

Monitoring of Bioremediation Efficacy at Five Sites with Mixed Hydrocarbon Contamination by Mineralization and Functional Gene Assays

Kirsten Jørgensen, Jani Salminen¹, Pirjo Tuomi²

*Finnish Environment Institute
Research Programme for Contaminants and Risks
Helsinki, Finland*

Current affiliation:

¹*Vrije Unversiteit, Amsterdam*

²*Golder Associates Oy, Helsinki*



S Y K E

Aim

- To compare activity measurements of petroleum hydrocarbon degradation with DNA-based methods to assess efficacy of bioremediation at 5 sites



S Y K E

Background

- Petroleum hydrocarbons
 - Biodegradable both aerobically and anaerobically
 - Biodegradability widespread among bacteria
 - Mixture of hundreds of single compounds
 - Estimation of biodegradation efficiency needed



S Y K E

Methods used for aerobic petroleum hydrocarbon degradation in this study

- Mineralization of ^{14}C -labelled specific compounds in soil samples (trapping CO_2)
- Abundance of specific aerobic degradation genes in soil samples
 - MPN-PCR (Replicate limited dilution RLD-PCR)
 - Real-time PCR



S Y K E

Other methods

- Soil respiration to estimate the total microbial activity in soil samples
- Total bacterial number by epifluorescence microscopy (DAPI staining)
- Petroleum hydrocarbon concentrations in soil by GC-FID and GC-MS



S Y K E

Model compound



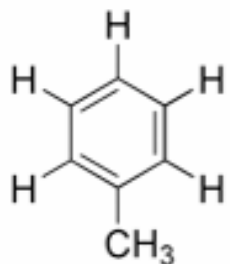
14,15-¹⁴C-octacosane

Target gene

alkB

Target enzyme

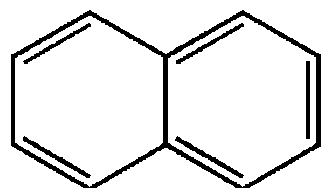
Alkane hydroxylase



UL-¹⁴C-toluene

xylE

Catechol-2,3-dioxygenase



UL-¹⁴C-naphthalene

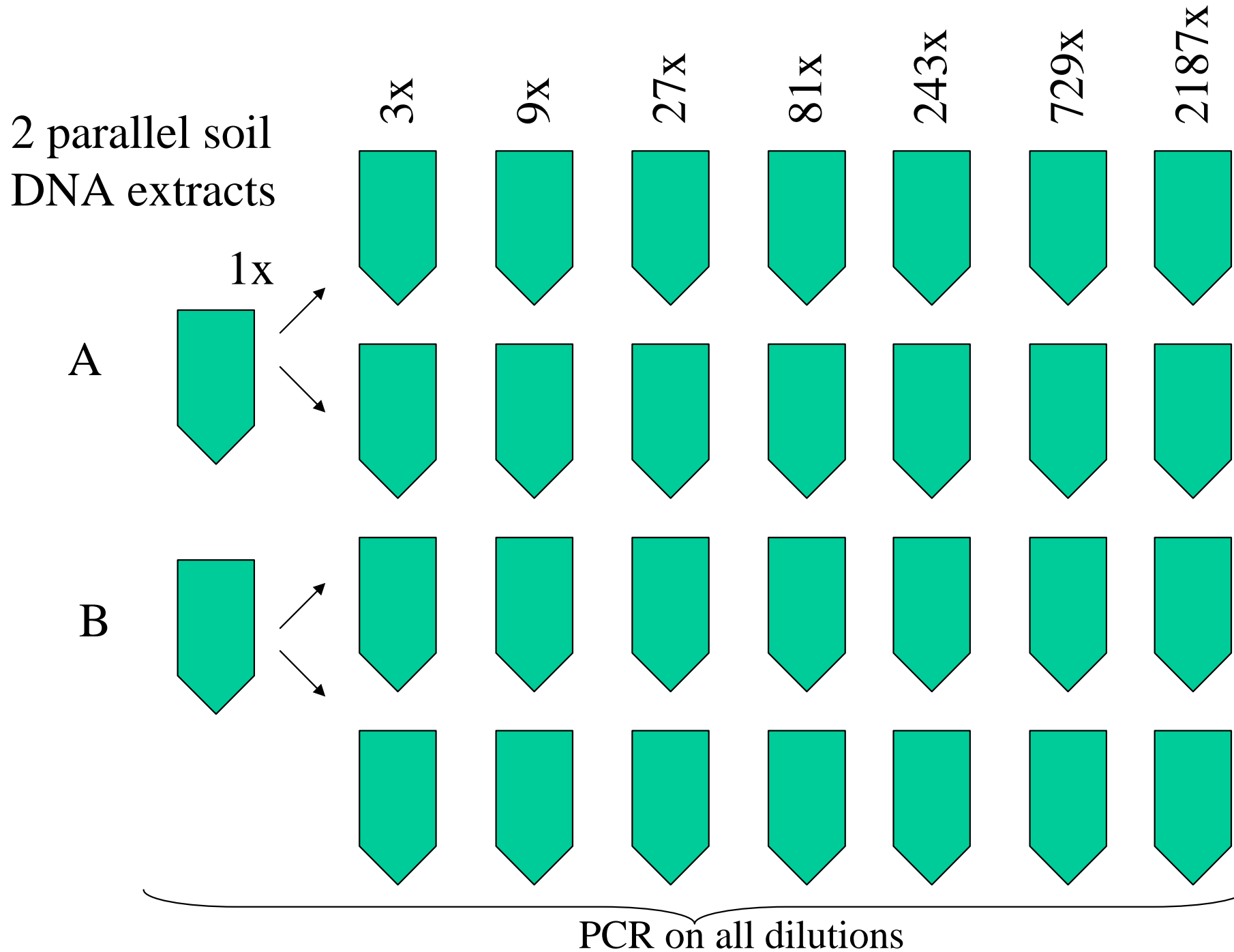
nahAc

Naphthalene dioxygenase

Primers used in RLD-PCR

Target gene	Target compound degradation	Primer name	Sequence	Product size (bp)
<i>alkB</i>	Alkane degradation			
(Whyte et al. 1996)		alkB703for	5'-TGGCCGGCTACTCCGATGATCGGAATCTGG-3'	869
		alkB1572rev	5'-CGCGTGGTGATCCGAGTGCCGCTGAAGGTG-3'	
<i>nahAc</i>	Naphthalene degradation			
(Wilson et al. 1999)		Ac114F	5'-CTGGC(T/A)(T/A)TT(T/C)CTCAC(T/C)CAT-3'	482
		Ac596R	5'-C(G/A)GGTG(C/T)CTTCCAGTTG-3'	
<i>xylE</i>	Toluene degradation			
(Jussila et al. 2007)		xylEbf (482)	5'-AGGTATGGCGGCTGTGCGTTTC-3'	469
		xylEbr (950)	5'-TTCGTTGAGAATGCGGTCGTGG-3'	

Replicate limited dilution RLD-PCR



Sites and samples investigated

					Sampling information			
Site	Name	Contamination	Oxic layer (m)	Soil type	Time	Point	Profile depth (m)	No. of Samples in profile
1	Trollberget	Diesel fuel, lubrication oil	0-1	Sand, filling	Jul 2000	G1	0-3	5
			0-1.5	Sand, filling	"	G15	0.5-3.5	6
			0-1.2	Sand, silt	May 2001	G17	0.5-3.5	6
			0-1.5	Sand, filling	"	G18	0.5-5.5	10
2	Etna	Heating oil	0-11	Sand, till, silt	Nov 2000	P51	2-11	7
3	Vuosaari	C ₁₀ -C ₃₀ PHs, wastewater sludge	0-1	Deposited waste water	Mar 2002	2	1.5-2.5	2
			0-1	sludge		3	1.5-2.5	2
			0-1			4	1.5-2.0	1
4	Lempäälä	Gasoline	0-1.5	Silt, clay	May 2002	7	2.5-3.5	2
			0-1.5			9	2.0-4.5	3
5	Sköldvik	Refinery waste	0-0.3	Agricultural soil + waste	Aug 2002		0-0.3	3 (no profile)
Clean soil	Trollberget	None	0-4	May 2001	Sand	G4	0.5-1.5	2

Site 1 Trollberget, closed dumpsite, soil profile





Site 1 Trollberget, subsurface soil sampling

Site 2, Etna, Hanko, heating oil leakage



Subsurface soil sampling at site 2, Etna



Average gene abundances measured by RLD-PCR and mineralization rates in Finnish soil samples. Data from Salminen et al. 2008.

Site	Gene abundance (10 ³ gene copies g ⁻¹ DW)			¹⁴ C-Mineralization rate (day ⁻¹)		
	<i>alkB</i>	<i>xylE</i>	<i>nahAc</i>	Octacosane	Toluene	Naphthalene
1 (n=27)	0.01	0.08	6.8●	0.0012	0.0005	0.0030●
2 (n=7)	0.94●	0.13	0.30	0.0015●	0.0007	0.00003
3 (n=5)	0.31	0.20	<0.20	0.0003	0.0021	0.0023
4 (n=5)	<0.15	0.13●	<0.15	0.0008	0.0031●	0.0020
5 (n=3)	34●	624●	14●	0.048●	0.009●	0.0776●
Clean (n=2)	<0.05	<0.12	<0.12	0.0001	0.00003	0.0003

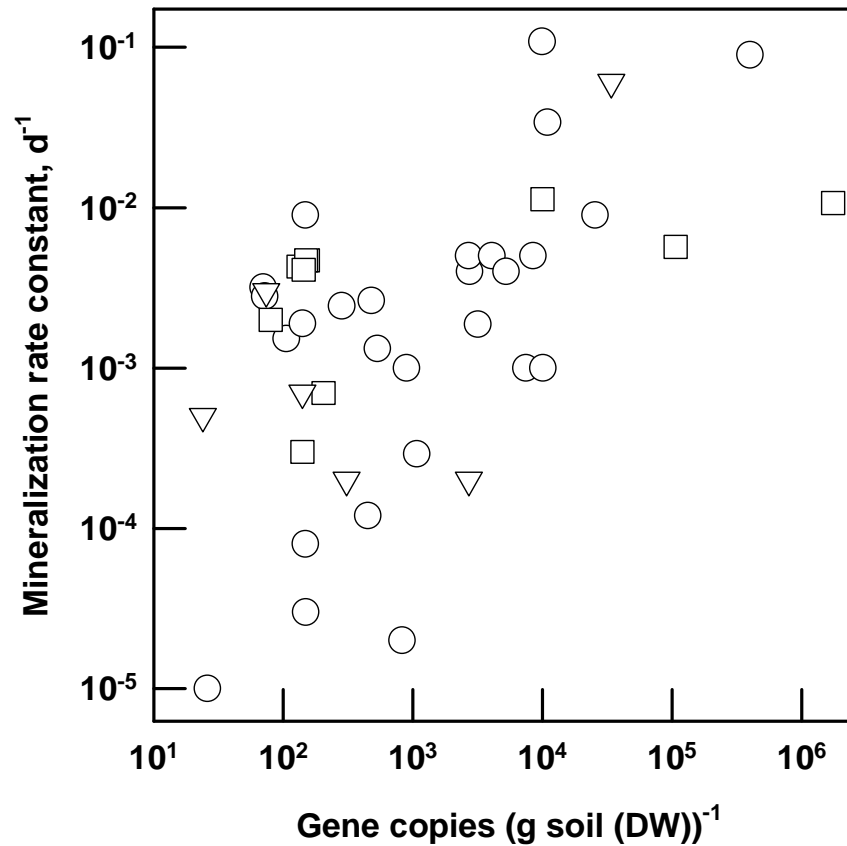
● Max within site

● Max between sites

Correlation between the measured parameters in aerobic samples

	Naphthalene mineralization rate	Toluene mineralization rate	Octacosane mineralization rate	Mineral oil concentration	Organic matter content	Respiration rate	Bacterial cell number
<i>NahAc</i> gene abundance RLD-PCR	+	+		+			+
<i>XylE</i> gene abundance RLD-PCR		+	+		+		
<i>AlkB</i> gene abundance RLD-PCR							
Naphthalene mineralization rate		+		+	+	+	+
Toluene mineralization rate				+	+		+
Octacosane mineralization rate					+		
Mineral oil concentration					+		
Organic matter content						+	+
Respiration rate							
Bacterial cell number							

Relation between gene abundances measured by RLD-PCR and mineralization rates in the samples from sites 1-5.



- *nahAc* vs naphthalene
- *xylE* vs toluene
- ▽ *alkB* vs octacosane

Comparison of RLD-PCR and real-time PCR for selected samples

Site	Sample	<i>nahAc</i>				<i>xylE</i>	
		qPCR	RLD-PCR	qPCR/RLD-PCR		qPCR	RLD-PCR
		Gene copies g ⁻¹ DW				Gene copies g ⁻¹ DW	
1 (Trollberget)	G18, 0.8-1.3 m	60900	100	575	400	<110	
	G18, 1.3-1.8 m	287500	1100	268	100	<110	
	G18, 1.8-2.3 m	885100	3200	278	30	<140	
2 (Etna)	P51, 3.0-3.5 m	473000	800	573	14100	<150	
	P51, 4.5-5.0 m	120200	30	4623	151600	130	1157
3 (Vuosaari)	P2, 1.3-1.8 m	184800	<150		5400	<150	
	P3, 1.3-1.8 m	32100	<210		2700	210	13
4 (Lempäälä)	P7, 3.0-3.5 m	25400	<160		12400	160	80
	P9, 1.8-2.3 m	4300	<150		34500	150	230
5 (Sköldvik)	0.0-0.3 m	1385000	1000	1394	Not determined	1755000	
	0.0-0.3 m	561700	1100	512	5677300	106600	53

Conclusions 1

- Octacosane, toluene and naphthalene were all mineralized at the 5 contaminated sites
- The highest mineralization rates and gene abundances were obtained at a landfarming site (Sköldvik)
- Gene abundances reflected the contamination type



S Y K E

Conclusions 2

- The gene abundances for aerobic toluene and naphthalene were correlated with the corresponding mineralization rates in aerobic samples
- Real-time PCR was much more sensitive than RLD-PCR and is recommended in future studies for assessment of gene abundances



S Y K E

Future prospects

- Abundances of genes involved in anaerobic petroleum hydrocarbon degradation should be included in the analyses
- Many samples and many genes need to be assessed due to the heterogeneity of the subsurface and the contaminants
- Optimized PCR-methods for functional gene abundances can be used in the evaluation of bioremediation efficiency



S Y K E