Hungary-Serbia
IPA Cross-border Co-operation Programme

Good neighbours creating common future

# PHANETRI

## *Development of an in field, ecologically safe, continuously detoxifying technology for producing bio-vegetables*

## Project Workshop:
## *„Bioremediation for agriculture and healthy environment"*

**June 28, 2013**

Educons University, Faculty of Environmental Protection, Lecture Hall (3th Floor), Vojvode Putnika 85, Sremska Kamenica, Serbia

| | |
|---|---|
| 9.00-9.30 | **Registration of the participants** |
| 9.30-9.35 | **Welcome speech**, Prof. Dr. Dejana Panković, Faculty of Environmental Protection, Educons University, Sremska Kamenica, Serbia |
| 9.35-10.00 | Dr. László Manczinger: "**Remediation potential of the white-rot fungus *Phanerochaete chrysosporium***", Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary |
| 10.00-10.30 | Dr. András Szekeres: "**GC-MS method for dithiocarbamate analysis using liquid injection technique**", Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary |
| *10.30-11.00* | *Coffee break* |
| 11.00-11.15 | MSc. Gordana Danilović: "**Variability of *Trichoderma* strains isolated from different soil types and their tolerance to copper** ", Faculty of Environmental Protection, Educons University, Sremska Kamenica, Serbia |
| 11.15-11.30 | Prof. Dr. Dejana Panković: "**The effect of selected *Trichoderma* strain on drought response of tomato plants**", Faculty of Environmental Protection, Educons University, Sremska Kamenica, Serbia |
| 11.30-11.50 | Dr Vladimir Beškoski: "**Bioremediation, technology of choice for soil and groundwater polluted industrial sites with petroleum derivatives**", Faculty of Chemistry, University of Belgrade, Serbia |
| 11.50-12.10 | MSc. Vera Gujaničić: "**The contributions of soil microorganisms in sustainable agricultural production**", Faculty of Agriculture, University of Belgrade, Serbia |

12.10-13.30 Laboratory visit at the Faculty of Environmental Protection, Educons University, Sremska Kamenica, Serbia

13.30-15.30 *Lunch break*

15.30-16.30 Round table discussion with workshop participants

***

Participation of the representatives of the project target groups is highly welcomed:

1. *Researchers participating in the project (HU/SRB).*
2. *PhD students participating in the project (HU/SRB).*
3. *Undergraduate students participating in the project (HU/SRB).*
4. *Non-participating scientists with interest to project achievements (HU/SRB).*
5. *Non-participating PhD students with interest to project achievements (HU/SRB).*
6. *Non-participating undergraduate students with interest to project achievements (HU/SRB).*
7. *Farmers in the target (cross-border) region (HU/SRB).*
8. *Representatives of SMEs in the target (cross-border) region (HU/SRB).*
9. *Representatives of agriculture-connected authorities, official bodies, self-organised organisations in the target (cross-border) region (HU/SRB).*
10. *Regional (political) decision makers (HU/SRB).*
11. *Representatives of common public with interest to sustainable agriculture, environmental protection and life sciences (HU/SRB).*
12. *Representatives of the media.*

*This document has been produced with the financial assistance of the European Union. The content of the document is the sole responsibility of the Faculty of Environmental Protection, Educons University, Sremska Kamenica, Serbia and the Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary and can under no circumstances be regarded as reflecting the position of the European Union and/or the Managing Authority.*

**dr Vladimir P.BEŠKOSKI**
**Faculty of Chemistry**
**University of Belgrade**
**Belgrade, Serbia**

Hungary-Serbia
IPA Cross-border Co-operation Programme

**PHANETRI**

## INVITATION LETTER

Dear Dr Beškoski,

We would like to invite you to give oral presentation in duration of thirty minutes at the workshop ***„Bioremediation for agriculture and healthy environment"***, *to be held on the 28th of June 2013 in Lecture Hall of* Educons University, (3th Floor), Vojvode Putnika 85 in Sremska Kamenica.

*This workshop is activity in the frame of* ***IPA*** *project entitled* ***„Development of an in field, ecologically safe, continuously detoxifying technology for producing bio-vegetables".***

The work of your group has been recognized as significant and innovative for the multidisciplinary area covered with our program, so we hope that you will accept this invitation and contribute to successful progress of the workshop.

Prof. Dejana Pankovic
Vice rector for science

Hungary-Serbia
IPA Cross-border Co-operation Programme

EDUCONS UNIVERSITY

**Project Workshop:**

***„Bioremediation for agriculture and healthy environment"***

# BIREMEDIATION, TECHNOLOGY OF CHOICE FOR TREATMENT OF SOIL AND GROUNDWATER FROM INDUSTRIAL SITES POLLUTED WITH PETROLEUM DERIVATIVES?


Vladimir P. BEŠKOSKI[1,2] Srdjan MILETIĆ[2], Mila ILIĆ[2], Gordana GOJGIĆ-CVIJOVIĆ[2], Miroslav M. VRVIĆ[1,2]

[1]Faculty of Chemistry, University of Belgrade, Serbia
[2]Department of Chemistry, IChTM, University of Belgrade, Serbia



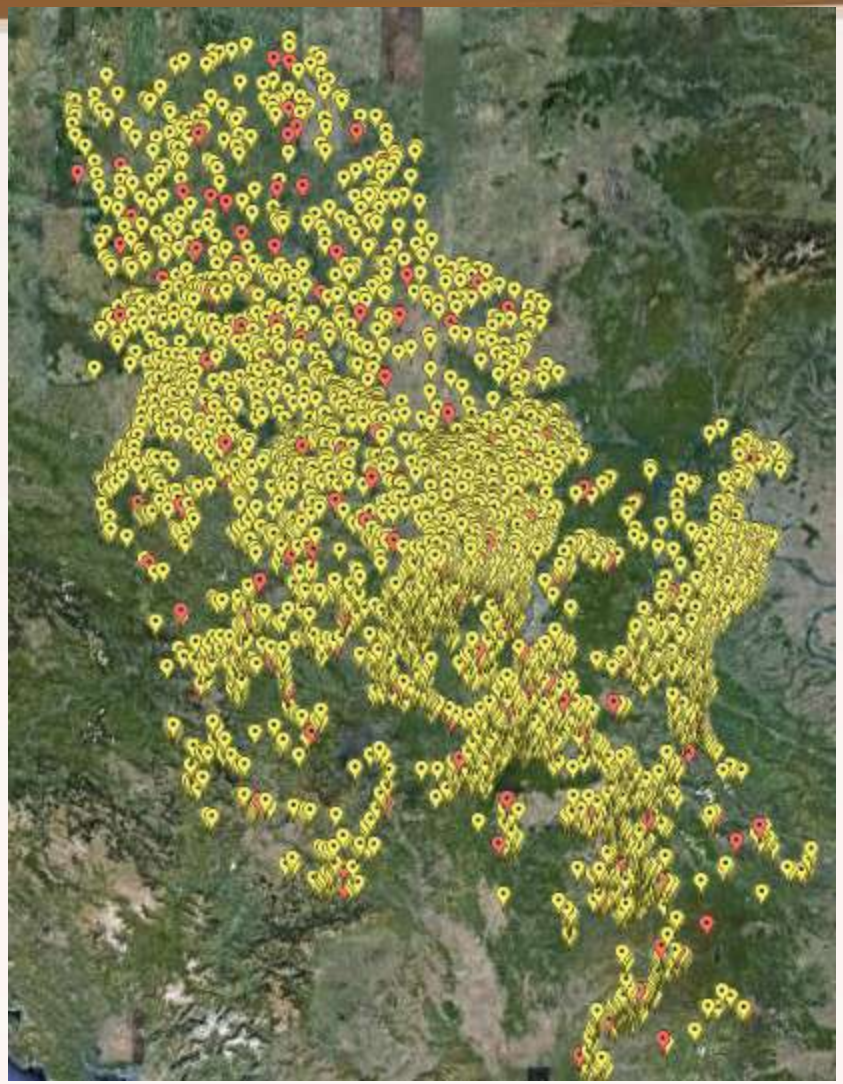
Educons University, Sremska Kamenica, Serbia
June 28, 2013

# Polluted Environment in Serbia






Europe ~ 1 800 000 potentially polluted areas / 240 000 site requires remediation!
Serbia 357 heavily polluted sites!



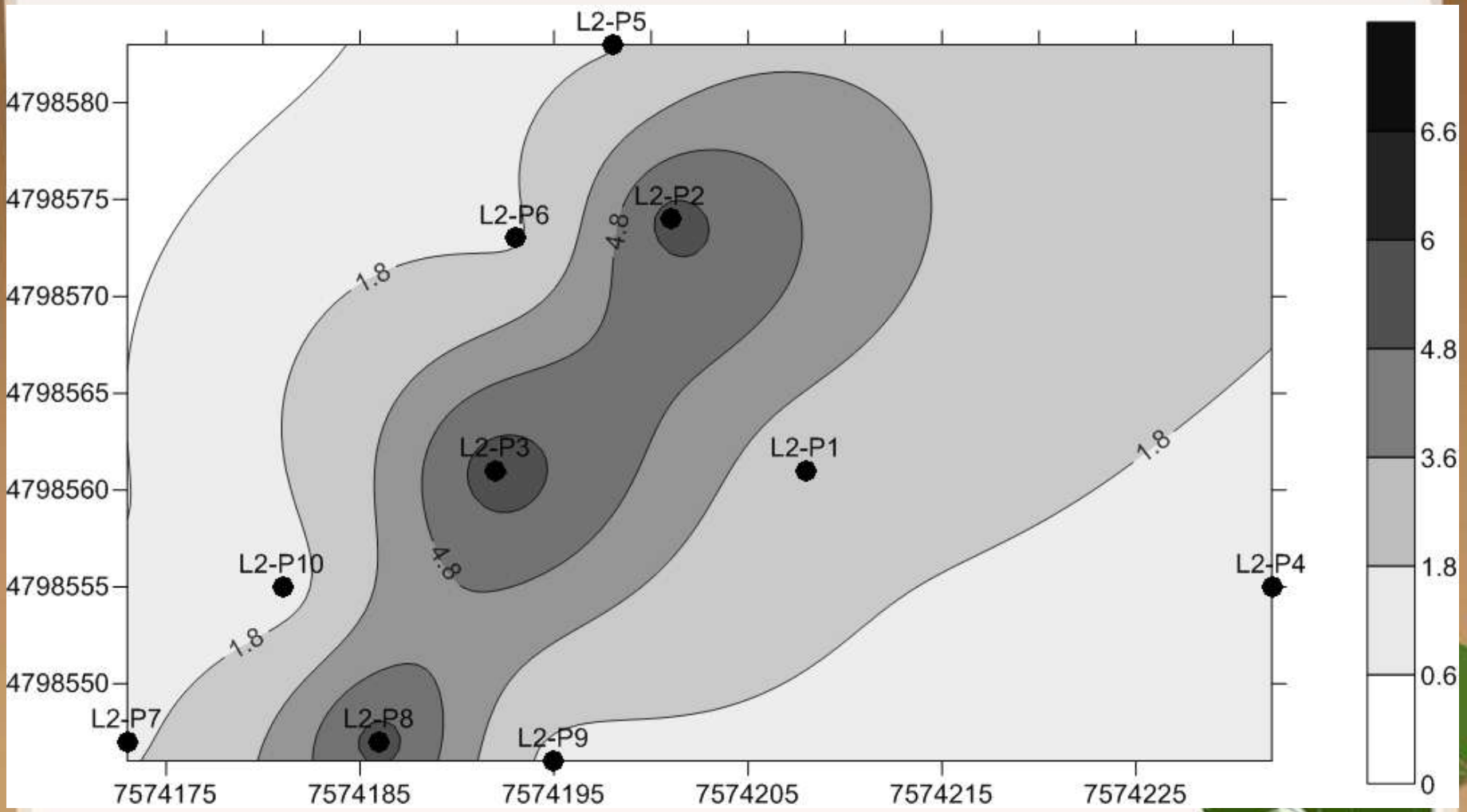
**Locations of <u>known illegal landfills</u> in the Republic of Serbia**
"Report on the State of the Environment in Republic of Serbia for 2009.". Serbian Environmental Protection Agency and Ministry of Environment and Spatial Planning of the republic of Serbia

# Niteks-Benetton Niš, Serbia


# Mazut reservoirs in Niteks-Benetton Niš, Serbia

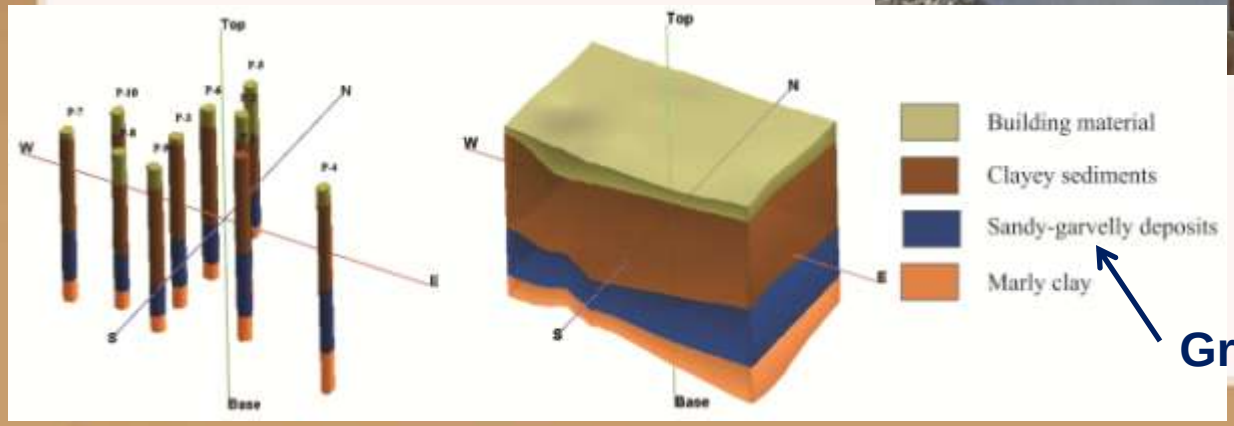

The locality of the company Niteks-Benetton in Niš (Serbia) was contaminated over several years with petroleum products due to the continuous pollution from leaking tanks.

# Concentrations of Total Petroleum Hydrocarbons (TPH) in groundwater determined after sampling from piesometers P-1 to P-9 from location L-2




TPH **700 - 8440** µg/L (600 µg/L remediation value)

3D model of the location L-2!




0.2 to 1.7 m

2.6 to 5.6 m

1.5 to 3.0 m

**Groundwater**

# *Ex situ* bioremediation of the polluted soil from Nitex, Niš

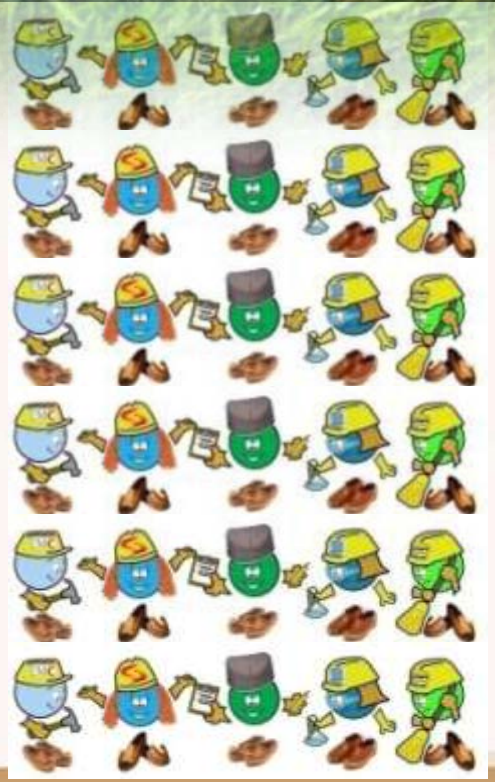
Total Volume ~ 4.400 $m^3$

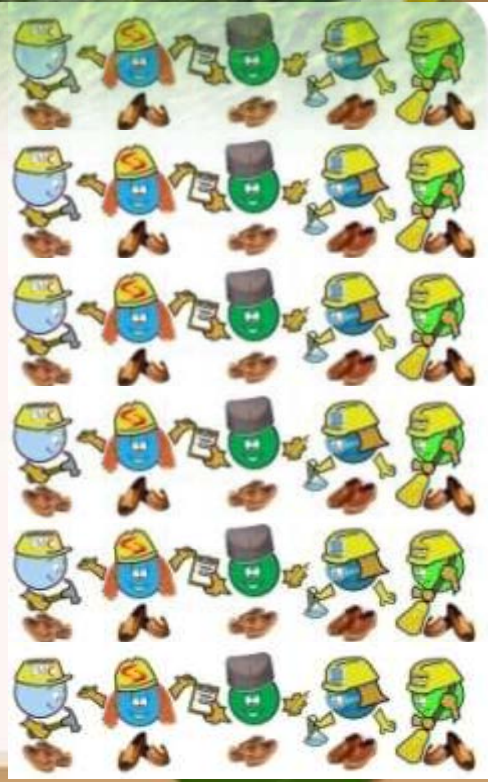
TPH 144 mg/kg-max **6.918** mg/kg

(5000 mg/kg Intervention value)

ISOLATION AND SELECTION OF ZYMOGENOUS MICROORGANISMS

BIOMASSS PRODUCTION





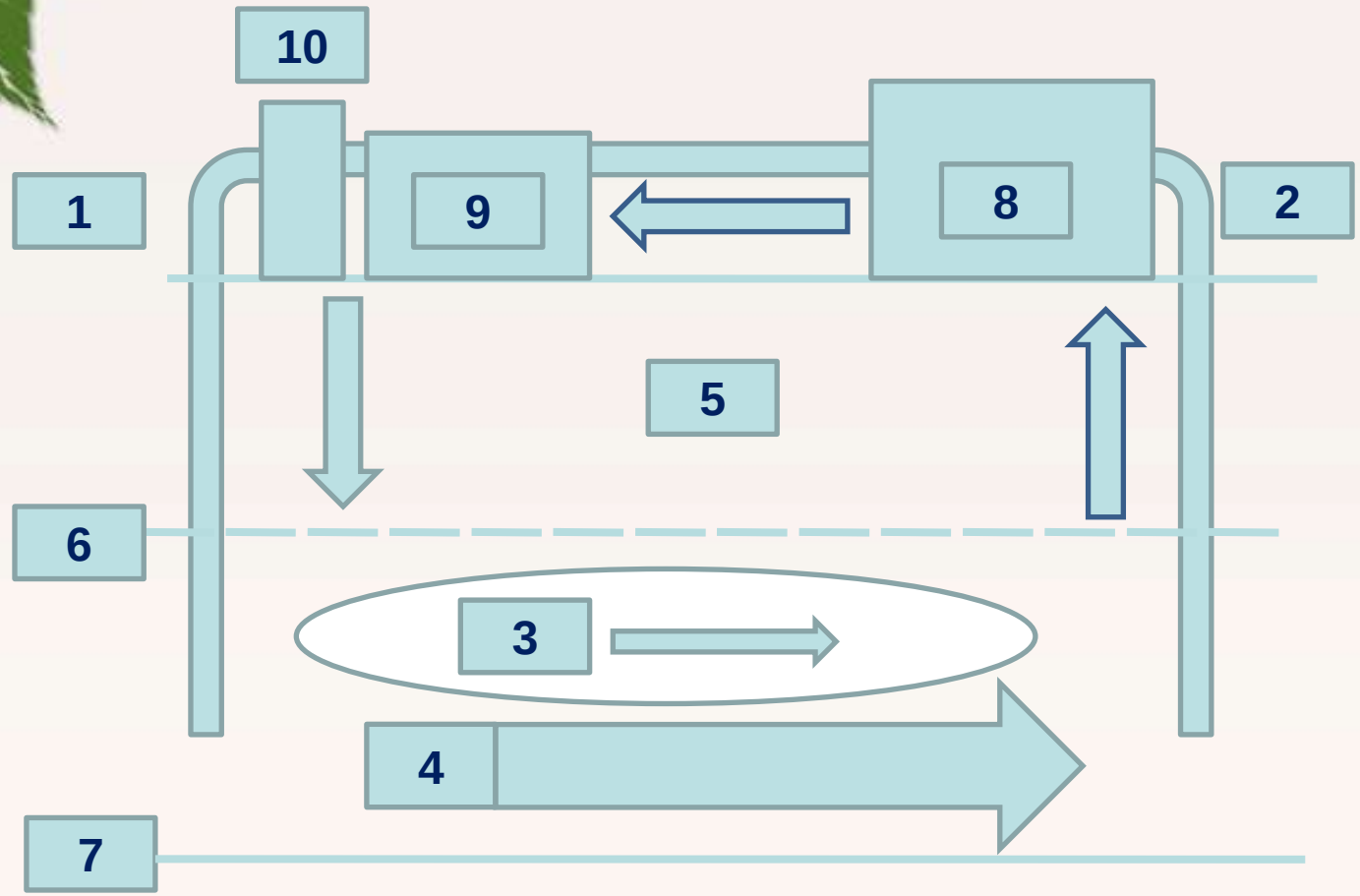
# Ground waters

- Ground waters (GW) which contained dissolved hydrocarbons and a floating layer of an oil pollutant (Light Non-Aqueous Phase Liquid - LNAPL) were treated with **filtration-adsorption remediation technique**, using the columns filled with natural inorganic hydrophobic adsorbents, and *in situ* bioremediation based on the principle of "bipolar" model.

- ***In situ* bio/remediation of GW and soil layers** in contact with groundwater was accomplished by chemical and biological **stimulation**, **augmentation** and **aeration** in closed "bipolar" system (pumping out – pumping in), with adsorption in the "external unit".

- Natural microbial processes in groundwater were additionally stimulated by chemical or physical increase in the aeration capacity.

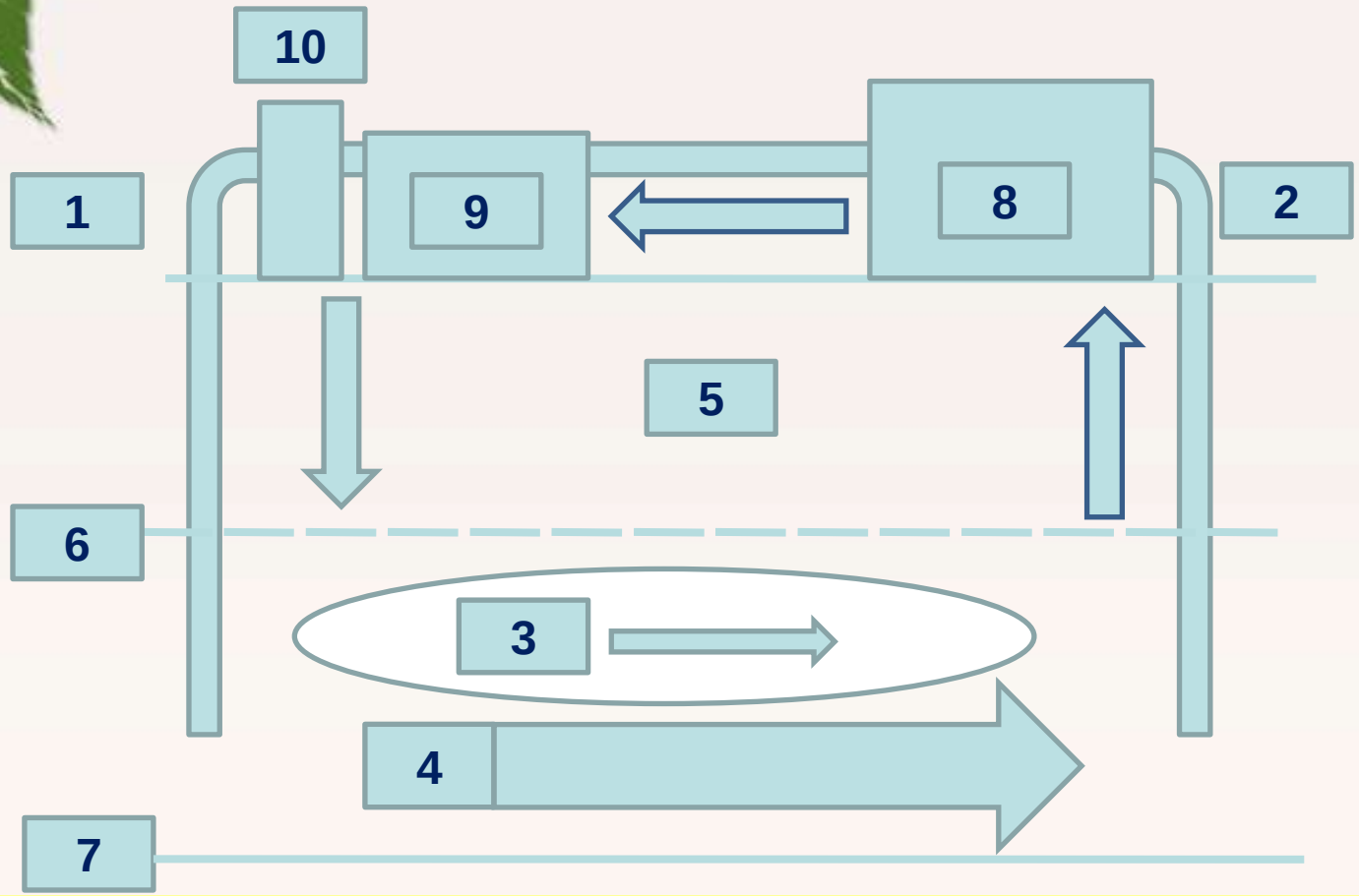
- Bioaugmentation was achieved by injection of biomass of **zymogenous microorganisms** isolated from treated polluted GW.




**Closed bipolar model for water-solution flows (marked with small arrows):**

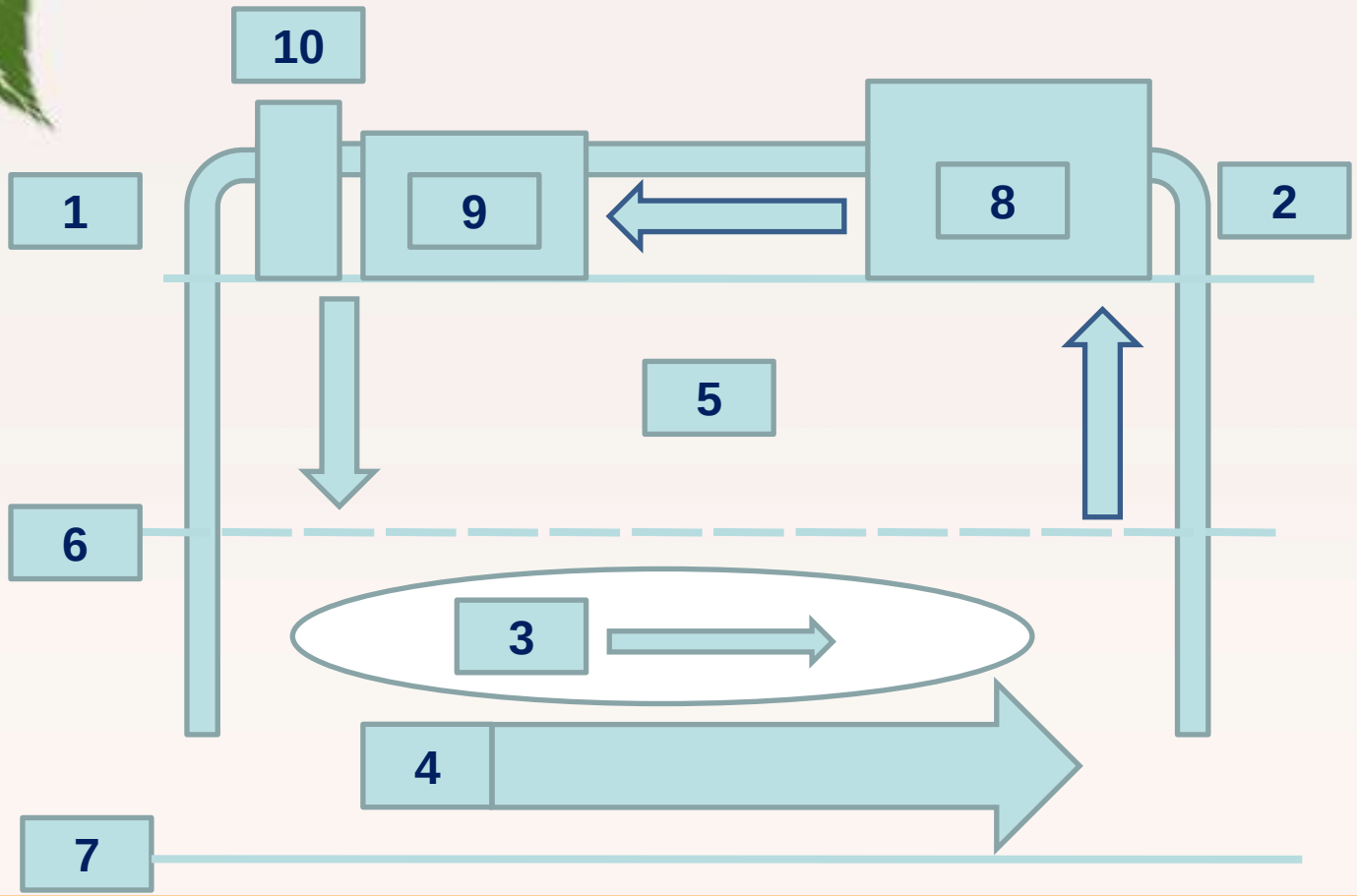
cohesive fluid between the **contaminated soil** (**5**) and the **pollutant** (**3**) is the **groundwater** flowing in the indicated direction (**4**), which level is (**6**) and the water-impermeable layer (**7**).

*(It is not scaled and the ratios are not the same as in the reality)*




**The purification procedure:**

from the collection **tank** (**9**) to the infusion-**injection well** (**1**) and further to the flow of the polluted groundwater, it was introduced a solution with microorganisms (bioaugmentation) and nutritive components (biostimulation). Aeration was done by compressor or by adding chemical generators of molecular oxygen (bioaeration) using appropriate device (**10**).




**<u>In this way began a bioremediation cycle of groundwaters:</u>**

“reactive solutions” – groundwaters, which were dynamically decontaminated, were transported to the **filtration-adsorption unit** (**8**) by the submersible pump (**2**). The filtration-adsorption unit - column filled with natural inorganic hydrophobic adsorbents (separation of pumped LNAPL).

# FILTRATION-ADSORPTION UNIT

**Total mass transfer of groundwater was 2317 $m^3$**

# Basic characteristics of the GW during the treatment

| Date [2012 Y] | $t_{water}$ [°C] | pH | NTU | $O_2$* [mg/L] |
|---|---|---|---|---|
| May, 1 | 15.4 | 6.9 | 80 | 3.4 |
| June, 1 | 16.1 | 6.9 | 22 | 8.5 |
| July, 1 | 16.9 | 7.0 | 5 | 8.8 |

*NTU - Nephelometric Turbidity Unit*
** Dissolved $O_2$*

At the beginning TPH **700 – 8440** µg/L

(600 µg/L remediation value)

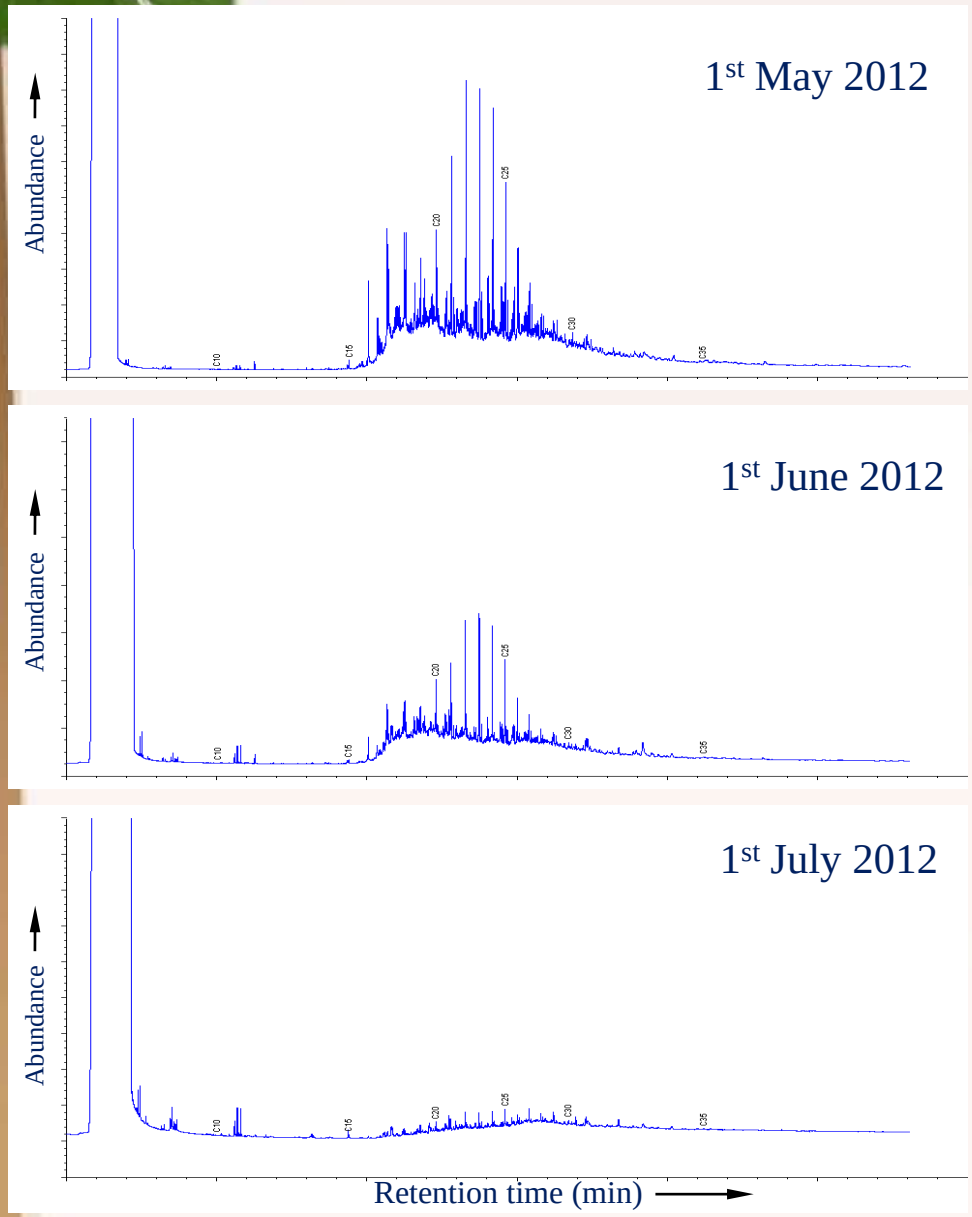
After 60 days L-2/B-1<**0.05**; L-2/B-3<**0.05** i L-2/B-4<**0.05** µg/L


B1
B2
B3
0 Day


L2
B3
L2
B3
30 Day


L2 B1
22.6.12.
L2 B3
22.6.12.
L2 B4
22.6.12.
60 Day

# *In situ* Bioremediation

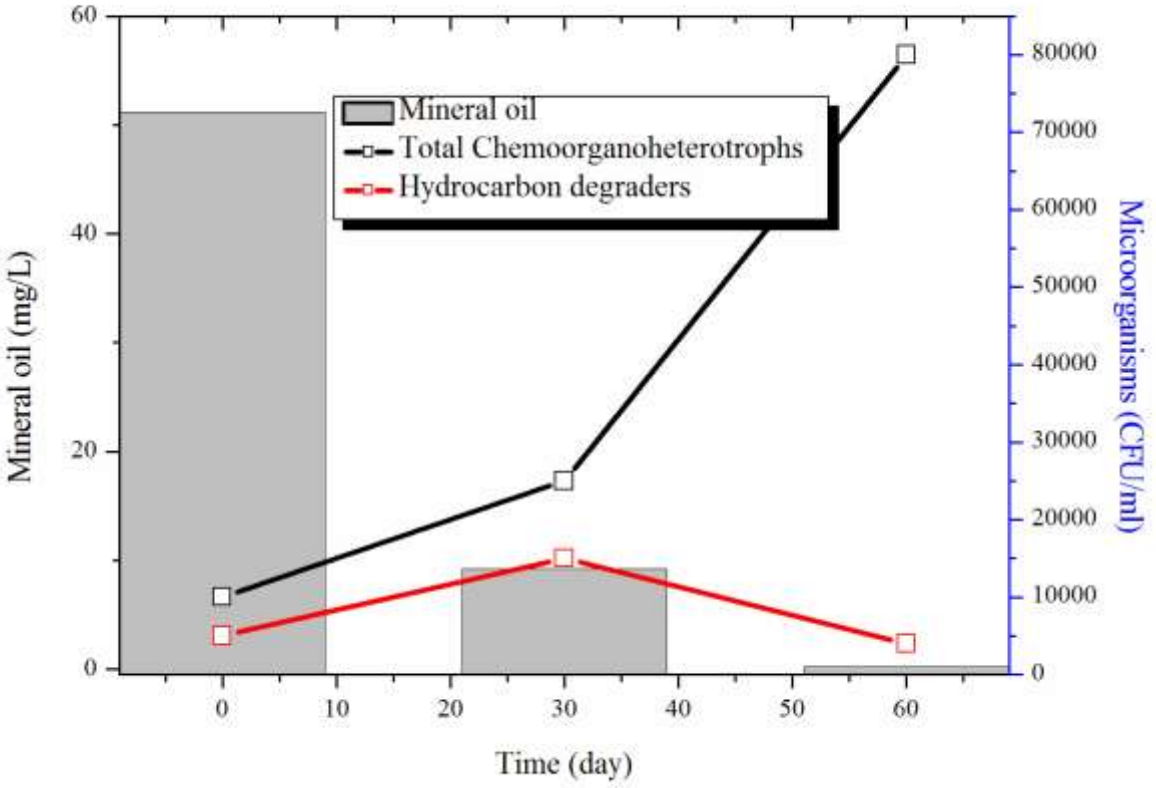



The change in the content of the "mineral oil" – total petroleum hydrocarbons (TPH): at the beginning of the experiment, after 30 days and after 60 days.

*TPH was determined according to the international standard ISO 9377-2:2000: method using solvent extraction and gas chromatography.*

*Isolated hydrocarbon degraders:*

*Pseudomonas sp.,*
*Achromobacter sp.,*
*Bacillus sp.,*
*Micromonospora sp.,*
*Rhodococcus sp.,*
*Acinetobacter sp.*



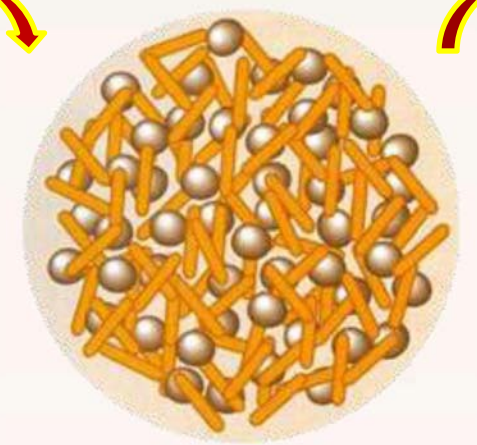

The change in the content of the "mineral oil" – total petroleum hydrocarbons (TPH) and the consortium of chemoorganoheterotrophic and hydrocarbon degrading microorganisms in the samples of water treated by bipolar system.

# Biodiversity

- Number of CFU/g
- Number of different species

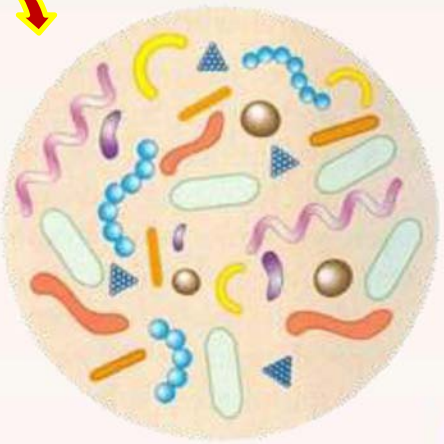
Nonpolluted environment

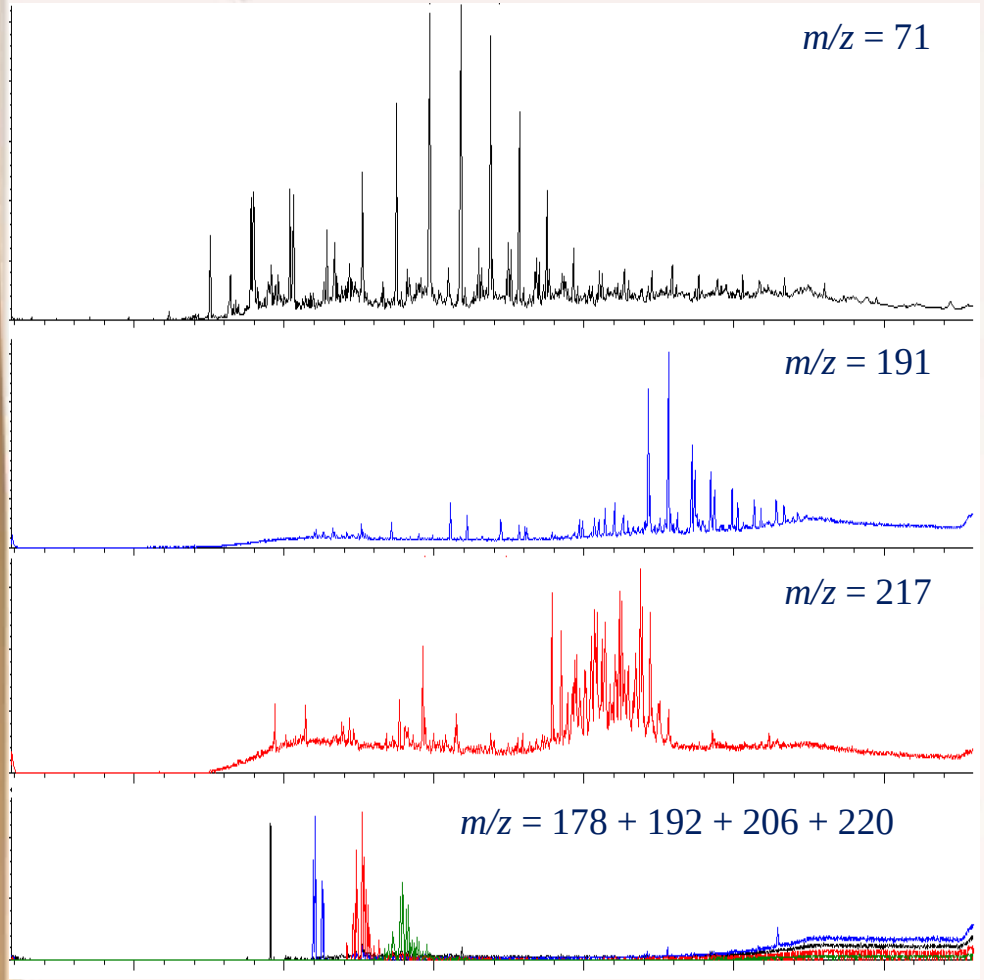
Polluted environment



Zymogenous microorganisms

Nonpolluted environment



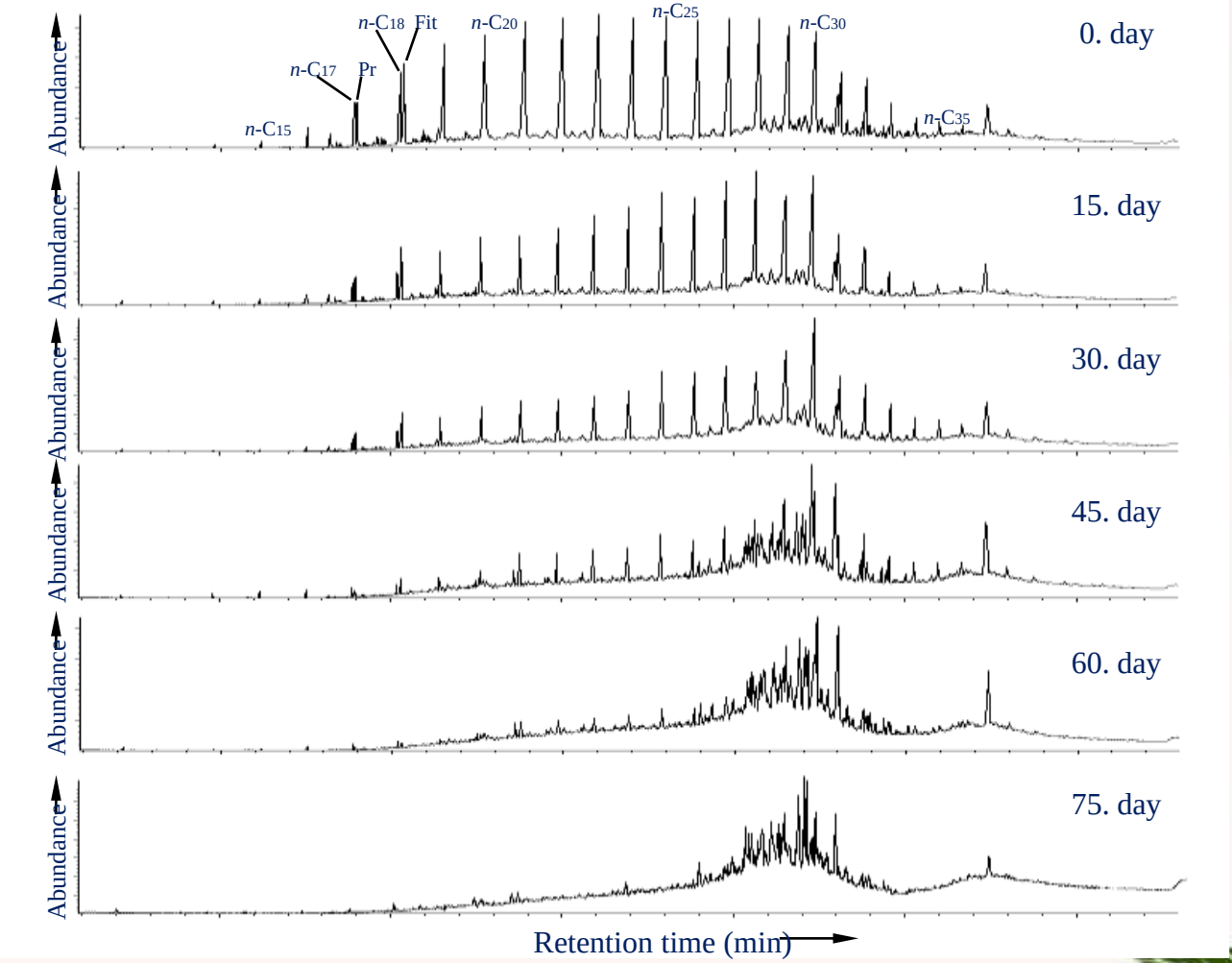



# ***In situ* Bioremediation**

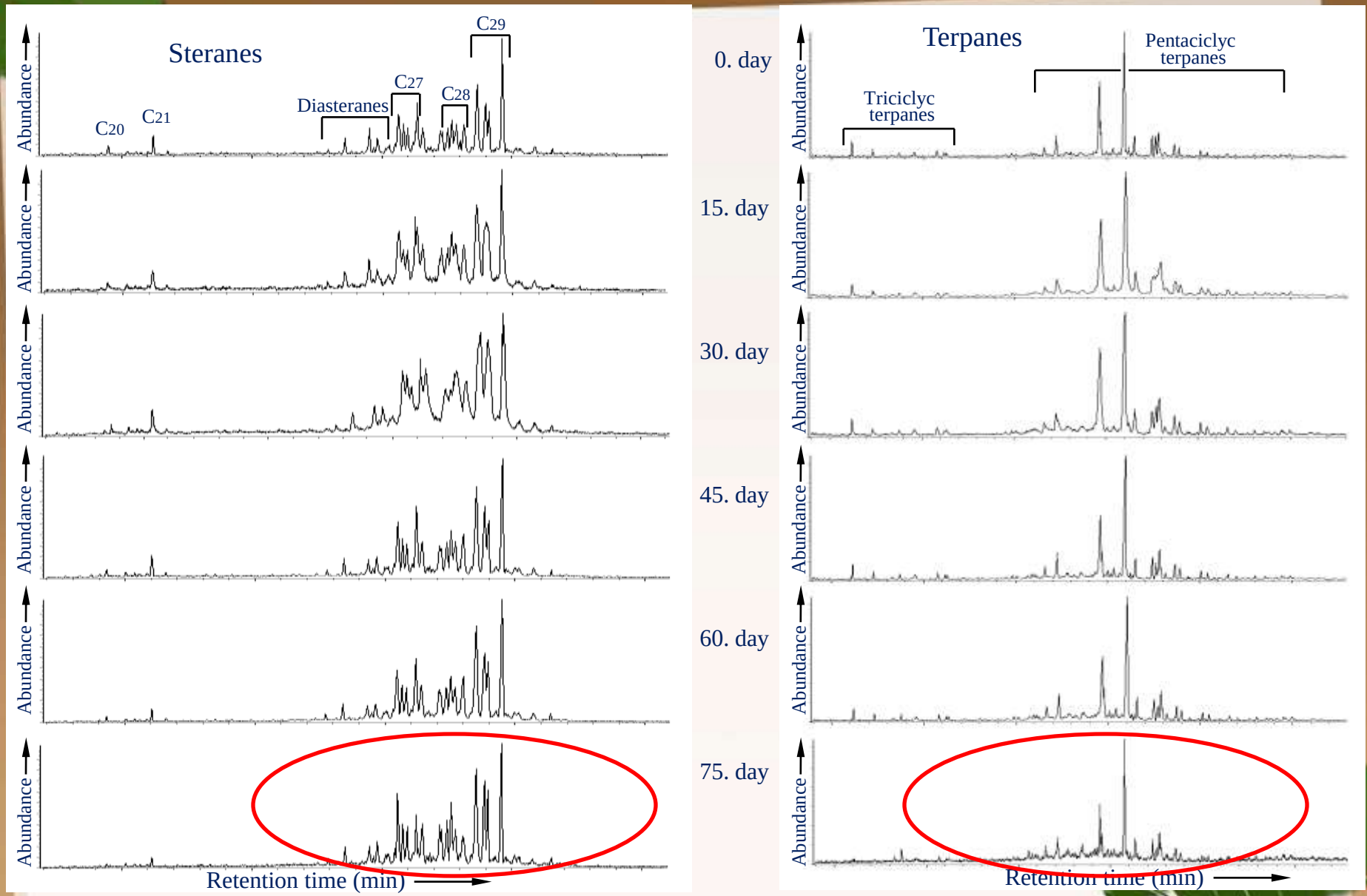
Detailed analyses of the target compounds were conducted in the single ion monitoring mode (SIM), comprising the following ion chromatograms:

*m/z* = 71 (***n*-alkanes** and **isoprenoids**),
*m/z* = 191 (**terpanes**),
*m/z* = 217 (**steranes**),
*m/z* = 178 (**phenanthrene**),
*m/z* = 192 (**methyl-phenanthrenes**),
*m/z* = 206 (**dimethyl-phenanthrenes**) and
*m/z* = 220 (**trimethyl-phenanthrenes**).

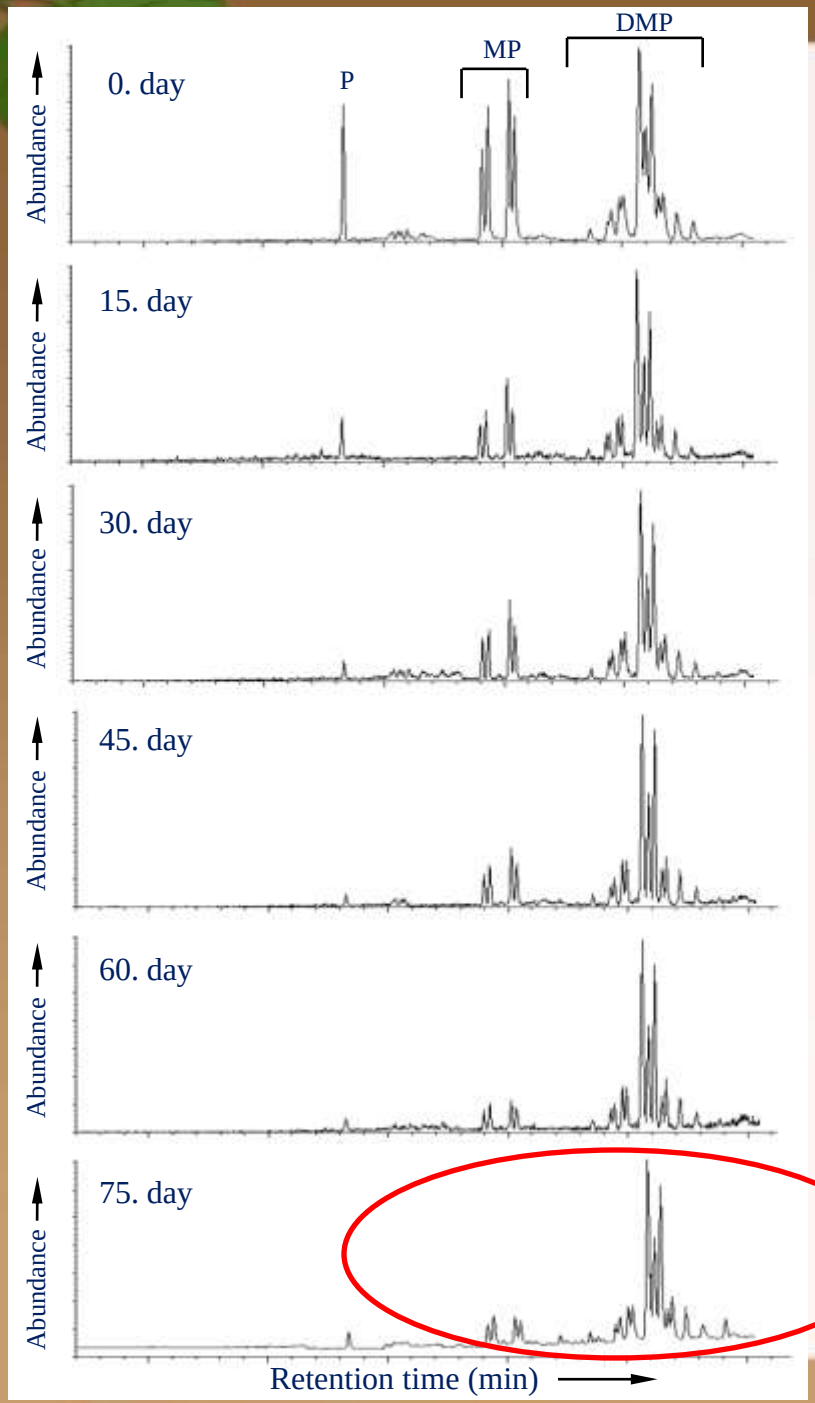
# Biodegradation of the oil pollutant in the laboratory




Total ion chromatograms (TIC) of **saturated fractions** after the experiment of simulated biodegradation with consortium of bacteria and fungi.



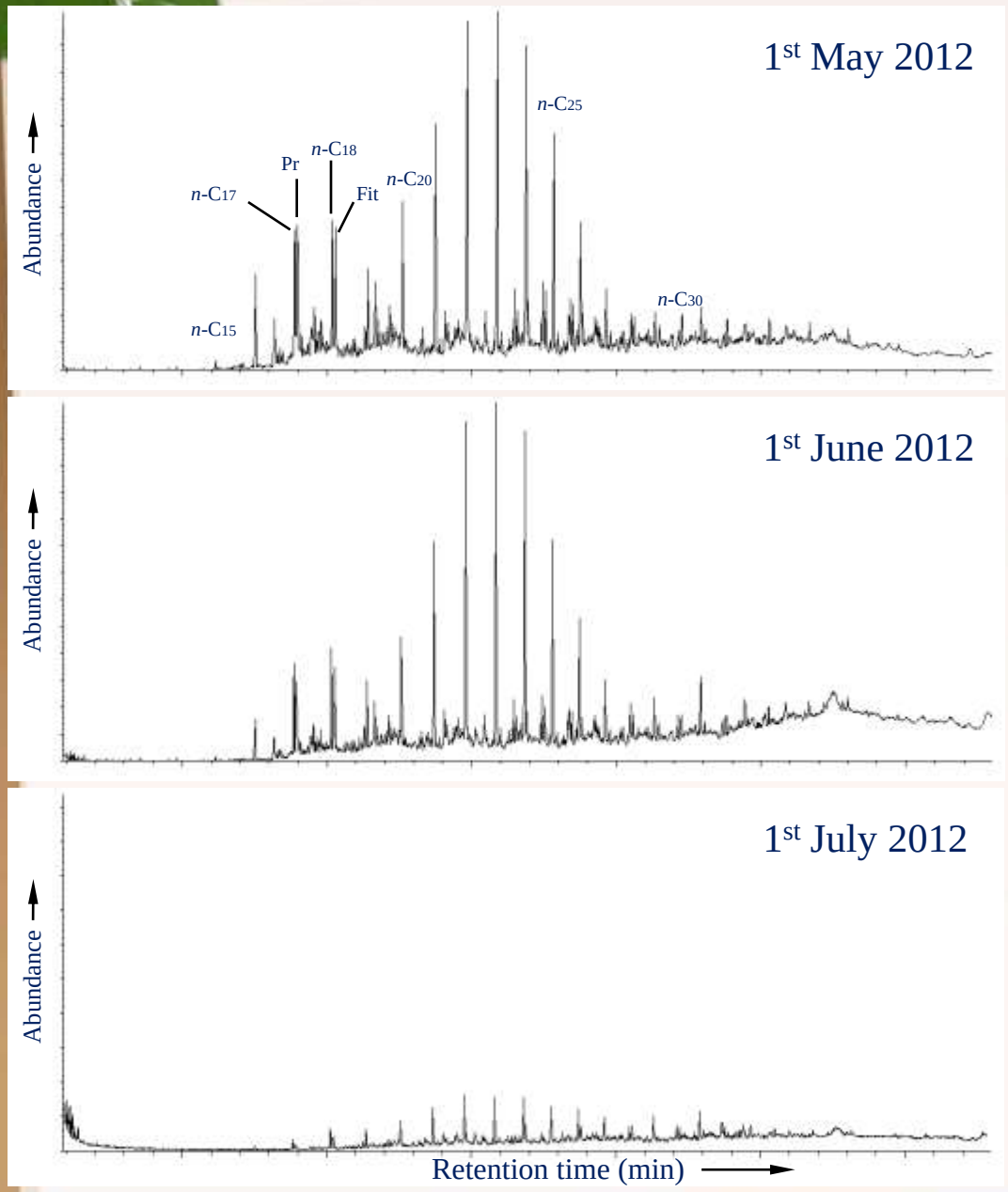

**<u>Biodegradation of the oil pollutant in the laboratory:</u>** fragmentograms of **steranes** (*m/z* = 217) and **terpanes** (*m/z* = 191) after simulated biodegradation with **<u>consortium of bacteria and fungi.</u>**




## Biodegradation of the oil pollutant in the laboratory

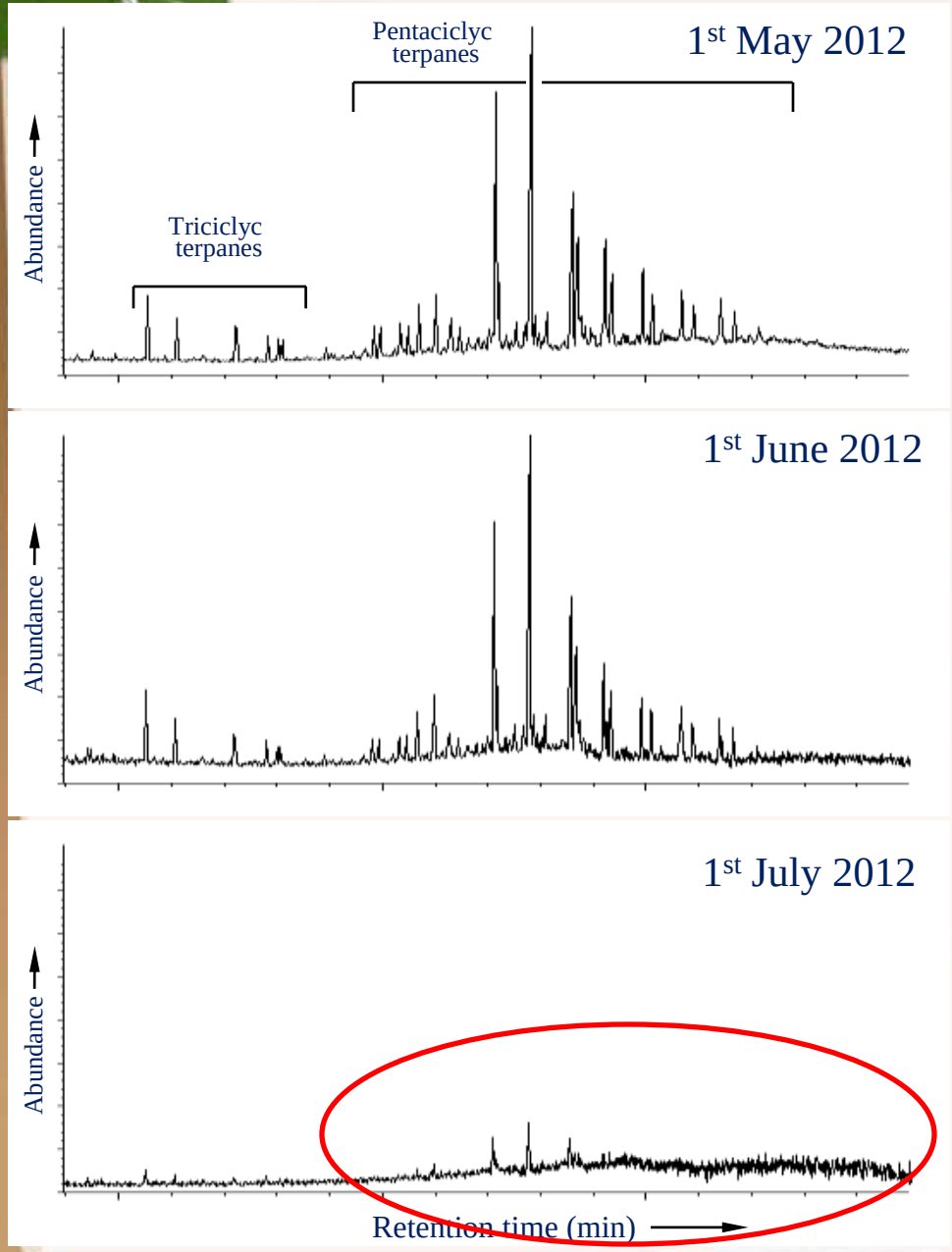
Fragmentograms of **phenanthrene** (P; *m/z* = 178), **methylphenanthrenes** (MP; *m/z* = 192) and **dimethylphenanthrenes** (DMP; *m/z* = 206) after the experiment of simulated biodegradation with consortium of bacteria and fungi.

# *In situ* Bioremediation



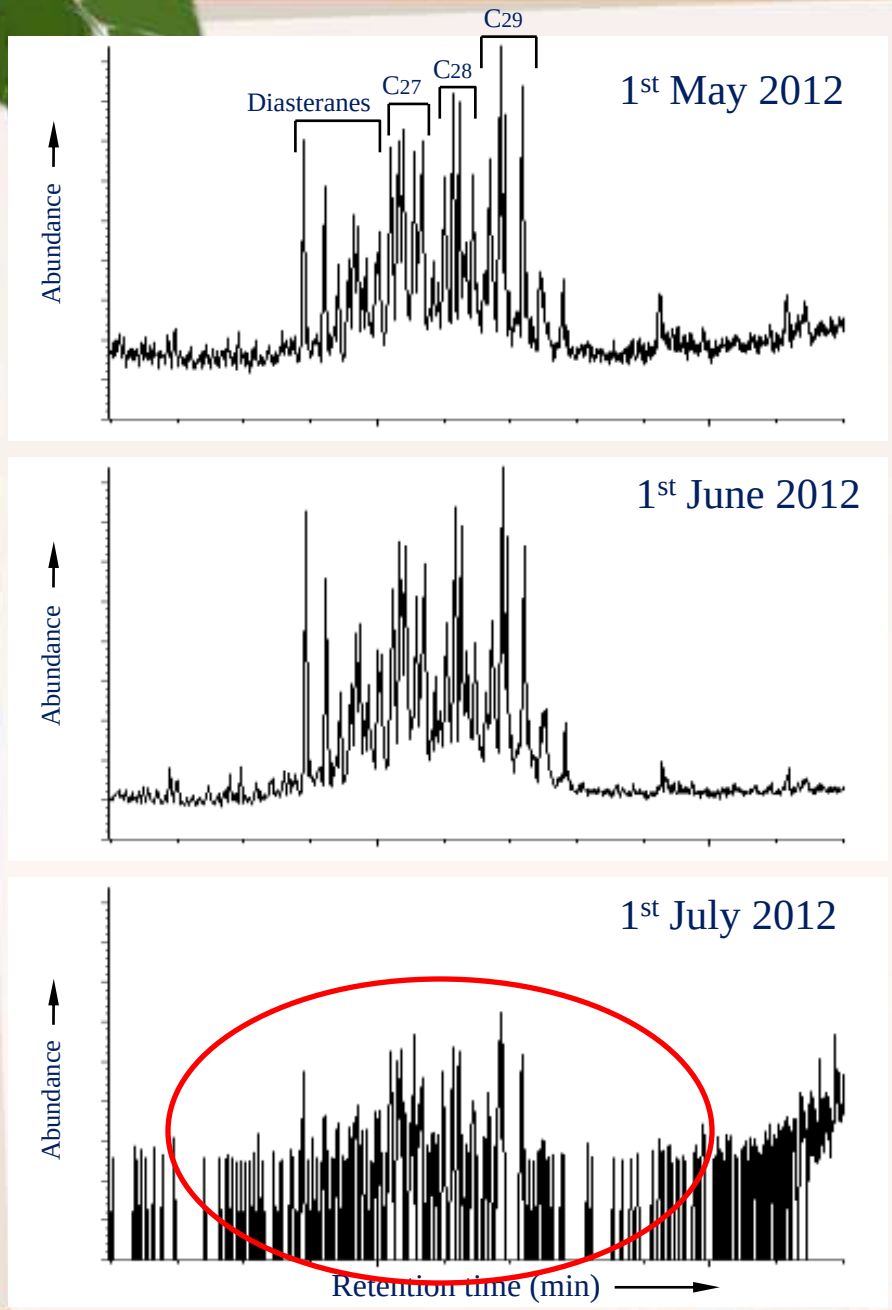

Fragmentograms of ***n*-alkanes** and **isoprenoids** (*m/z* = 71) obtained by GC-MS analysis of the extracts isolated from the samples at the beginning of the experiment, after 30 days and after 60 days.

# *In situ* Bioremediation



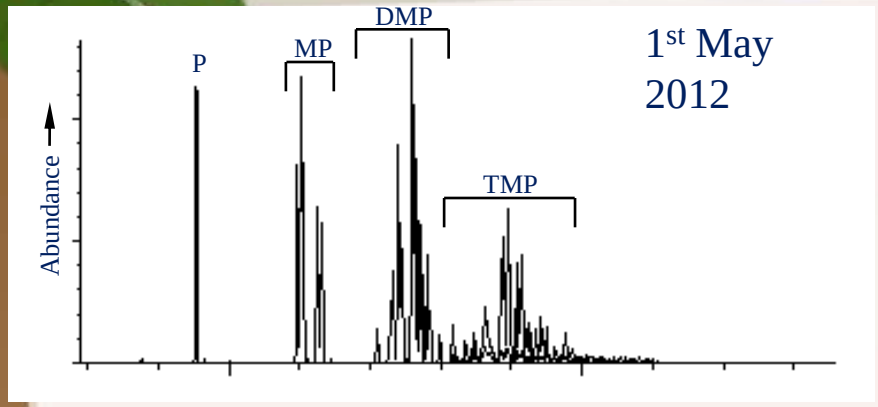

Fragmentograms of **terpanes** (*m/z* = 191) obtained by GC-MS analysis of the extracts isolated from the samples at the beginning of the experiment, after 30 days and after 60 days.

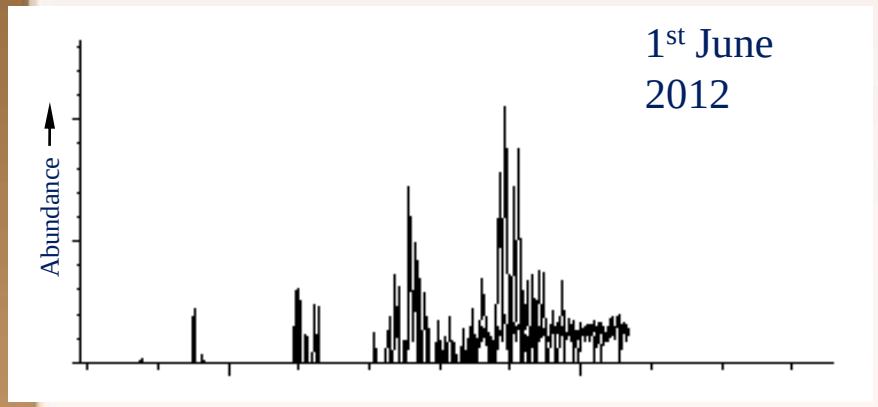
# *In situ* Bioremediation

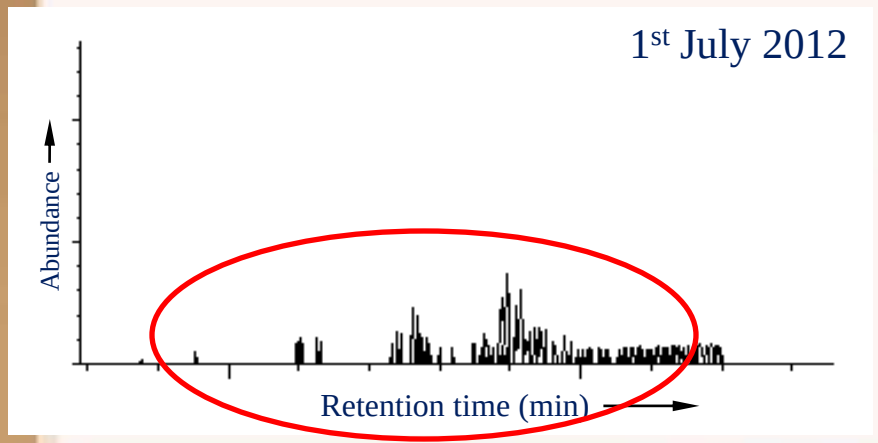



Fragmentograms of **steranes** (*m/z* = 217) obtained by GC-MS analysis of the extracts isolated from the samples at the beginning of the experiment, after 30 days and after 60 days.

# *In situ* Bioremediation




Fragmentograms of **phenanthrene** (P; *m/z* = 178), **methylphenanthrenes** (MP; *m/z* = 192), **dimethylphenanthrenes** (DMP; *m/z* = 206) and **trimethylphenanthrenes** (TMP; *m/z* = 220) obtained by GC-MS analysis of the extracts isolated from the samples at the beginning of the experiment, after 30 days and after 60 days.

*In situ* industrial scale is more efficient then laboratory biodegradation tests!?

**Laboratory - Shake flasks test technique – Limited Biodiversity**

**Environment – unlimited bidiversity!!!**

# Conclusion

A comparison of results from the laboratory study and those from the nature shows a **more intense effect on polycyclic hydrocarbons** during *in situ* bioremediation.

Namely, the consortium of zymogenous microorganisms which was used in the laboratory experiment had limited effect (steranes and triterpanes were not degraded) and the products of metabolism were accumulated. **In Erlenmeyer flasks** consortium does not change (**diversity can not be increased**), only the ratio of individual microorganisms in it.

**In nature, the composition of consortium changes quantitatively (total number), but qualitatively (the diversity of microorganisms) as well.** As a result, the microorganisms which were not dominant at the beginning might prevail in the end.

# Conclusion

**Q:** Bioremediation, technology of choice for treatment of soil and groundwater from industrial sites polluted with petroleum derivatives?

**A: Bioremediation is technology of choice for industrial sites contaminated with petroleum derivatives!!!**

- *Ex situ* and *in situ* treatment of soil and groundwater are visible – depends on the situation and needs!

- Zymogenous microorganisms consortia has limitless possibilities thanks to the unlimited and unimaginable genetic apparatus!

- Activities of the consortia of zymogenous microorganisms in the environment comparing to activities on similar substrates in the laboratory conditions is more intensive.

- Value of this biotechnology depends on our imagination and creativity!

## Acknowledgments

We thank the Research Fund of the Republic of Serbia for supporting this research

(Project: III 43004)

# Research group

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Thank you very much
for the attention!