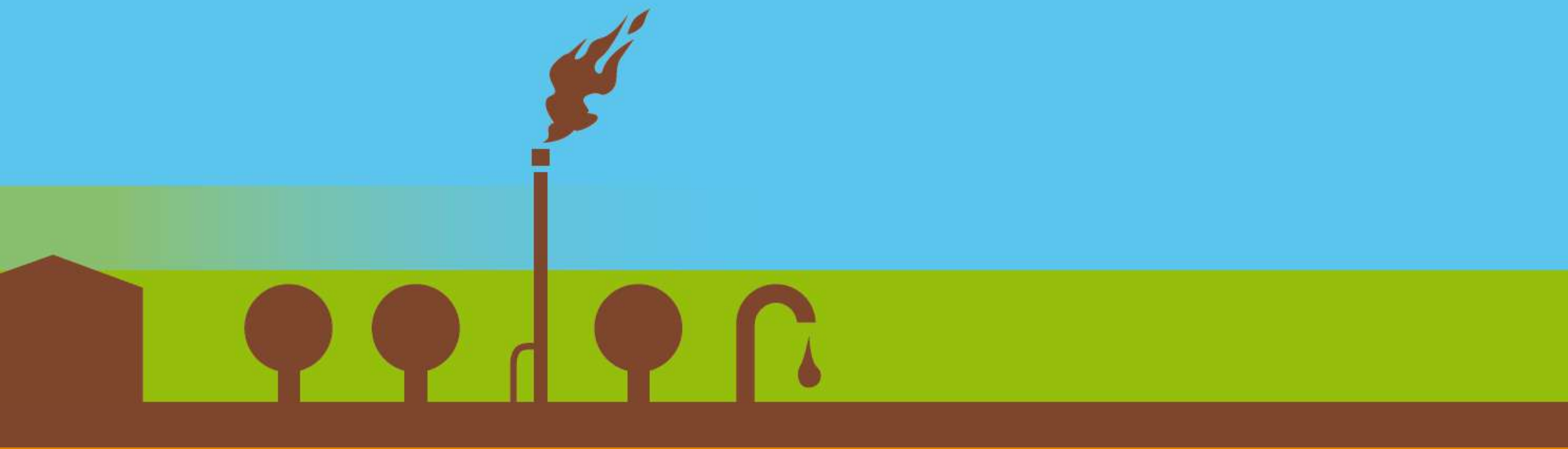
13th International SedNet Conference, Lisbon
07.09.2023

Isodetect Umweltmonitoring GmbH



Assessing *Remediation* of Polluted Marine and Soil Sediments with Advanced in Situ Monitoring Tools

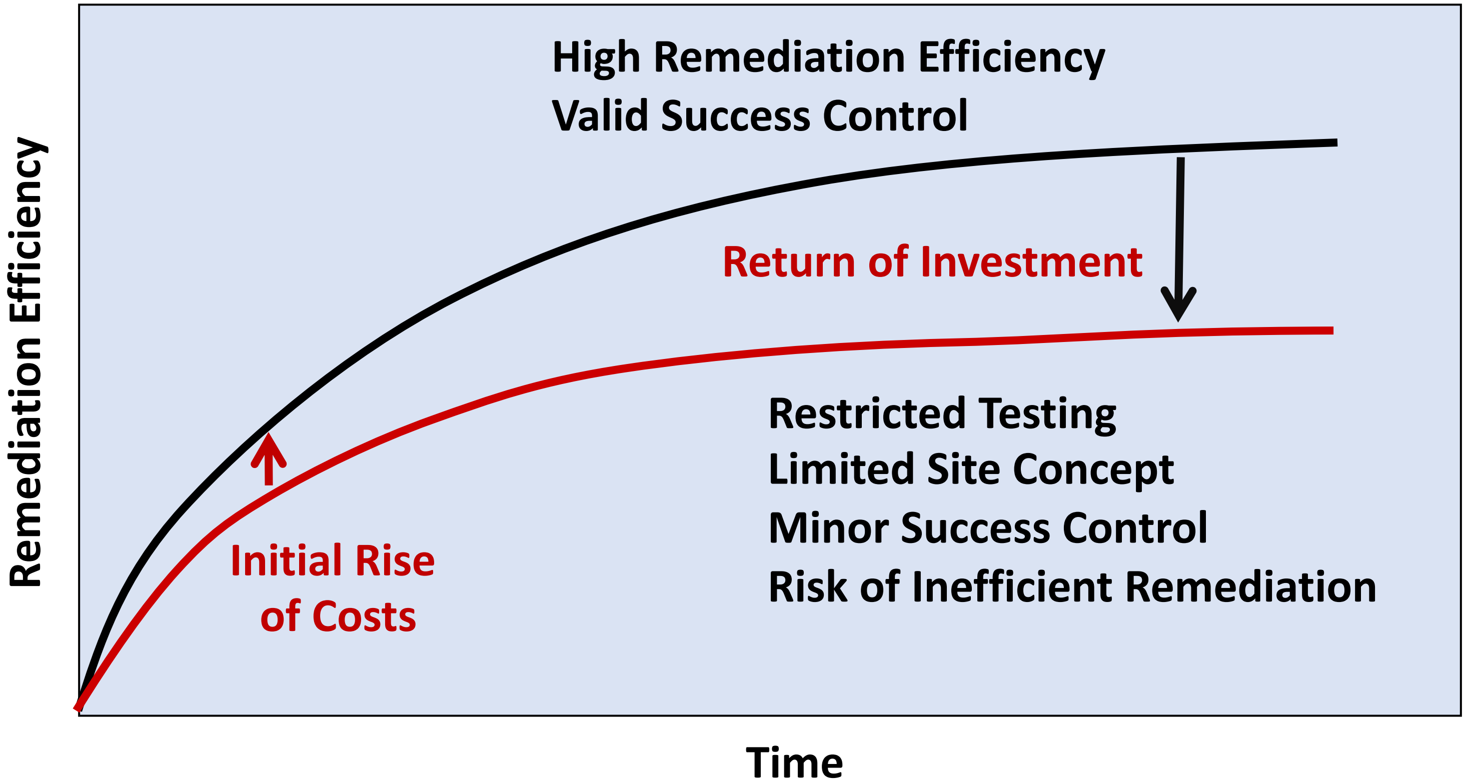
Heinrich Eisenmann, Kevin Kuntze, Anko Fischer (Isodetect GmbH, Munich & Leipzig, Germany)

Jofre H. Ferran (Water, Air and Soil Unit, Eurecat, Manresea, Spain)

Chiara Melchiorre (Stazione zoologica di Napoli A. Dohrn, Napoli, Italy)

SedNet · MYSOIL · LIFE sedremed · Life

Why Monitoring Investments?



Advanced Monitoring Tools for Polyaromatic and Aliphatic Pollutants

- **Application in marine sediments (SEDREMED): Bagnoli/Naples**
  Electrochemical and biological remediation

- **Application in soil (LifeMySoil): Industrial contamination**
  Mycopiles augmented with TPH degrading fungi

Industrial Area 1904 - 1990
8 km west from Naples

Nowadays: Abandoned Site

Abandoned Industrial Area of Bagnoli
Need for Innovative Coastal Remediation

LIFE sedremed

Matrix:
- **Marine sediment polluted with PAH & heavy metals**

Proposed technologies:
- **Electrochemical stimulation (Ekogrid) and bacterial amendment (Idrabel BioVase)**

We are replacing (baseline):
- **Excavation**

Lab Tests ⇨ Mesocosms ⇨ Pilot test fields ⇨ Extended Area

Today 15:15 – 15:35, here: Raffaele Vaccaro, Nisidia Environment
"Policy solutions for management of contaminated sediments in the EU"

Tools to Investigate Contaminant Degradation

Quantitative
Semi-quantitative
In situ

- ***qPCR*** **Genetic Analysis**
- **BACTRAPS** **^{13}C *in situ* Microcosms**
- **Contaminant Isotopes** **$^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{37}Cl/^{35}Cl$**

Quantitative
Laboratory Assays

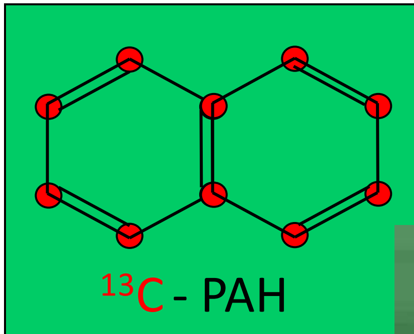
- **Percolated Columns** **Batch Microcosms**
- **^{13}C-labeled** **Batch Microcosms**

Qualititative
In situ

- **Metabolite Analysis**
- **Redox Isotopes** **z.B. SO_4, H_2S, CH_4, CO_2, NO_3**
- **GC/MS-Screening** **Concentrations Profiles**

For more details
visit our two-day yearly workshop at Isodetect Leipzig

BACTRAPs
Labeling, Loading & *in situ* Exposition



Isotope Labeled Pollutants

Sorbed to Carrier Material

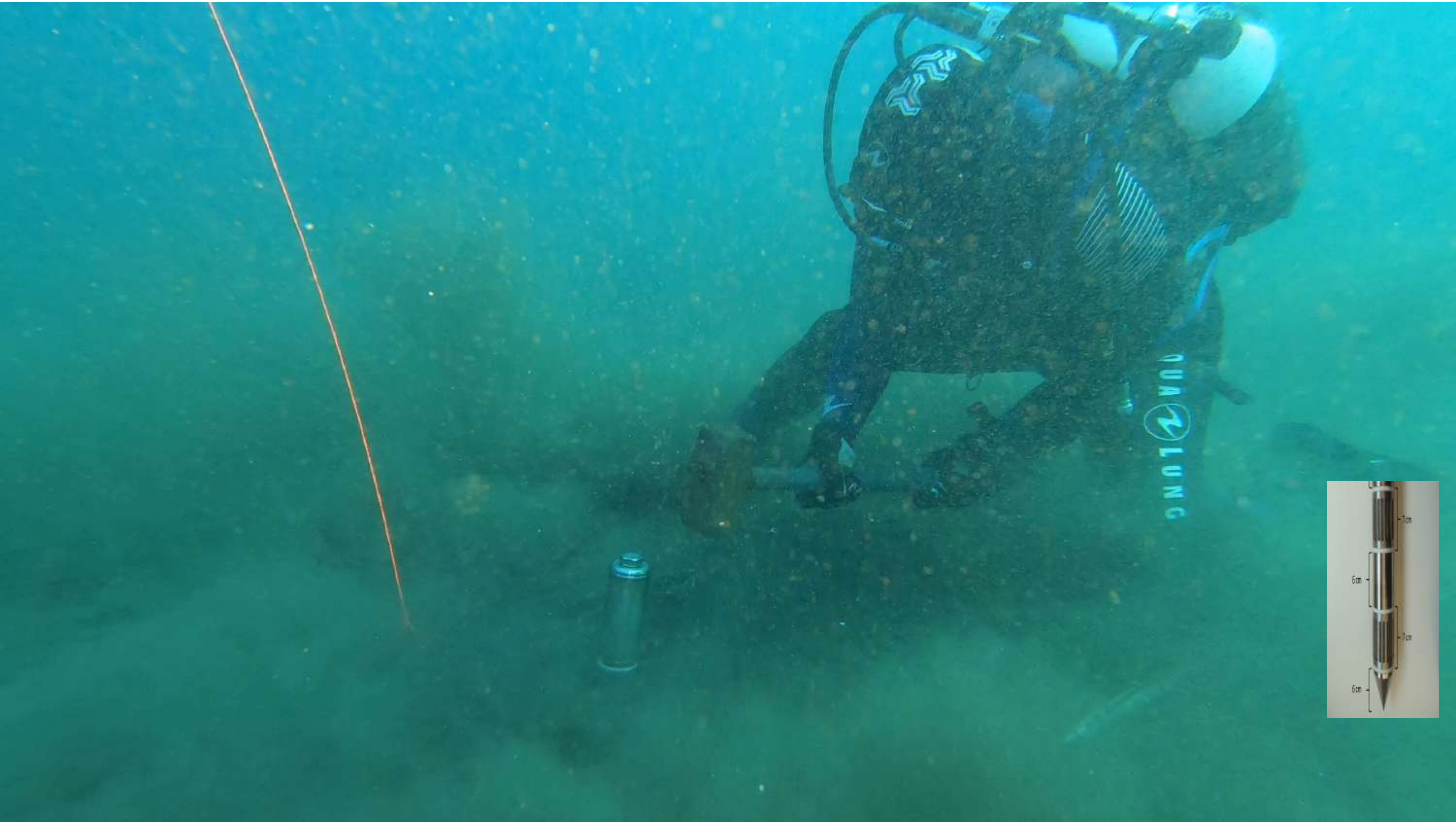
Exposition

Geyer et al. (2005) Environ Sci Techn 39: 4983-4989
Stelzer et al. (2006) Org Geochem 37: 1394-1410
Bombach et al. (2010) Appl Microbiol Biotechnol 86:839–852

New BACTRAP Installation Systems for Coastal Sediments

Highly Robust in Harsh Environments

BACTRAP Exposition in Coastal Sediments



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A Tough Job for Divers

BACTRAPs
^{13}C-Label from Contaminants Incorporated into Biomass

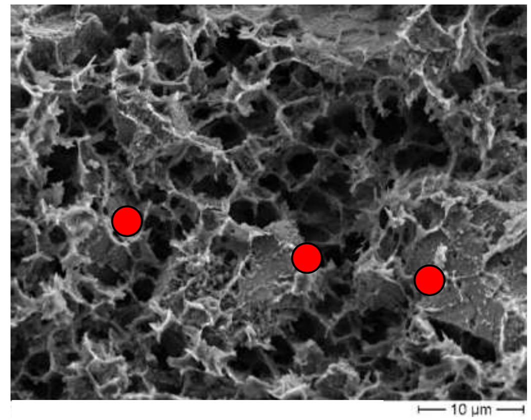
Microbial Colonization of Carrier Surface

↓

Recovery after 2-4 months

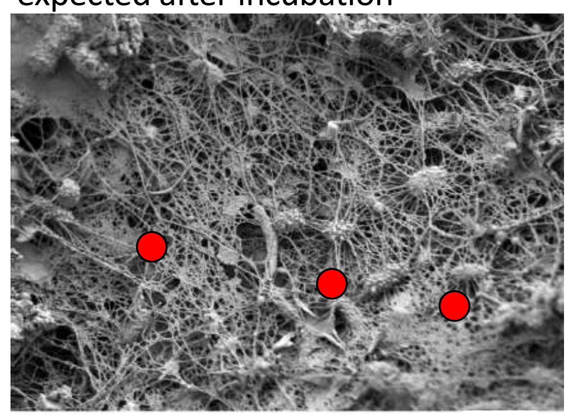
^{13}C Incorporation into Amino Acids (AA) and Phospholipid Fatty Acids (PLFA)

before incubation



^{13}C-Naphthalene or ^{13}C-Acenaphthene

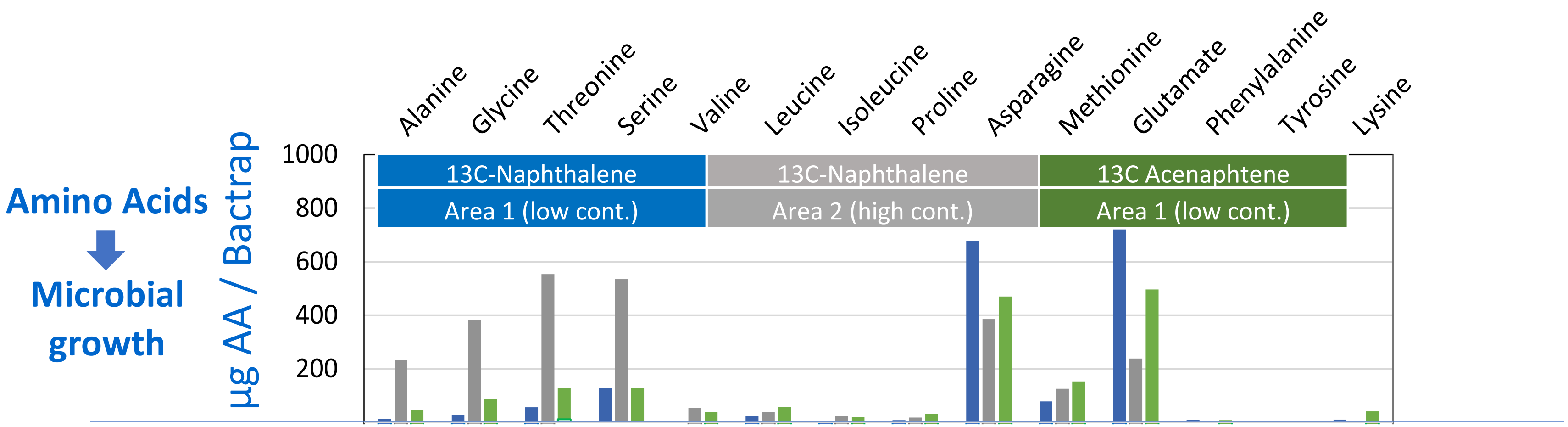
expected after incubation



4 µm

BACTRAPS from Natural Marine Sediments

13C-Naphthalene or 13C-Acenaphthene from High/Low Contaminated Areas



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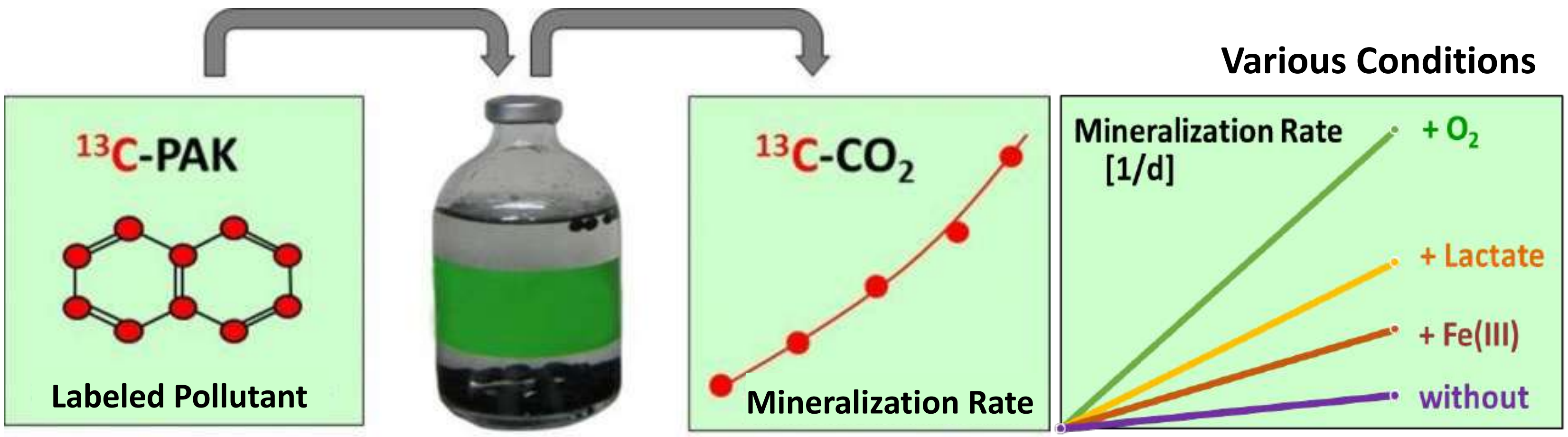
Laboratory Assays: ^{13}C-Label from Contaminant Appears in Final Mineralization Product ^{13}C-CO_2



- Quantification of complete degradation (metabolization)
- Check of most efficient *in situ* stimulation approach
- Highly sensitive and also quantitative (however: *ex situ*)

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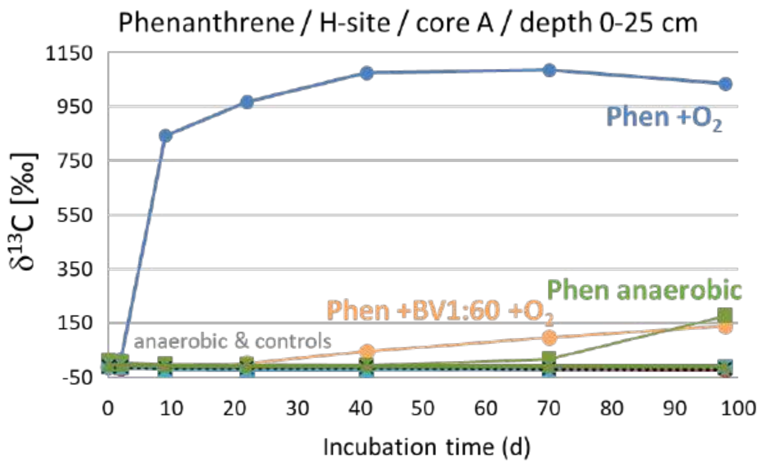
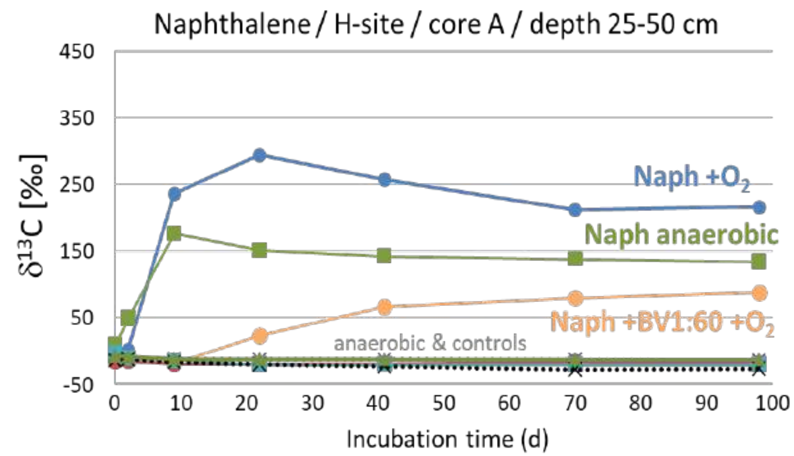
52 (!) Laboratory Assays





- Immediate PAH degradation by <u>oxygen amendment</u>
- Rapid <u>anaerobic degradation</u> of Naph, after 70 days also of Phen
- Assays with <u>BioVase+O_2 show a long lag-phase</u> and exhibit high O_2 consumption (inhibition?)

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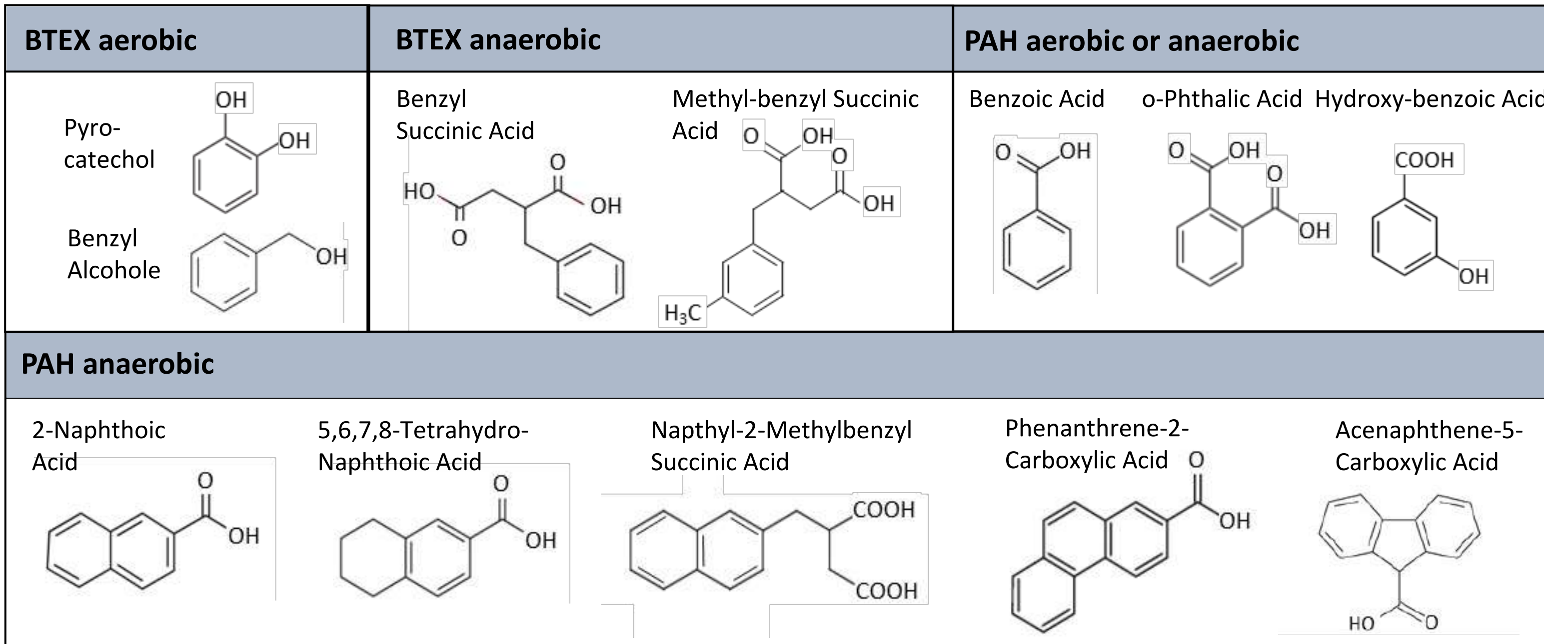
Some Specific Metabolites

that Indicate Aerobic or Anerobic Biodegradation of BTEX & PAH



- Clear evidence of degradation, but not quantitative
- Discrimination of anaerobic/aerobic degradation
- Preferably suitable for PAH and BTEX

Sediment Cores: Metabolite Analysis

sample ID	HA-1a	HA-2a	HA-3a	HA-4a	LA-4a	LB-1a
unit	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
Metabolites of aerobic BTEX biodegradation						
Catechol (1,2-dihydroxybenzene)	nd	nd	nd	nd	nd	nd
Methylcatechol (Dihydroxytoluene)	nd	nd	nd	nd	nd	nd
Ethylcatechol	nd	nd	nd	nd	nd	nd
Benzyl alcohol	nd	nd	nd	nd	nd	nd
Metabolites of aerobic Naphthalene biodegradation						
1-Naphthol (1-Hydroxynaphthalene)	nd	nd	nd	nd	nd	nd
2-Naphthol (2-Hydroxynaphthalene)	nd	nd	nd	nd	nd	nd
1,2 or 2,3-Dihydroxynaphthalene	nd	nd	nd	nd	nd	nd
Metabolites of anaerobic BTEX biodegradation						
Benzylsuccinic acid	nd	nd	nd	nd	nd	nd
(1-phenylethyl)benzylsuccinic acid	nd	nd	nd	nd	nd	nd
(2 and/or 3 and/or 4)-Methylbenzylsuccinic acid	nd	nd	nd	nd	nd	nd
Metabolites of anaerobic Naphthalene biodegradation						
(1 and/or 2)-Naphthoic acid	nd	nd	nd	nd	nd	nd
5,6,7,8-tetrahydro-2-Naphthoic Acid	nd	nd	nd	nd	nd	nd
Metabolites of anaerobic PAH biodegradation						
Naphthylmethylsuccinic acid	nd	nd	nd	nd	nd	nd
Phenanthrene-4-carboxylic acid or 9-Anthracencaboxylic aci	nd	nd	nd	nd	nd	nd
Flouren-9-carboxylic carboxylic acid	nd	nd	nd	nd	nd	nd
Acenaphthene- 5 and/or 3 -carboxylic acid	nd	nd	nd	nd	nd	nd
Metabolites of aerobic and anaerobic mono- and polycyclic aromatic hydrocarbon biodegradatior						
Benzoic acid	**+/-**	**10**	**32**	**24**	**25**	**62**
(2 and/or 3 and/or 4)-Hydroxybenzoic acid	nd	nd	nd	nd	nd	nd
2,5-Dihydroxybenzoic acid (Gentisic acid)	nd	nd	nd	nd	nd	nd
3,4-Dihydroxybenzoic acid (Protocatechuic acid)	nd	nd	nd	nd	nd	nd
o/m/p-Toluic acid (methylbenzoic acid)	nd	nd	nd	nd	nd	nd
Phenol (probably co-contamination)	nd	nd	nd	nd	nd	nd
o/m/p-Cresol (probably co-contamination)	nd	nd	nd	nd	nd	nd
Metabolites of anaerobic alkane biodegradation						
Alkylsuccinic acids	nd	nd	nd		nd	nd

n.d. = not detected, below limit of detection

+ = detected, but quantification not possible due to peak overlay

+/- = detected, but below limit of quantification

Beside benzoate, no specific metabolite of the aerobic/anaerobic PAH degradation were detected
→ marginal natural attenuation of PAH

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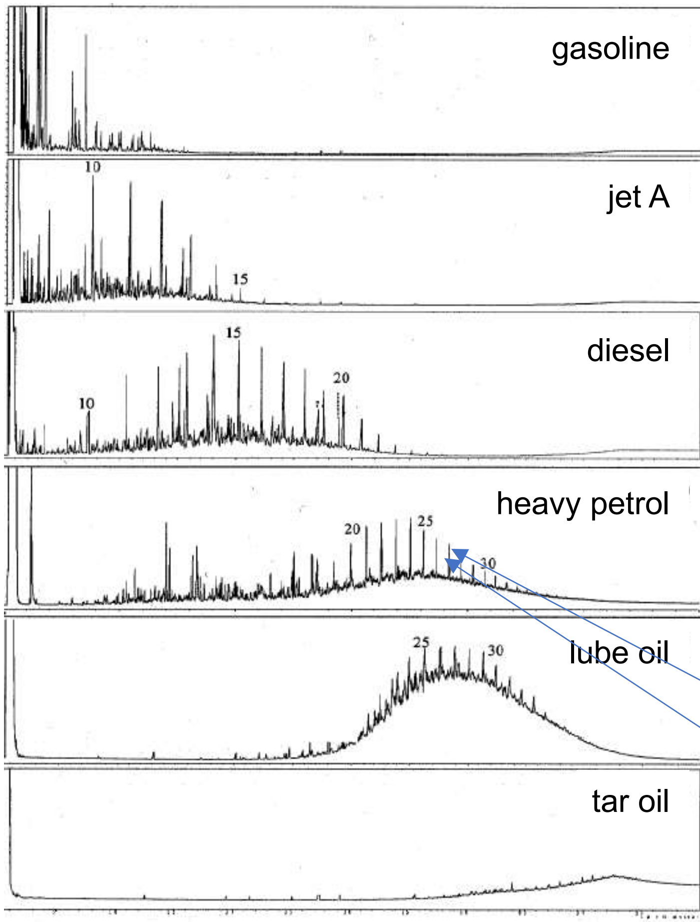
GCMS Screening of Petroleum



<u>Characteristic Chromatograms</u>

- Patterns change through degradation or aging (e.g. n-alkanes, cyclohexanes, isoprenoids, ...)

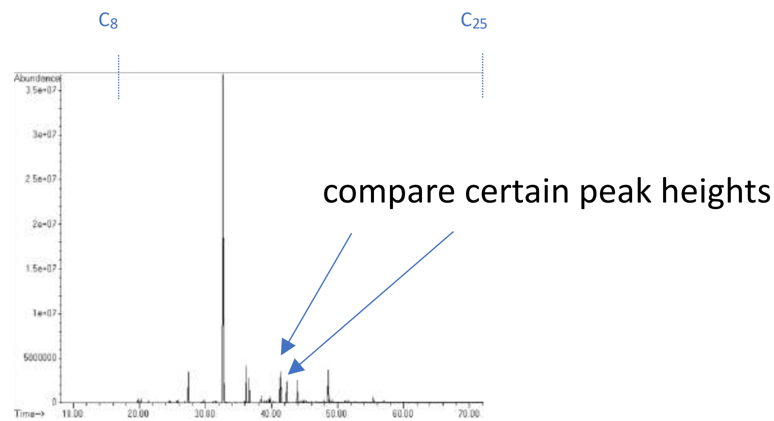
- Indication of release period (e.g. MTBE, ETBE, lead)

- Degradation is indicated by diagnostic ratios of specific compounds (e.g. heptadecane/pristane or octadecane/phytane)

Diagnostic ratios: compare certain peak heights

Sediment Cores: GCMS Diagnostic Ratios

Sample	acenaphthene/fluorene ←	dibenzofurane/fluorene ←	phenanthrene/anthracene →	pyrene/methylpyrene →
LA-1α				
LB-1α				
LA-2α			1.7	
LB-2α			1.7	
LA-3α			2.1	
LB-3α			1.7	
LA-4α			1.3	
LB-4α			2.1	
HA-1α	0.92	0.44	1.1	12.01
HB-1α	0.77		2.0	
HA-2α	0.51	0.57	2.1	3.53
HB-2α		0.43	1.9	2.82
HA-3α	0.43	0.44	1.3	3.58
HB-3α			1.3	0.98
HA-4α			1.1	4.61
HB-4α			1.4	



Some ratios would indicate degradation....

- but there is a high analytical uncertainty due to low peaks and high background
- carefully inspect your analytical results, data not reliable!

BACTRAPs (^{13}C-Phenanthrene) in Mesocosms

Preliminary Outcome

- BACTRAPS and ^{13}C lab microcosms were the most suitable monitoring methods
- Marginal natural attenuation at the site
- Very high potential for microbial elimination of contaminants at aerobic conditions
- Microbial amendment might inhibit degradation at an initial stage of treatment
- Technological treatments will be modified
  e.g. currency, pulsing, intensity, treatment periods, stimulation cocktail

Improvement of Technologies

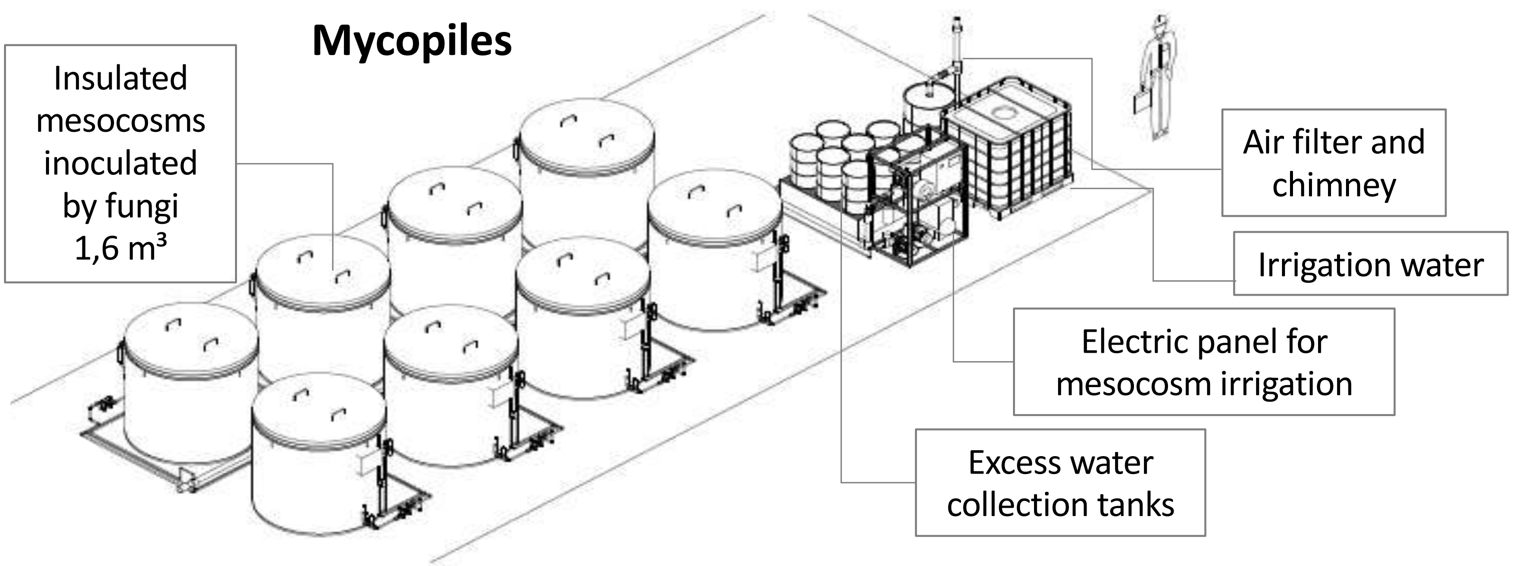
Better Remediation Efficiency

MySoil: Enhanced Soil Remediation by Auxiliary Fungi

Matrix:
- **Industrial soil polluted with TPH/PAH**

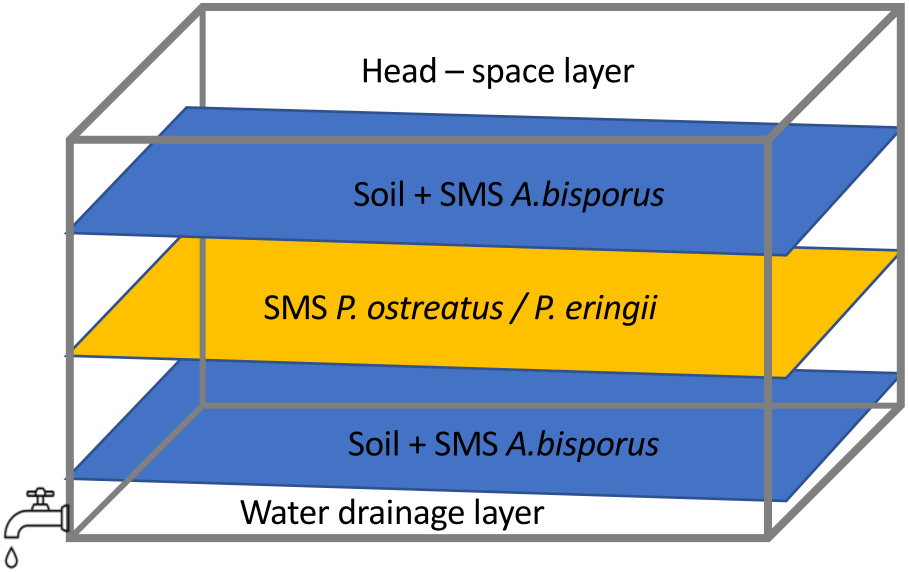
Proposed technology:
- **Fungal bioremediation**

We are replacing (baseline):
- **Thermal desorption**

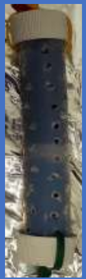


Design of Mycopile Mesocosm

Layers with Specific Fungal Species



Testing of the MYCOTRAP prototypes in Mesocosms

	2 Casks	Two Carriers	One ^{13}C-labeled contaminant
	**Teflon tube** Length: 2,5 cm Ø: 2,5 cm 	**BioCoal** 	**13C-Hexadecane**
		Soil 	**13C-Hexadecane**
	**Stainless steel cylinder** Length: 2,5 cm Ø: 2,5 cm 	**BioCoal** 	**13C-Hexadecane**
		Soil 	**13C-Hexadecane**

Installation: 14th of November 2022

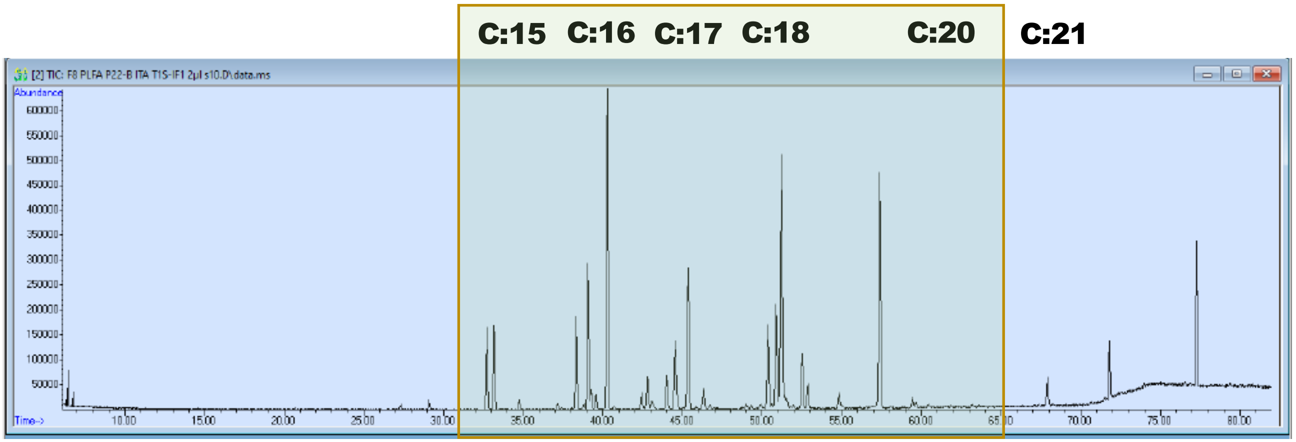
Retrieval: 3rd of February 2023

Adaptation of in situ microcosms (MYCOTRAPS) to specific environmental conditions

- **Teflon tubes filled with original soil loaded with 13C-Hexadecane**

MYCOTRAPS with Teflon and Natural Soil: Phospholipid Fatty Acids Representative for Fungi

Teflon and Soil
Fungi Inoculated

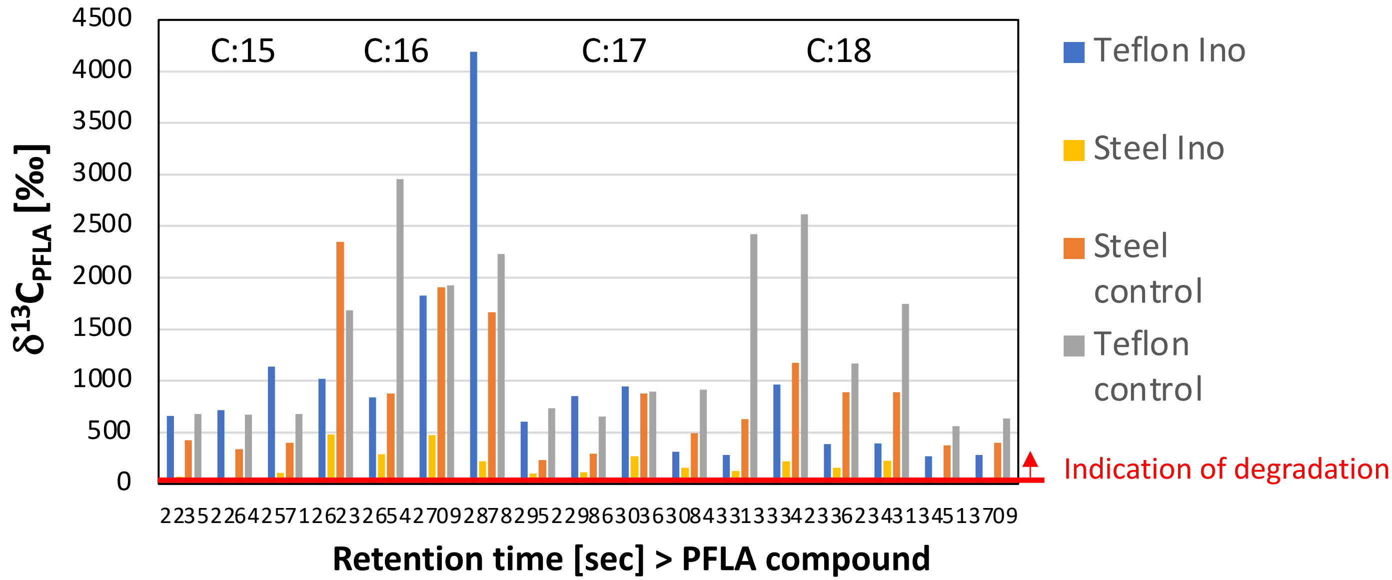


12.3 $\mu g_{PFLA}/g_{Soil}$

- Vital <u>microbial & fungal community</u> on MYCOTRAPS indicated by group-specific PLFA

- But: Exactly the same community in untreated mesocosms
  → up to now <u>no stimulation effect discernible</u>

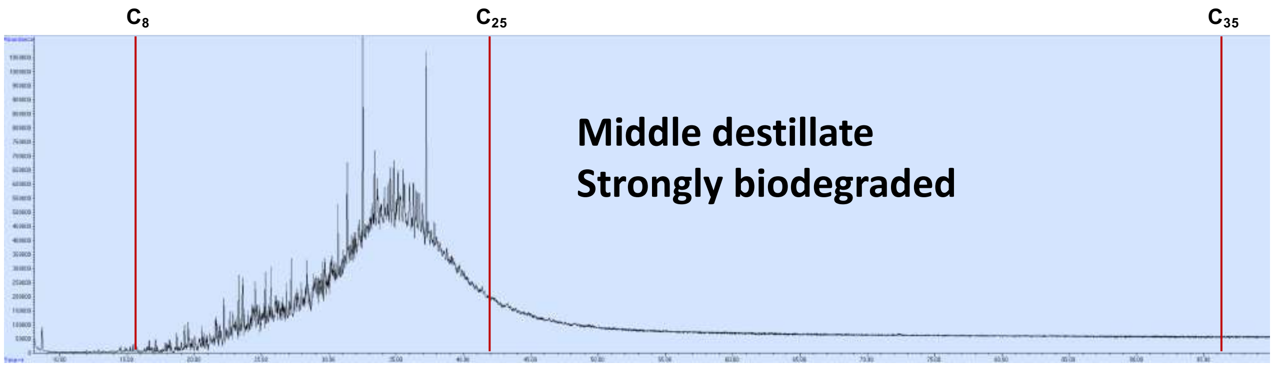
^{13}C-Hexadecane Incorporation into Phospholipid Fatty Acids (PLFA)



- ^{13}C-PLFA patterns show substantial ^{13}C-hexadecane elimination performed by intrinsic community
- Successful validation of mycotraps
- So far no significant difference between untreated control and mycopiles

GCMS Fingerprinting:
Prescreening of untreated contaminated soil samples

S5-11-1-3

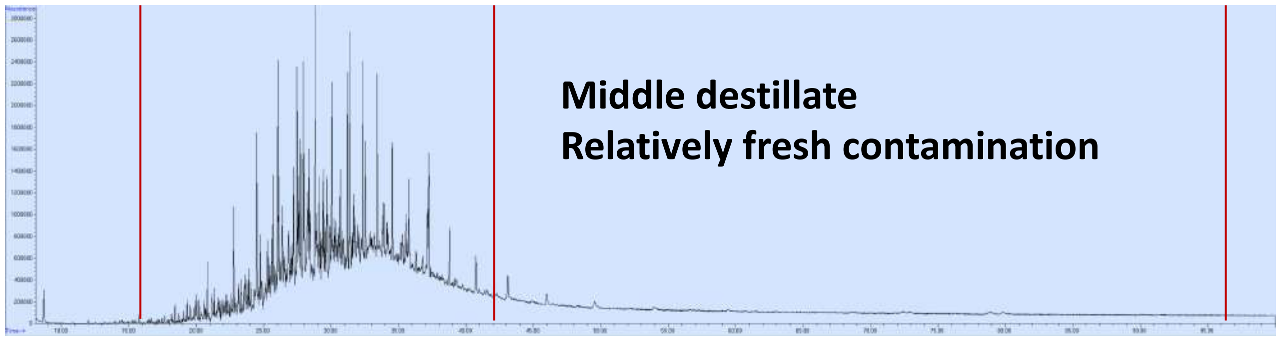


Diagnostic Ratios

	Measured ratio	Ratio of fresh sample
n-C_{17}/Pr	<0.1	1.5-2.5
n-C_{18}/Ph	<0.1	1.5-2.5

Degraded

LIFE-ACoPio2



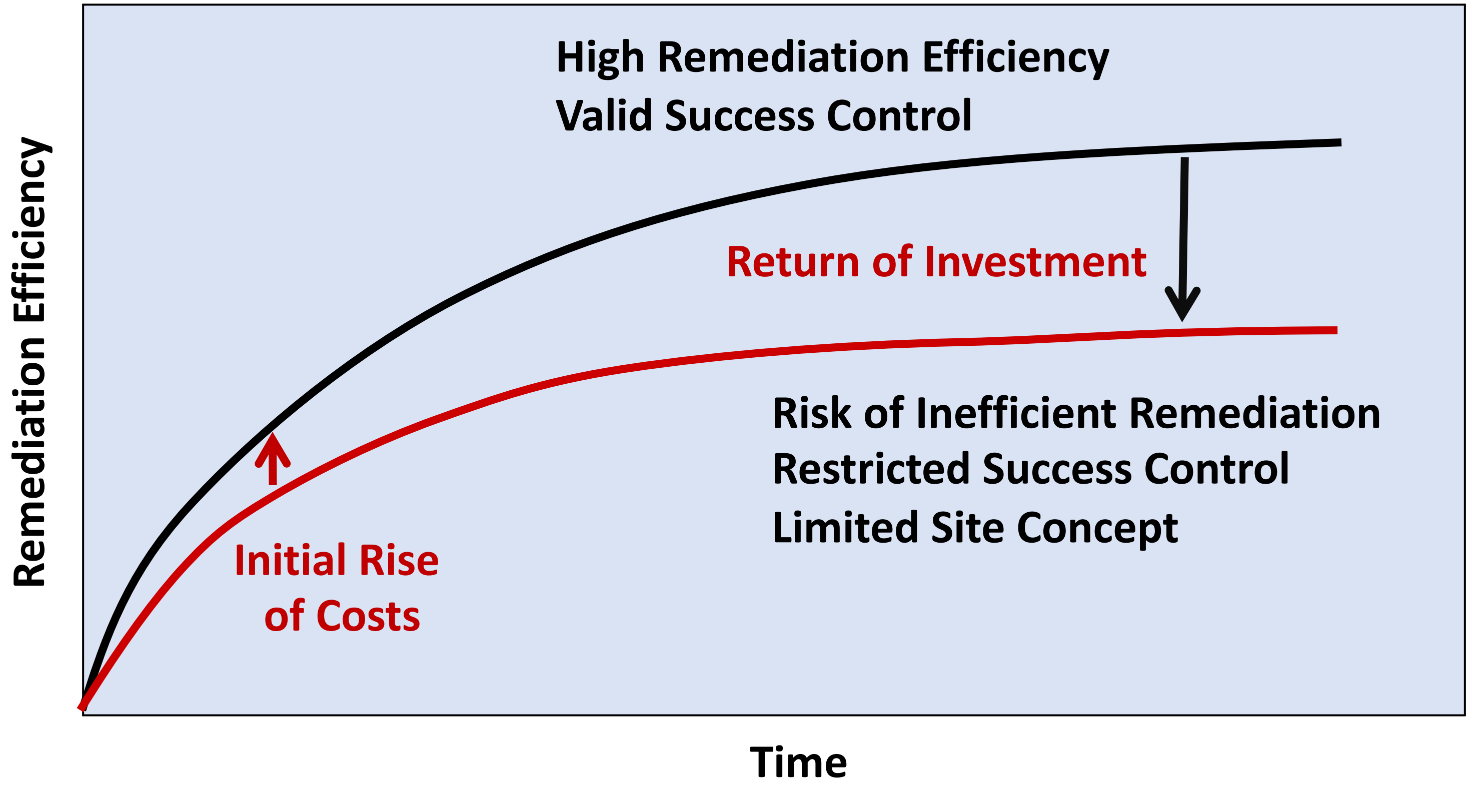
	Measured ratio	Ratio of fresh sample
n-C_{17}/Pr	0.7	1.5-2.5
n-C_{18}/Ph	1.5	1.5-2.5

- **Next step: Treated soil samples**
  → Change of diagnostic ratios

General Conclusions

- The application of new and efficient remediation technologies is a step-by-step procedure and requires adequate prearrangement and monitoring.
- Testing and success control of contaminant elimination in laboratory assays or mesocosms is an essential element of innovative remediation treatments.
- Don't expect a great success on the first trial.
- Advanced monitoring tools (e.g. BACTRAPS) can be applied and adapted according to environmental conditions (soil, coastal sediments).
- In two LIFE projects (Sedremed, MySoil) ongoing monitoring activities are expected to reveal the feasibility of advanced remediation technologies.

Why Monitoring Investments?



- ➢ Tools to improve remediation technologies

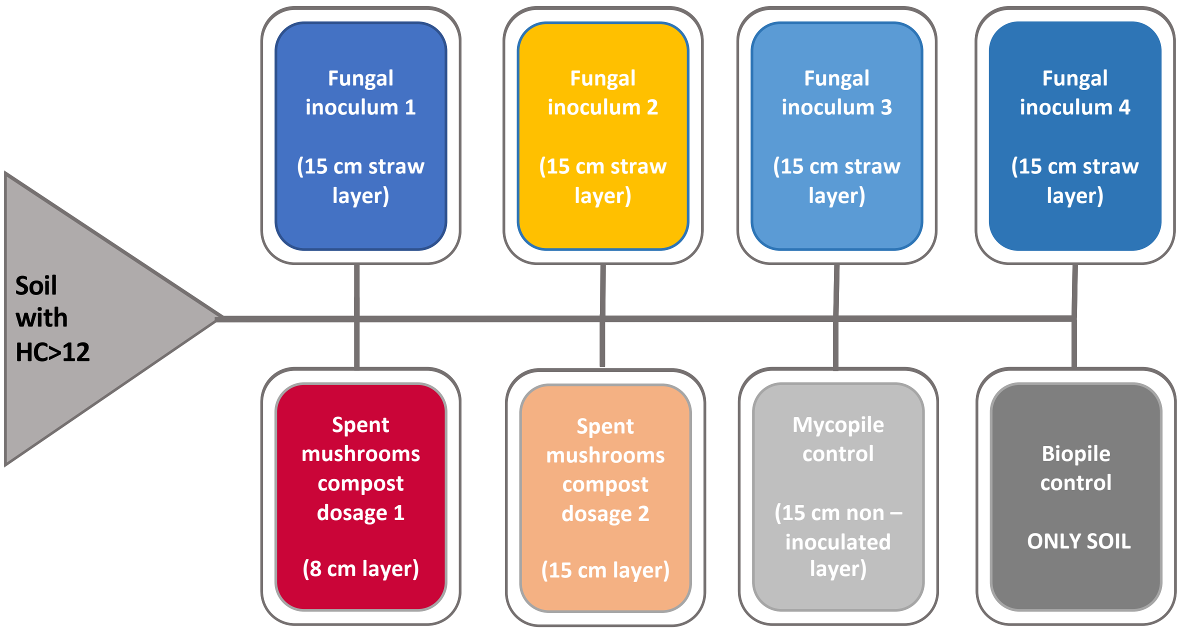
PILOT SITES

	Spain 1	France	Italy	Spain 2
Location	Huelva (CEPSA)	Rouen, Petit-Couronne	To define (Near Rome?)	Burgos
Activity	Refinery	Refinery (oil and gas activities)	Petrol station	Solar panels
Pollutants	TPH (10-18 g/kg)	TPH (25-30 g/kg)	TPH (~ 8 g/kg)	HTF (biphenil and biphenil ether)
Research partner	UAM	novobiom	Università degli Studi della Tuscia	UAM
Industrial partner	KEPLER	VALGO	eni rewind	KEPLER
Picture of the site				

Mesocosms design

n. 8 mesocosms of 1 m^3 (8 m^3 total).

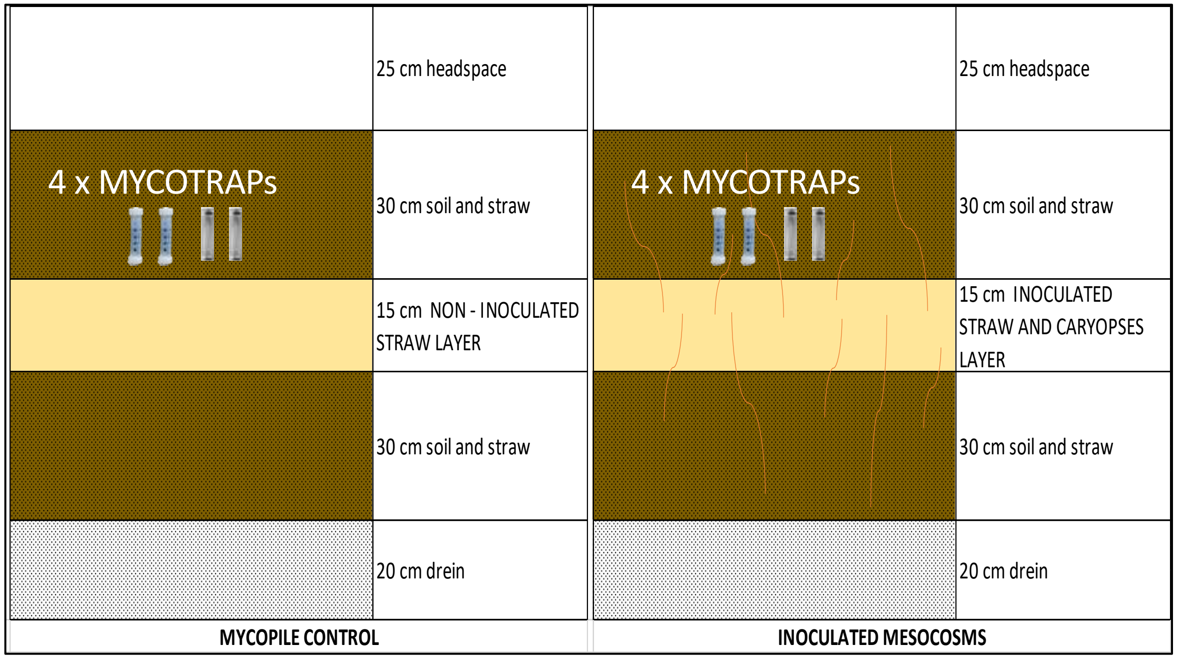
Approx. **7 m^3 of HC>12 contaminated soil** will be used.



Soil as-is and mixed with straw

Spent mushrooms compost bale

MYCOTRAP prototypes for the Italian mesocosm experiment



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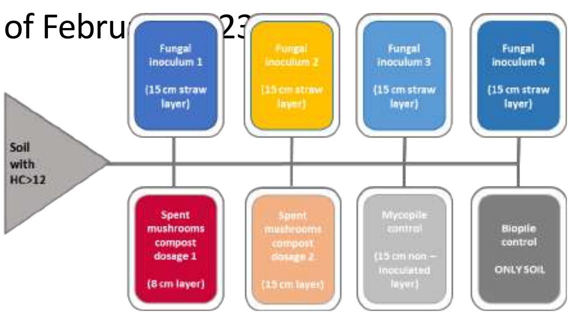


Sediment, Seawater and BACTRAP Sampling

16x Sediment
2 sites, High contaminated site (H; old name site 43) amd Low contaminated site (L; old name site 62) each sampled in
2 cores (A and B) --> 4 cores in total. Each one split in 2 equal parts (longitudinally) and in
4 depths
(transversal cuts at 0-25; 0-50; 50-100; >100 cm).
For each depths the two longitudinal parts were carefully homogenised.

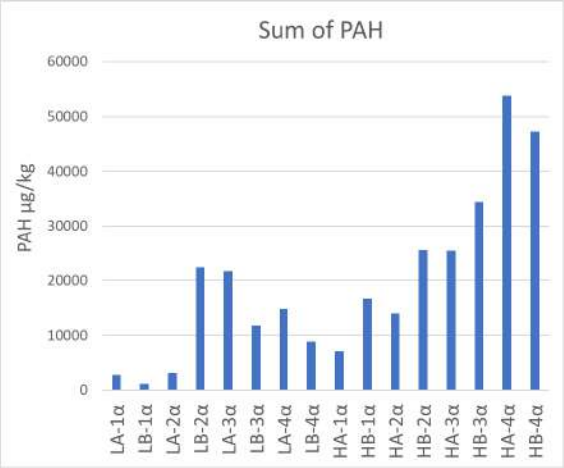
2x Seawater
- Bottle Sea Water 3 x 1L from each site (H & L)

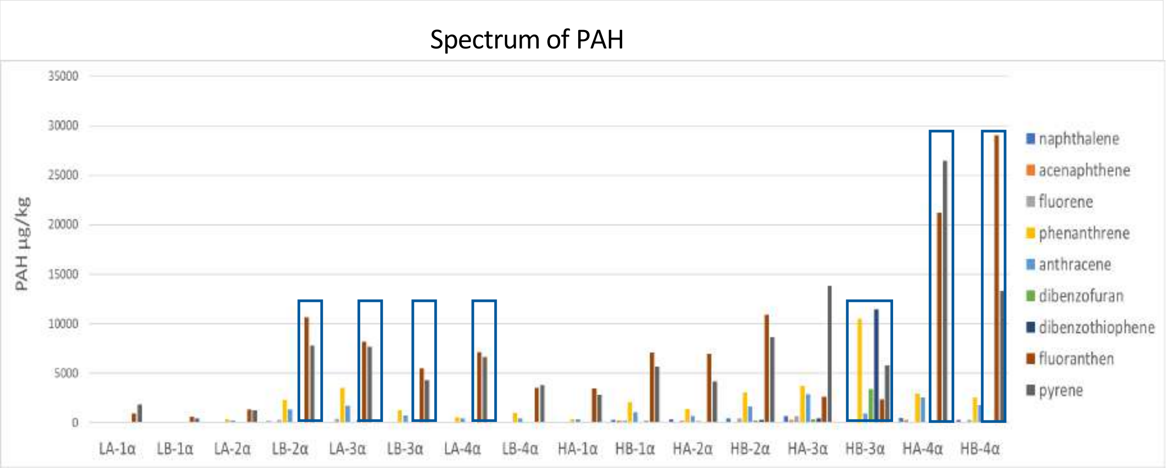
8x BACTRAPs
2 lancets (one at each site) covering
2 depths (0-15 cm, 35-50 cm) each with
2 pollutants (^{13}C-Acenaphthene, -Naphthalene)

Samples received at Isodetect
23rd of Dec 2022 (sediments & seawater)
27th of Feb 2023 (BACTRAPs)

Site	Core	Fraction ID	DEPTH FRACTION (cm)	amount received	storage		metabolite	13C-CSIA	GCMS-Fingerprint
L	A	LA-1α	0-25	250 ml bottle	-20°C		1	1	1
		LA-1β	0-25	250 ml bottle	+4°C				
		LA-2α	25-50	250 ml bottle	-20°C		1	1	1
		LA-2β	25-50	250 ml bottle	+4°C				
		LA-3α	50-100	250 ml bottle	-20°C		1	1	1
		LA-3β	50-100	250 ml bottle	+4°C				
		LA-4α	100-130	beutel	-20°C		1	1	1
		LA-4β	100-130	beutel	-20°C				
	B	LB-1α	0-25	250 ml bottle	-20°C		1	1	1
		LB-1β	0-25	250 ml bottle	+4°C				
		LB-2α	25-50	250 ml bottle	-20°C		1	1	1
		LB-2β	25-50	250 ml bottle	+4°C				
		LB-3α	50-100	250 ml bottle	-20°C		1	1	1
		LB-3β	50-100	250 ml bottle	+4°C				
		LB-4α	100-130	beutel	-20°C		1	1	1
		LB-4β	100-137	beutel	-20°C				
seawater L site				3 x 1L	+4°C		1		
H	A	HA-1α	0-25	250 ml bottle	-20°C		1	1	1
		HA-1β	0-25	250 ml bottle	+4°C				
		HA-2α	25-50	250 ml bottle	-20°C		1	1	1
		HA-2β	25-50	250 ml bottle	+4°C				
		HA-3α	50-100	250 ml bottle	-20°C		1	1	1
		HA-3β	50-100	250 ml bottle	+4°C				
		HA-4α	100-154	beutel	-20°C		1	1	1
		HA-4β	100-154	beutel	-20°C				
	B	HB-1α	0-25	250 ml bottle	-20°C		1	1	1
		HB-1β	0-25	250 ml bottle	+4°C				
		HB-2α	25-50	250 ml bottle	-20°C		1	1	1
		HB-2β	25-50	250 ml bottle	+4°C				
		HB-3α	50-100	250 ml bottle	-20°C		1	1	1
		HB-3β	50-100	250 ml bottle	+4°C				
		HB-4α	100-170	beutel	-20°C		1	1	1
		HB-4β	100-170	beutel	-20°C				
seawater H site				3 x 1L	+4°C		1		
							18	16	16

Sediment Cores: Spatial Patterns of PAH Concentrations





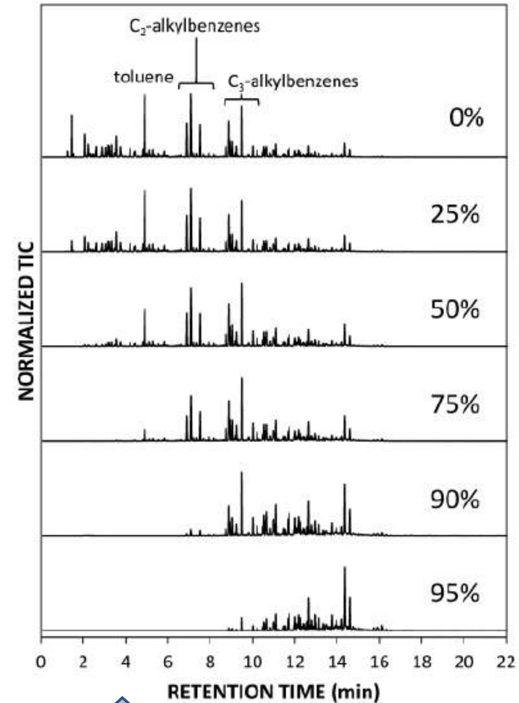
- Major pollution at H-site (50 mg/kg)
- Depths with major pollution:
  L-site 25-100 cm, H-site >100 cm
- Small-scale heterogeneity of amounts

- Major components at all depths: fluoranthene, pyrene, (50 mg/kg)
- Punctual highs of phenanthrene, dibenzofuran, dibenzothiophene
- Small-scale similarity of PAH patterns

GCMS-Fingerprints

Fuel Type	Level of Biodegradation	Chemical Composition	
Gasoline, Diesel, Bunker C fuel	1	Abundant n-alkanes	Increasing level of biodegradation
	2	Light-end n-alkanes removed	
	3	Middle range n-alkanes, olefins, benzene & toluene removed	
	4	More than 90% of n-alkanes removed	
	5	Alkylcyclohexanes & alkylbenzenes removed Isoprenoids & C_0-naphthalene reduced	
	6	Isoprenoids, C_1-naphthalenes, benzothiophene & alkylbenzothiophenes removed C_2-naphthalenes selectively reduced	
	7	Phenanthrenes, dibenzothiophenes and other polynuclear aromatic hydrocarbons reduced	
	8	Tricyclic terpanes enriched Regular steranes selectively removed C_{31} to C_{35}-homohopanes reduced	
	9	Tricyclic terpanes, diasteranes & aromatic steranes abundant	

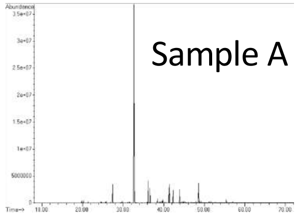
Weathering Stages

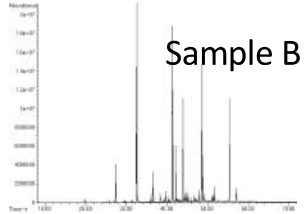


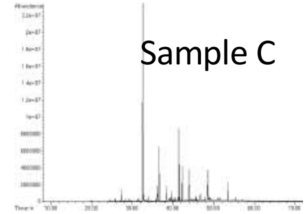
Altering Patterns of PAH

GCMS-Fingerprinting by GC-MS analysis (Single Ion Mode SIM)

Soxhlet-Extraction



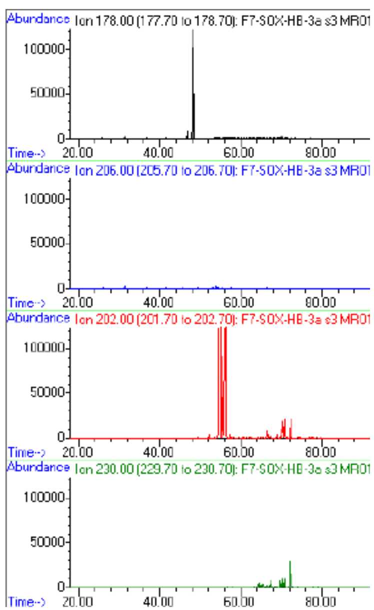




Evaluation of Natural Attenuation by Diagnostic Ratios of Compounds

PAHs exhibit different affinities for biodegradation, e.g. 2-ring PAH vs 3-ring PAH;
General trend that microbial persistence increase with increasing alkylation, e.g. PAH/(C2-PAH+C3-PAH) ↓

Single Ion Mode Analyses



Ratio decreasing through degradation, e.g.
Anthracene & Phenanthrene (m/z 178)
versus
C2-Anthracenes & Phenanthrenes (m/z 206)

Ratio decreasing through degradation, e.g.
Pyrene & Fluoranthene (m/z 202)
versus
C2-Pyrenes & Fluoranthenes (m/z 230)