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NEW APPROACHES FOR ASSESSMENT AND IMPROVEMENT OF ENVIRONMENTAL STATUS IN BALKAN REGION: INTERACTIONS BETWEEN ORGANISMS AND ENVIRONMENT

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May 28-30, 2012.

ABSTRACTS

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[P17]

Change of biodiversity of petroleum polluted soil during *ex situ* bioremediation process

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Industrial development has led to the utilization of substances of various origin and composition. Crude oil, oil derivatives and products of petrochemical industry are major power sources and raw materials used in manufacturing and as such, they are most predominantly used in all domains of life and work. Having such a wide spectrum of use they often cause soil and water contamination due to inadvertent spill outs during their exploitation, transportation, processing, storage and use. Bioremediation is the technology which deals with the cleaning and remediation of soil by biological methods, i.e. non-pathogenic microorganisms that feed on pollutants.

The purpose of this study is to detect the changes of biodiversity of soil resulting from the pollution caused by oil and oil derivatives, and monitoring the changes in the composition of bacterial biomass during the process of *ex situ* bioremediation. The analysis of chemical and microbiological indicators of unpolluted soil and soil contaminated by oil and oil derivatives has revealed a decrease in the biodiversity, followed up by an increase in the number of hydrocarbon degrading microorganisms and their share in the total biomass. With the reduction of contamination, selection pressure of the environment and abiotic factors are reduced, which results in increased biodiversity. In the polluted environment microorganisms that can use pollutants as the sole source of energy and carbon are dominantly bacteria of the following species: *Pseudomonas, Achromobacter, Bacillus, Micromonospora, Rhodococcus*. Isolated bacteria can use aliphatic, aromatic, polycyclic aromatic and sulphurous polycyclic aromatic hydrocarbons as the sole source of carbon. With the increased contamination by nonpolar pollutants, an increase in the total number of biosurphactant producing microorganisms has been detected.

With the completion of bioremediation and approximation of concentration of polluting substances to their values in unpolluted soil, increased microbial biodiversity is detected with the reduced share of microorganisms that are capable to use hydrocarbons as the sole source of carbon.

Acknowledgement

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CHANGE OF BIODIVERSITY OF PETROLEUM POLLUTED SOIL DURING *EX SITU* BIOREMEDIATION PROCESS

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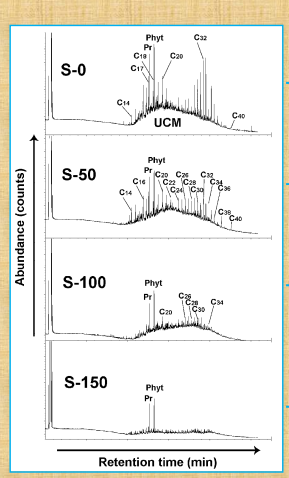
INTRODUCTION

Industrial development has led to the utilization of substances of various origin and composition. Crude oil, oil derivatives and products of petrochemical industry are major power sources and raw materials used in manufacturing and as such, they are most predominantly used in all domains of life and work. Having such a wide spectrum of use they often cause soil and water contamination due to inadvertent spill outs during their exploitation, transportation, processing, storage and use. Bioremediation is the technology which deals with the cleaning and remediation of soil by biological methods, i.e. non-pathogenic microorganisms that feed on pollutants [1].

Time [day]	0	50	100	150
TC[a] [CFU/g]	2.0×10^6	2.2×10^6	1.3×10^7	8.0×10^6
HD[b] [CFU/g]	7.2×10^4	1.5×10^6	9.9×10^6	2.0×10^6
HD [%]	4	68	76	25

[a]TC-total chemoorganoheterotrophs
[b]HD-hydrocarbon degraders

Reduced biodiversity at the expense of increasing the number of HD within the number of TC!



Dominant bacterial genera:

- *Pseudomonas, Achromobacter, Sphingomonas, Acinetobacter, Bacillus, Micrococcus, Mycobacterium, Micromonospora, Rhodococcus* (9)
- *Pseudomonas, Achromobacter, Sphingomonas, Bacillus, Mycobacterium, Micromonospora, Rhodococcus* (7)
- *Pseudomonas, Achromobacter, Bacillus, Micromonospora, Rhodococcus* (5)
- *Pseudomonas, Achromobacter, Sphingomonas, Acinetobacter, Bacillus, Staphylococcus, Micrococcus, Mycobacterium, Micromonospora, Rhodococcus, Penicillium, Aspergillus* (12)

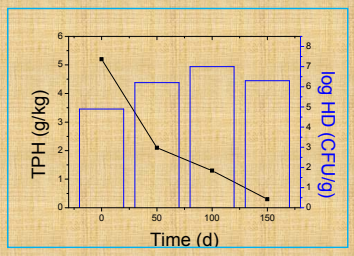
CONCLUSION

Petroleum and petroleum products consist of thousand compounds and therefore the biodegradation of such a complex mixtures requires the participation of multiple cultures. Some defined bacterial species are able to degrade, to a limited extent, all hydrocarbons present in heavy fuel oil or oil sludge. Some of the polluting components may be dissolved only by the joint metabolic activity of multiple genera of microorganisms. An advantage to the use of mixed cultures is a broader degradation capacity, synergic effect and co-metabolism. A consortium of microorganisms can conduct processes of degradation, while at the same time, being more resistant, on average, to changes in the ecosystem than just a single microbial species.

With the completion of bioremediation and lowering of concentration of polluting substances almost to their values in unpolluted soil, increased microbial biodiversity is detected with the reduced share of microorganisms that are capable to use hydrocarbons as the sole source of carbon.

RESULTS AND DISCUSSION

The purpose of this study is to detect the changes of biodiversity of soil resulting from the pollution caused by oil and oil derivatives, and monitoring the changes in the composition of bacterial biomass during the process of *ex situ* bioremediation. The analysis of chemical and microbiological indicators of unpolluted soil and soil contaminated by oil and oil derivatives has revealed a decrease in the biodiversity, followed up by an increase in the number of hydrocarbon degrading microorganisms and their share in the total biomass. With the reduction of contamination, selective pressure of the environment and abiotic factors are reduced, which results in increased biodiversity. In the polluted environment microorganisms that can use pollutants as the sole source of energy and carbon are dominantly bacteria of the following species: *Pseudomonas, Achromobacter, Bacillus, Micromonospora, Rhodococcus*. Isolated bacteria can use aliphatic, aromatic, polycyclic aromatic and sulphurous polycyclic aromatic hydrocarbons as the sole source of carbon. With the increased contamination by nonpolar pollutants, an increase in the total number of biosurphactant producing microorganisms has been also detected [2-7].



Average rate of TPH (Total Petroleum Hydrocarbons) degradation:

0-50 day: **62** mg/kg/day

0-100 day: **16** mg/kg/day

100-150 day: **20** mg/kg/day

	Bacillus sp. NS026	Rhodococcus NS032	Micromonospora sp. NS096	Pseudomonas sp. NS009	Achromobacter sp. NS014
Diesel fuel	+[a]	+	+	+	+
n-Hexane	+	+	+	+	+
n-Hexadecane	+	+	+	+	+
n-Octadecane	+	+	+	+	+
Benzene	+	+	+	+	+
Toluene	+	+	-	-	-
Xylene	-	+	-	-	-
Phenantrene	-	+	+	-	-
Pyrene	+	+	+	+	+
Etylbenzene	+	+	-	-	-
Octylbenzene	+	+	+	+	+
Hexadecylbenzene	+	+	+	+	+
Sodium-benzoate	+	+	+	-	+
2-Phenylphenol	+	+	+	+	-
Dibenzothiophene	+	+	+	+	+

[a] +viable growth observed in 1×10^{-5} dilution

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