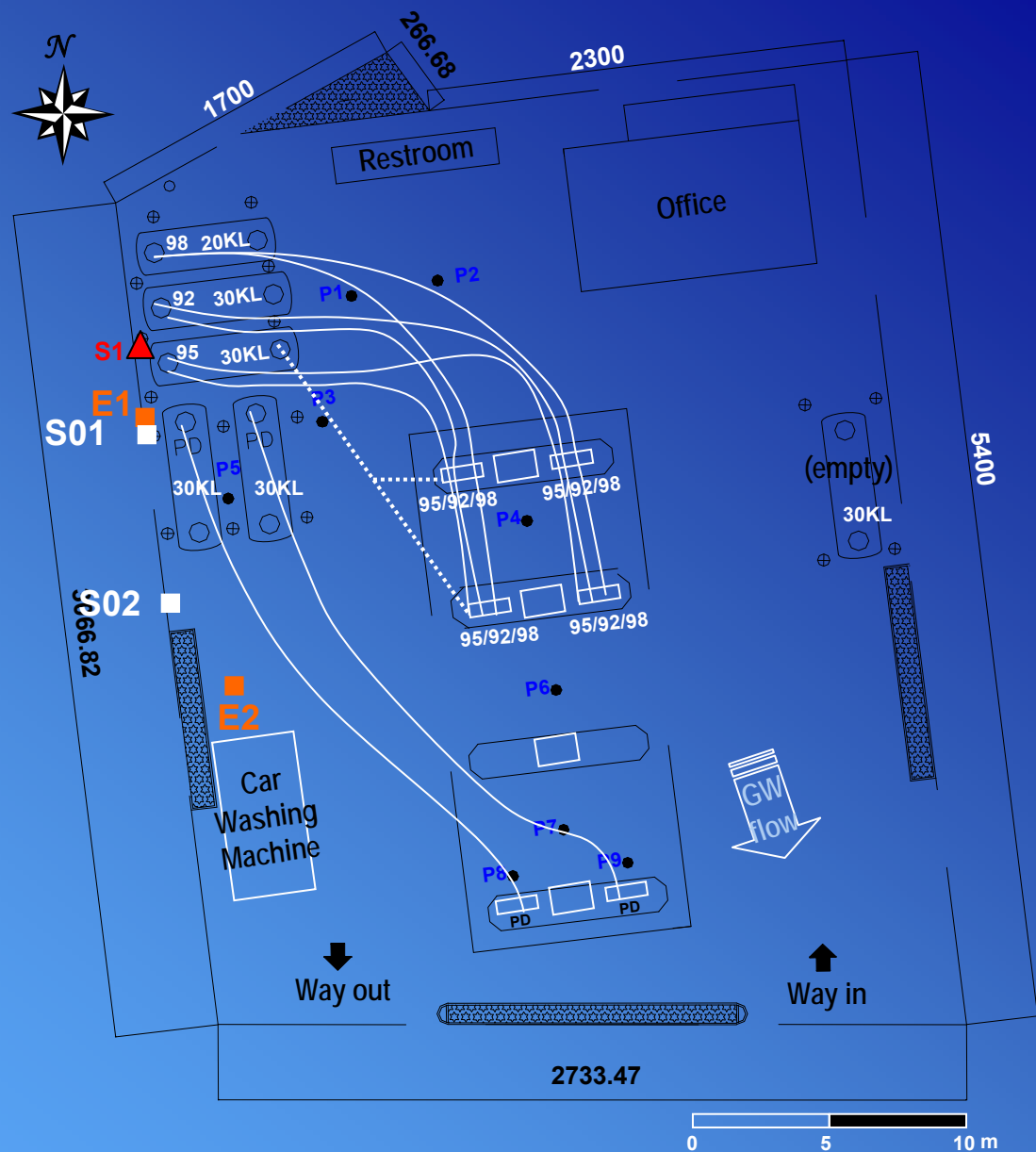
1st Injection April 15, 2009

- Two injection points
E1 : S1 Source Area
E2 : Downgradient



Initial Injection Performance Monitoring

- June 24th 2009 (2 months after 1st injection)
- Collected samples at **S01 & S02**
- Analyzed for:
 - TPH
 - BTEX



■ Injection point ■ Sampling point



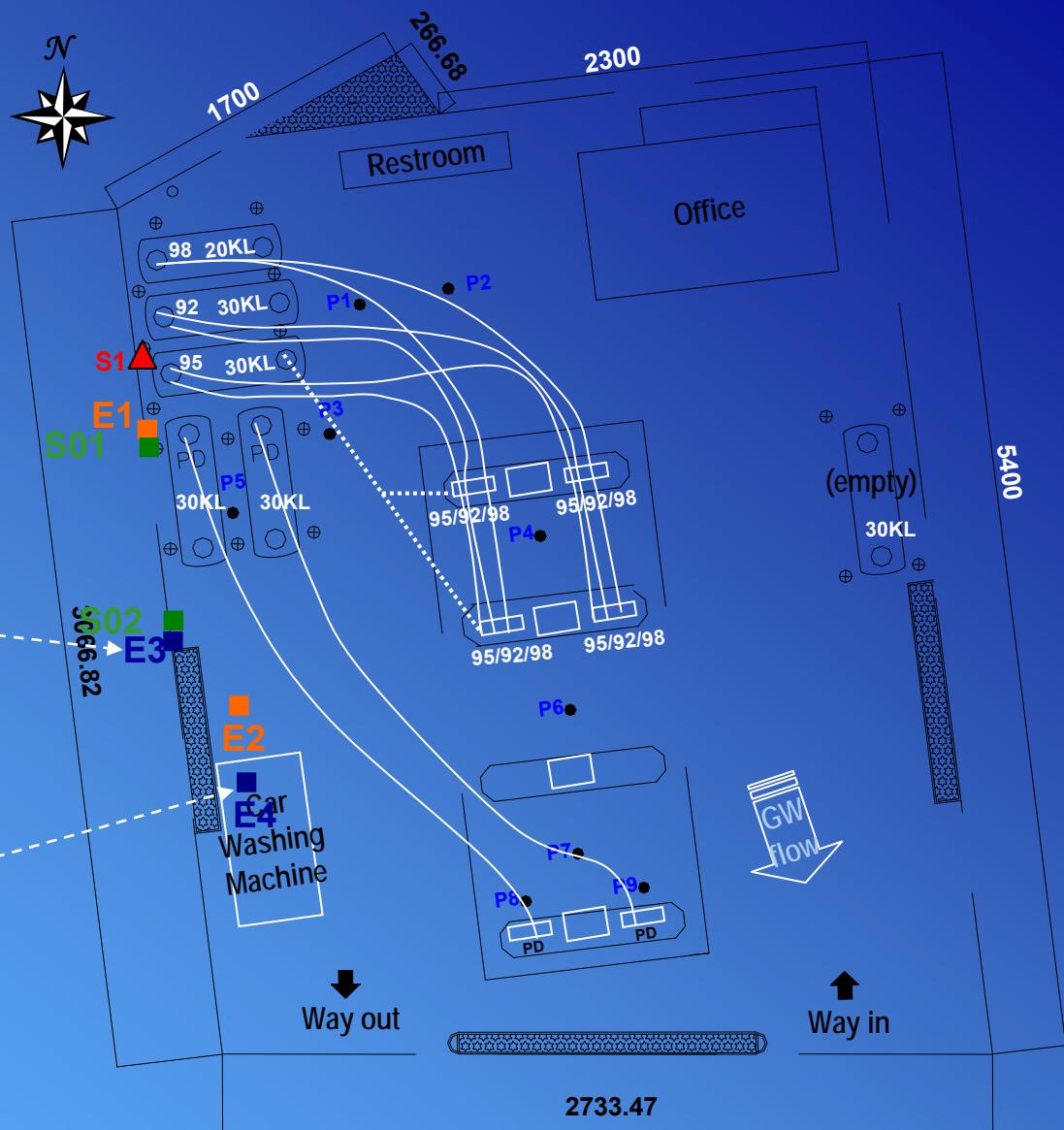
1st Round Results

- TPH at both S01 and S02 are below the standard.
 - S01: TPH has been reduced from 2,303 mg/kg to non-detective (<60 mg/kg).
 - S02: TPH was able to meet the standard but still contaminant was there (<474mg/kg).
- **Implement the 2nd round injection work at S02 area.**

Analytes & Soil Sample No.		Analytical values (mg/kg)					
		Benzene	Toluene	Ethyl- benzene	Xylene	TPH-g	TPH-d
Soil Pollution Control Standards		5	500	250	500	1000	
Jun 24 th 2009	S01	<0.10	<0.10	<0.10	<0.30	<10.0	<50.0
	S02	<0.10	<0.10	<0.10	<0.30	<10.0	464

2nd Injection Septemebr 16th, 2009

- Two injection points
 - E3** : S02 Area
 - E4** : Downgradient



■ Injection point (1st)

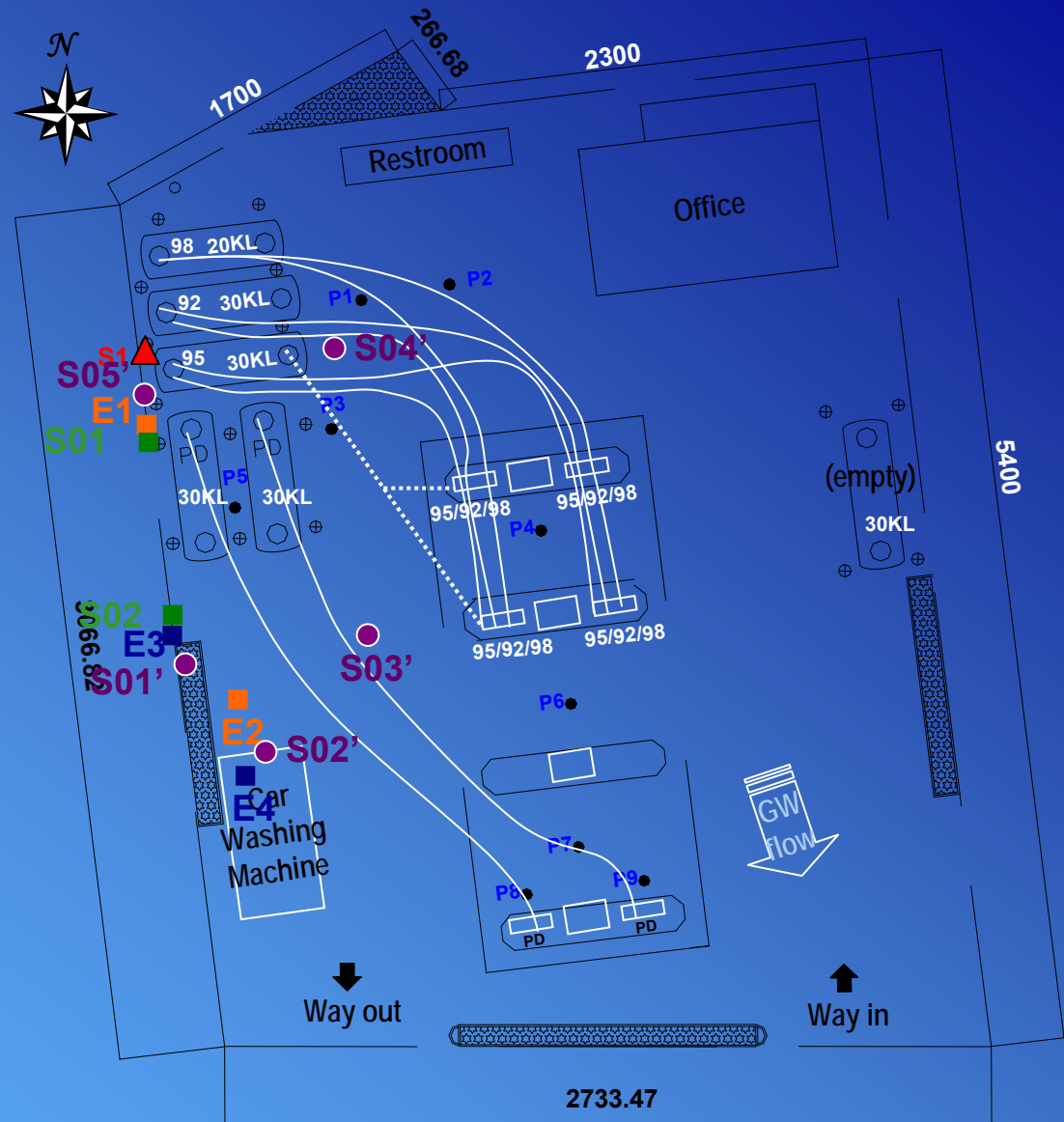
■ Sampling point (1st)

■ Injection point (2nd)



Second Injection Performance Monitoring

- December 9th 2009 (3 months after 2nd injection)
- Collected samples at S01', S02', S03', S04' & S05'
- Analyzed for:
 - TPH
 - BTEX



- Injection point (1st)
- Injection point (2nd)
- Sampling point (1st)
- Sampling point (2nd)



2nd Round Results

- TPH at all the five sampling points are reduced to non-detective (<60mg/kg).
- EOX™ helped clean the soil and reach site closure in an year.

Analytes & Soil Sample No.		Analytical values (mg/kg)					
		Benzene	Toluene	Ethyl-benzene	Xylene	TPH-g	TPH-d
Soil Pollution Control Standards		5	500	250	500	1000	
Dec 9 th 2009	S01'		<0.10	<0.10	<0.30	<10.0	<50.0
	S02'	<0.10	<0.10	<0.10	<0.30	<10.0	<50.0
	S03'	<0.10	<0.10	<0.10	<0.30	<10.0	<50.0
	S04'	<0.10	<0.10	<0.10	<0.30	<10.0	<50.0
	S05'	<0.10	<0.10	<0.10	<0.30	<10.0	<50.0

Summary

- Total 4 drums of EOx™ (50kg per point) were used for remediation.
- No rebound was observed.
- EOx™ helped clean the soil and reach site closure in a year.

EOS Remediation

For more information, please visit:
www.EOSRemediation.com



Thank you for attending ...
Questions?

YOUR NATURAL SOLUTIONS
Patented Methods for *In Situ* Bioremediation

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