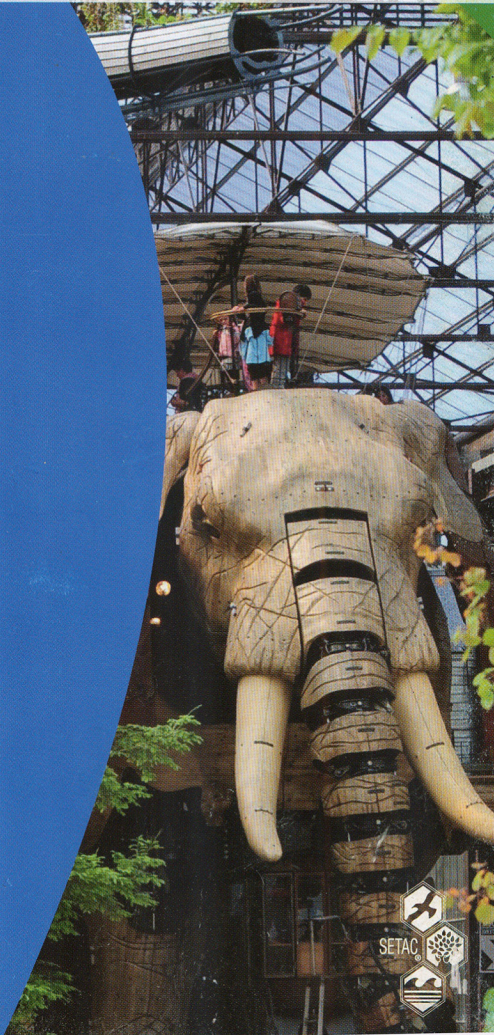# SETAC Europe 26th Annual Meeting
# 22-26 May 2016, Nantes, France

## Environmental contaminants from land to sea: continuities and interface in environmental toxicology and chemistry

Nantes 2016
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munities and environmental variables
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barthelmebs

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M. Posselt, A. Ribbenstedt, J. Benskin

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**TH026** | Microbial turnover of PAH: analy-
sis of degradation and dissolution kinetics
and simulation of remediation options
| Stefan Trapp (Technical University of
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**TH027** | enviPath - The environmental
contaminant biotransformation pathway
resource | Kathrin Fenner (ETH Zürich/
Eawag, Switzerland) | J. Wicker, M.
Guetlein, E. Schmid, D. Latino, S. Kramer,
K. Fenner
**TH028** | Evaluating and validating a Quan-

completely degrade another β-triketone herbicide, mesotrione and producing already known metabolites (AMBA and MNBA). Microbial toxicity of sulcotrione and mesotrione and their related metabolites in bacterial cultures was estimated by measuring 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme inhibition. Our results indicate that β-triketone herbicides toxicity linked to HPPD inhibition was due to parent molecules, and not to the formed metabolites. Attempts were done to identify the genetic localization of sulcotrione degradation in *Pseudomonas sp.* 1OP and *Bradyrhizobium sp.* SR1. Plasmid profiles of both strains revealed the presence of large plasmids (>12 kb and >50 kb, respectively). Curing experiments showed that *Pseudomonas sp.* 1OP lost its ability to degrade sulcotrione under non-selective conditions, therefore degradation capacity may be attributed to the presence of this plasmid. On the contrary, under the same conditions, *Bradyrhizobium sp.* SR1 plasmid was not cured and the sulcotrione-degrading ability of the strain was maintained. Furthermore, a 14 000 Tn5 mutant library was constructed using a Tn5 mutagenesis approach conducted on *Bradyrhizobium sp.* SR1. Among this library, two mutants affected in their biodegradation capacity were identified. Full sequencing of SR1 and Tn5 mutants is ongoing to identify possible degrading gene candidates. Keywords: biodegradation, β-triketone, herbicides, metabolites.

### TH020
**Fingerprinting Micropollutant Transformation in Hyporheic Zones**

M. Posselt, Stockholm University / Department of Environmental Science and Analytical Chemistry; A. Ribbenstedt, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; J. Benskin, Stockholm University / Environmental Science and Analytical Chemistry
Hyporheic zones are key compartments for the functioning of aquatic ecosystems. As dynamic and complex transition regions between rivers and aquifers, they are characterized by the simultaneous occurrence of multiple physical, biological and chemical processes. Assessing persistence of environmental contaminants in the hyporheic zone is non-trivial. In addition to advection processes, transformation can be influenced by numerous biogeochemical factors, including physical characteristics of the sediments (e.g. porosity and bed morphology), chemical parameters (e.g. occurrence of electron donors or acceptors), and biological factors (number and type of microbial population). These variables can lead to difficulties in reproducing lab-based biodegradation experiments and in extrapolating estimates of persistence in the lab to field conditions. Benchmark chemicals (i.e. model substances which are co-incubated with a substance of interest in lab-based biodegradation experiments) have been proposed as a means of addressing inter-assay variability in sediment biodegradation experiments. These controls may aid in extrapolating amongst various lab-based experimental designs and conditions, and perhaps even to conditions encountered in the field. The present work takes an initial step towards characterizing a suite of benchmarking chemicals for use with sediment biodegradation experiments by measuring their degradation half-lives (DegT50) under a series of carefully controlled conditions (temperature, oxygen content, stirring) in closed-bottle experiments. Natural sediments from the river Erpe (Berlin, Germany) were collected and characterized prior to use. Marker chemicals (which included pharmaceuticals and industrial chemicals) were selected based on their environmental occurrence and to cover a range of physicochemical properties, transformation rates and pathways. We expect this approach to aid in the development of more appropriate assays specifically designed for probing micropollutant transformation in the hyporheic zones, for extrapolation of micropollutant half-lives between lab and field, as well as among diverse river sediments and within various locations within a river system.

### TH021
**The role of solar radiation on the photodegradation of PAHs in soils**

M. Marques, Rovira i Virgili University / Chemical Engineering; N. Domínguez, URV/IISPV; M. Mari, Rovira i Virgily University / Chemical Engineering; J. Sierra, Universitat Rovira i Virgili / Soil Science Unit; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering; M. Nadal, University Rovira i Virgili / Laboratory of Toxicology and Environmental Health; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health
Polycyclic aromatic hydrocarbons (PAHs) are a family of widespread environmental pollutants, whose chemical structure is based on two or more fused benzene rings. PAHs are released from natural and anthropogenic combustion processes. Although these chemicals are mostly released to air, soil is considered as one of the major sinks of atmospheric PAHs, undergoing wet and dry deposition processes. Since the environmental fate and transport of PAHs is highly influenced by temperature and solar radiation, photodegradation is likely to be the main process related to the loss of PAHs on soil surfaces, leading to the potential formation of more toxic metabolites than their parent compounds. This study was aimed at estimating the photodegradation rate of PAHs in soils under winter conditions in the Mediterranean region. Soil samples were taken from the A horizon of two common Mediterranean soils located in remote areas. Arenosol soil samples corresponded to Haplic Arenosol, an acidic and coarse-textured soil with granitic origin. In turn, Regosol soil samples belonged to Calcaric Regosol, which is formed of sedimentary materials and fine-textured soil. Ten grams of cleaned soil were deployed in uncovered glass Petri dishes. Every sample was

spiked with 250 μL of a stock solution containing 16 US EPA priority PAHs at a concentration of 100 μg/mL and exposed to sunlight radiation in a methacrylate box placed on the roof of the School of Chemical Engineering, Tarragona, Catalonia. Dark controls were also performed to assess slow sorption processes. Temperature and solar radiation intensity were simultaneously monitored during the experiment. The results showed that PAHs behaved differently according to their molecular weight, temperature, radiation and soil texture. A decreasing trend of PAH concentrations in both soils in samples exposed to daylight was observed. Low molecular weight PAHs are more influenced by volatilization and sorption, while photodegradation is more evident for medium and high molecular weight PAHs. Photodegradation rates were higher than those obtained previously in laboratory conditions, since the intensity of solar radiation is ten times higher than that emitted by fluorescents in a climate chamber.

### TH022
**Bioavailability-related effects of dissolved organic matter on biodegradation of PAHs**

J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo; C. Jimenez Sanchez, Inst de Recursos Naturales y Agrobiol. de Sevilla / Agroquimica y Conservacion de Suelos; R. Posada-Baquero, J. Garcia, M. Cantos, Instituto de Recursos Naturales y Agrobiologia de Sevilla CSIC
In environmental sciences, dissolved organic matter (DOM) is usually differentiated from particulate organic matter as the size fraction of organic matter smaller than 0.45 μm. This fraction typically consists of a multitude of structurally different compounds, all typically present at low concentrations, although it can differ significantly in quality (or biodegradability) and quantity in time and space. The environmental fate of organic pollutants, such as PAHs, can be affected by DOM via increased apparent solubility, desorption, transport and biodegradation. In our group, we have specifically addressed the influences of DOM quality on bioavailability-related phenomena: chemotaxis, attachment and solubilisation. We used, for our studies, different experimental models to assess the bioavailability of PAHs for biodegradation, and these included Tenax extraction, dual 14C/residue analysis of microcosm samples, dynamic passive dosing with PDMS, biphasic NAPL/water systems, and column systems. Different model DOM sources, of dissimilar quality but all with potential use in bioremediation were used, and included humic acids, root exudates, biosurfactants and organic fertilizers. We found that biodegradation of poorly bioavailable PAHs was enhanced by (bio)surfactants (Environ. Sci. Technol. 48:10869-10877, 2014), the targeted fertilization of free-oil phases or NAPLs (Environ. Sci. Technol. 45:1074-1081, 2011), by modulating the deposition and tactic motility of microbial degraders in porous media (Environ. Sci. Technol. 46:6790-6797, 2012), and by root exudates (Soil Biol. Biochem. 57:830-840, 2013; Environ. Sci. Technol. 49:4498–4505, 2015). However, a negative influence on biodegradation of PAHs by humic acids (Environ. Pollut. 184:435-442, 2014) and biosurfactants (Environ. Pollut. 205:378-384, 2015) was found if they prevented cell attachment to the PAH-loaded PDMS sources. These influences of DOM on bioavailability are relevant not only for innovation efforts in bioremediation but they have also connections with the determination of bioavailability of organic chemicals in retrospective and prospective risk assessment and regulation (Environ. Sci. Technol. 49:10255-10264, 2015).

### TH023
**Investigation of motor oil biodegradation by different bacterial strains**

M. Ilic, IChTM / Department of Chemistry; S. Bulatovic, M. Ljesevic, Faculty of Chemistry, University of Belgrade; T. Šolević Knudsen, Institute of Chemistry, Technology & Metallurgy, University of Belgrade / Centre of Chemistry; J. Milic, Institute of Chemistry, Technology & Metallurgy / Centre of Chemistry; J. Avdalovic, S. Miletic, G. Gojgić-Cvijović, Institute of Chemistry, Technology and Metallurgy, University of Belgrade / Department of Chemistry; M.M. Vrvić. Faculty of Chemistry, University of Belgrade / Faculty of Chemistry
Large amount of motor oil is used in many branches of industry. The new generation of motor oils contains high percentages hydrocarbons (C16-C36), more than 75% cycloalkane and polycyclic aromatic hydrocarbons (PAH). However, used motor oils contain a higher percentage of aliphatic and aromatic hydrocarbons, nitrogen and sulfur compounds, and metals (Mg, Ca, Zn, Pb, etc.). In addition to these compounds, the presence of PAHs (naphthalene, benzo [a] pyrene and anthracene) is also expected. Therefore these oils represent a great danger to the environment, because all these compounds are known as mutagenic and carcinogenic [1, 2]. Globally, biodegradation is a common choice for remediation, because the pollutants become substrates for the growth of microorganisms [3]. The aim of this experiment was to investigate the biodegradation of motor oil by bacteria *Stenotrophomonas sp.* (NR 1), *Rhodococcus sp.* (UG 10) and *Bacillus sp.* (F 231) isolated from activated sludge of industrial wastewater treatment plant. The process of biodegradation of motor oil was monitored for 45 days. Each 15 days the process was stopped, the hydrocarbons were extracted, and the samples were analyzed by gas chromatography. Comparison of motor oil concentration in the samples and in the abiotic controls provided insight into the biodegradation activity of bacteria. The results showed a reduction in concentration of motor oil comparing to the

beginning of the experiment (300 ppm). The growth of bacteria confirmed that the motor oil was the only source of carbon. The reduction of motor oil concentration was correlated with the number of bacterial cells. Based on these results, it was concluded that the biodegradation activity was highest in *Bacillus sp*. (F 231). After 45 days this bacterial strain degraded 95.4% of motor oil. Under the same conditions two other bacterial strains showed lower biodegradation activity: 84.9% by *Stenotrophomonas sp*. (NR 1), and 76.6% by *Rhodococcus sp*. (UG 10). Keywords: biodegradation, motor oil, bacterial stains References: [1] Bhat, M.M., Shankar, S., Shikha, Yunus, M., Shukla, R.N., *Adv. Appl. Sci. Res*. **2** (2011) 321-326. [2] Obayori, O.S., Salam, L.B., Ogunwumi, O.S., *J. Bioremed. Biodeg*. **5** (2014), 1-7. [3] Anastas P., Eghbali N. *Chem. Soc. Rev*., **39** (2010), 301-312.

## TH024
**Bacterial community structure and biogeochemical activity in an aquifer contaminated with pesticides**
a. Mauffret, BRGM / Environmental biogeochemistry and water quality; N. Baran, M. Charron, C. Joulian, BRGM
Our objective was to assess the effect of cocktails of pesticides on groundwater microbial abundance, community structure and their nitrate reducing activity. We used two complimentary approaches: a 2-year *in situ* monitoring at the Ariege alluvial plain (France) and microcosms with groundwater with contrasted contamination history spiked with selected herbicides having a high occurrence in this aquifer, atrazine (ATZ), desethylated atrazine (DEA) and ATZ+DEA. Abundance of the universal marker (16S rRNA) and of nitrate-reducing bacteria (*narG* and *napA*) was assessed by qPCR. Presence of the ammonium oxidizer was monitored by the *amoA* gene by PCR. Biodiversity was assessed using a fingerprinting technic (CE-SSCP). Pesticides in water were analyzed by LC-MS/MS. In microcosms, biodiversity was higher in historically contaminated water than in pristine-like one and remained higher under laboratory incubations. Biodiversity was significantly affected by both the chemical concentration and the incubation duration (not the chemical type) in the pritine-like water while in historically contaminated water, it was affected by the incubation duration only (not the chemical type or concentration), suggesting a community tolerance to the pesticides induced by chronic exposure. Biomass and denitrification gene abundance was in most cases higher at 10 $\mu$g/L than at 1 $\mu$g/L or in control, especially at 30-d incubation in both water types. During the two-year *in situ* monitoring, tendencies between chemical and microbial criteria were similar to the microcosm outcomes however they did not result in significant relationships. More specifically, it was not observed a significant relationship between nitrate concentration and microbial biomass and abundance of denitrifying genes (*narG* and *napA*). Microbial end points based on molecular indicators should be proposed to complete the biodiversity objective under the European water directive framework with the microbial compartment.

## TH025
**Analysis of the microbial community of the river Tiber in different contamination points along its course**
A. Barra Caracciolo, National Research Council of Italy / Water Research Institute; P. Grenni, National Research Council of Italy (CNR) / Water Research Institute; S.M. Ludovica, National Research Council / Water Research Institute; D. Martina, Natonal Research Council / Water Research Institute; N. Ademollo, National Research Council of Italy / Water Research Institute; L. Patrolecco, Water Research Institute Italian National Research Council; V. Ferrero, LGC; R. Loos, European Commission Joint Research Centre / Institute for Environment and Sustainability; S. Tavazzi, European Commission Joint Research Centre / Institute for Environment and Sustainability IES; D. Mastroianni, F. Falconi, National Research Council / National Water Research Institute; t. lettieri, European. Commission - Joint Research Centre / Institute for Environment and Sustainability IES
One of the major issues in freshwater quality assessment is finding a link between the chemical and ecological status of a water system. Filling this gap is a new challenge for finding new biological indicators, by integrating multiple stressors, to describe/assess water quality thoroughly. In this context, the main aim of the MicroCokit FP7-PEOPLE-2012-IAPP Project is to identify microbial community based indicators for monitoring and evaluating different types of anthropogenic and environmental pressures. The case study is the river Tiber in which four different sampling sites along its course have been chosen and sampled in Autumn and Spring for 2 consecutive years. Except the pristine river source, the other three selected sites were differently exposed to anthropogenic pressures: Agricultural, Industrial and Anthropogenic one, respectively. Chemical analysis (inorganic elements, DOC, PAHs, organochlorine, triazine, chloroacetamide pesticides, perfluorinated compounds, pharmaceuticals, etc.) have been performed, together with the phylogenetic analysis of the bacterial community by Fluorescence In Situ Hybridization (FISH). Overall results of four sampling campaigns show how the changes in the microbial community structure reflect both natural environmental variations such as river course and seasonality, and the different sources of contamination.

## TH026
**Microbial turnover of PAH: analysis of degradation and dissolution kinetics**

## and simulation of remediation options
A. Rein, Technische Universität München / Chair of Hydrogeology; I.K. Adam, A. Miltner, K. Brumme, M. Kästner, Helmholtz centre for environmental research - UFZ / Department of Environmental Biotechnology; S. Trapp, Technical University of Denmark / DTU Environment
Polycyclic aromatic hydrocarbons (PAH) are hydrophobic compounds exhibiting high toxicity and carcinogenicity. Originating from tar, coking and incomplete burning processes, PAH have contaminated many industrial areas and most urban soils. Feasible strategies for minimizing related adverse effects include the utilization of microbial degradation processes, where hardly soluble compounds like PAH pose a particular challenge. For the prospective assessment of the turnover of PAH there is a research gap since only very limited kinetic data for different groups of PAH degrader bacteria are available. In particular for Mycobacteria the knowledge is limited due to the complex cell cycle with formation of cell clusters and aggregates with PAH. From experiments and inverse modelling, growth and affinity parameters have been determined and compared for well described phenanthrene and pyrene degrading Mycobacteria on both substrates. The PAH were present as microcrystals in suspension. We refined a recently developed numerical model for desorption and metabolism, taking simultaneously into consideration chemical activity, sorption and dissolution processes, metabolism and growth as well as cell maintenance and decay in non-steady-state. This model was applied to prospectively describe PAH turnover dynamics for various treatment options to remediate contaminated soils. The investigated Mycobacterium species were not superior in PHE degradation to strains investigated earlier with this method. The amount of PAH ultimately degraded rather depended on the ad/desorption rates, and hereby on the substrate flux to the microbes, than on the Monod and Michaelis-Menten parameters of the strains. Predictive simulations revealed that bioaugmentation would only have a small and short-term effect on biodegradation. The addition of adsorbing soil amendments would shift the remaining PAH to the adsorbed/sequestered pool leading to a decrease in toxicity (lower mobility and bioavailability), but also to a lower diffusive flux to microbial cells and thus to declining activity of the PAH degrading microbes. Mobilization of PAH by adding solvents or surfactants would foster microbial activity, but might also increase toxicity. As the most promising strategy, stimulating cometabolism (e.g. by adding compost) could maintain microbial mass and activity at a high level leading to a steady decline of PAH in all pools. The model can thus be a valuable tool for assessing remediation options.

## TH027
**enviPath - The environmental contaminant biotransformation pathway resource**
J. Wicker, University of Mainz; M. Gutlein, Johannes Gutenberg University Mainz; E. Schmid, ETH Zurich; D. Latino, Eawag Swiss Federal Institute of Aquatic Science and Technology; S. Kramer, Johannes Gutenberg University Mainz; K. Fenner, ETH Zürich/Eawag
The University of Minnesota Biocatalysis/Biodegradation Database and Pathway Prediction System (UMBBD/PPS) have been a unique resource covering microbial biotransformation pathways of primarily xenobiotic chemicals for over 15 years. This poster will introduce the successor system, enviPath (The Environmental Contaminant Biotransformation Pathway Resource), which is a complete redesign and reimplementation of UM-BBD/ PPS. enviPath uses the database from the UM-BBD/ PPS as a basis, extends the use of this database, and allows users to include their own biotransformation pathway data to support multiple use cases. As one new use case, we will present the package "DAR soil", which contains pathway information from soil degradation studies. This information has been extracted from pesticide registration dossiers (draft assessment reports, DAR) made publically available through the European Food Safety Authority. The package also contains information on different experimental conditions, which are stored as "scenarios". Compounds in the pathway are associated with a given scenario if they have been observed under the specific experimental conditions. If available, a biotransformation half-life (DT50) value is associated with a given compound in the pathway and a specific scenario. Much like the UM-PPS system, enviPath further provides different models to predict likely biotransformation pathways based on biotransformation rules. It supports relative reasoning for the refinement of predictions and allows its extensions in terms of previously published, but not implemented machine learning models. User access is simplified by providing a REST API that simplifies the inclusion of enviPath into existing workflows. An RDF database is used to enable simple integration with other databases. enviPath is publicly available at http://envipath.org with free and open access to its core data.

## TH028
**Evaluating and validating a Quantitative Structure Biodegradation Relationship (QSBR) model with experimentally determined biodegradation rates**
k. acharya, Newcastle University / Environmental Engineering; M. Barycki, University of Gdansk / Department of Chemistry; J. Dolfing, P. Meynet, Newcastle University / Civil and Environmental Engineering; W. Mrozik, Newcastle University / School of Civil Engieenering Geoscience; D. Werner, Newcastle University / Civil Eng and Geosciences; T. Puzyn, University of

# Investigation of motor oil biodegradation by different bacterial strains

Ilic M.[1], Bulatovic S.[2], Ljesevic M.[2], Solevic-Knudsen T.[1], Milic J.[1], Avdalovic J.[1], Miletic S.[1], Gojgic-Cvijovic G.[1], Vrvic M.[2]

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## Introduction & Objectives:

Large amount of motor oil is used in many branches of industry. The new generation of motor oils contains high percentages hydrocarbons (C16-C36), more than 75% cycloalkane and polycyclic aromatic hydrocarbons (PAH). However, used motor oils contain a higher percentage of aliphatic and aromatic hydrocarbons, nitrogen and sulfur compounds, and metals (Mg, Ca, Zn, Pb, etc.). In addition to these compounds, the presence of PAHs (naphthalene, benzo [a] pyrene and anthracene) is also expected. Therefore these oils represent a great danger to the environment, because all these compounds are known as mutagenic and carcinogenic [1, 2].
Globally, biodegradation is a common choice for remediation, because the pollutants become substrates for the growth of microorganisms [3].
The objective of this experiment was to investigate the biodegradation of motor oil by bacteria *Stenotrophomonas* sp. (NR 1), *Rhodococcus* sp. (UG 10) and *Bacillus* sp. (F 231) isolated from activated sludge of industrial wastewater treatment plant.

## Materials & Methods:

The process of biodegradation of motor oil was monitored for 45 days. Each 15 days the process was stopped, the hydrocarbons were extracted, and the samples were analyzed by gas chromatography Samples were analyzed on an Agilent 7890A gas chromatograph with a flame ionization detector (FID), equipped with a chromatographic column HP-5, length 30m and diameter 0,32mm, the thickness of the stationary phase 0,25μm. The carrier gas is hydrogen with a flow rate of 2 mL / min, injector temperature is 250 °C and the detector temperature is 320 °C. Temperature program: initial temperature of 40 °C during 1min., then heated to 100 °C at a rate of 15 °C / min., and then to a temperature of 310 ° C is 10 ° C / min and finally isothermal 310 °C for 15 minutes. The software that was used for data processing is ChemStation, Agilent Technologies.
Comparison of motor oil concentration in the samples and in the abiotic controls provided insight into the biodegradation activity of bacteria. The results showed a reduction in concentration of motor oil comparing to the beginning of the experiment (300 ppm). The growth of bacteria confirmed that the motor oil was the only source of carbon. The reduction of motor oil concentration was correlated with the number of bacterial cells.

## Results & Discussion:

Comparison of motor oil concentration in the samples and in the abiotic controls provided insight into the biodegradation activity of bacteria. The results showed a reduction in concentration of motor oil comparing to the beginning of the experiment (300 ppm) shown in Table 1. The growth of bacteria confirmed that the motor oil was the only source of carbon. The reduction of motor oil concentration was correlated with the number of bacterial cells (Table 2).

Table 1. Percentage of degraded motor oil.

| Microorganism | *Stenotrophomonas* sp. (NR 1) | | *Rhodococcus* sp. (UG 10) | | *Bacillus* sp. (F 231) | |
|---|---|---|---|---|---|---|
| Time / Effectiveness | remaining (mg)[a] | % biodegradation[b] | remaining (mg)[a] | % biodegradation[b] | remaining (mg)[a] | % biodegradation[b] |
| 15. day | 12,3 | 62,7 | 16,1 | 51,2 | 22,7 | 31,2 |
| 30. day | 9,6 | 70,9 | 15,1 | 70,9 | 21,0 | 36,3 |
| 45. day | 7,8 | 76,6 | 9,6 | 84,9 | 1,5 | 95,4 |

a* the values obtained by measuring the mass of the extracted remaining engine oil; b* % biodegradation is calculated to the initial amount of engine oil.

Table 2. Number of bacteria cells during the experiment .

| N° of bacteria cells | 0. day | 15. day | 30. day | 45. day |
|---|---|---|---|---|
| *Stenotrophomonas* sp. (NR 1) | 2x10^{13} | 1x10^{8} | 5x10^{8} | 2x10^{8} |
| *Rhodococcus* sp. (UG 10) | 1,4x10^{13} | 1x10^{8} | 3x10^{7} | 1x10^{7} |
| *Bacillus* sp. (F 231) | 7x10^{10} | 2x10^{8} | 8x10^{7} | 1x10^{8} |

## Conclusion:

Based on the results, it was concluded that the biodegradation activity was highest in *Bacillus* sp. (F 231). After 45 days this bacterial strain degraded 95.4% of motor oil (Fig. 1). Under the same conditions two other bacterial strains showed lower biodegradation activity: 84.9% by *Stenotrophomonas* sp. (NR 1) (Fig. 2), and 76.6% by *Rhodococcus* sp. (UG 10) (Fig. 3).
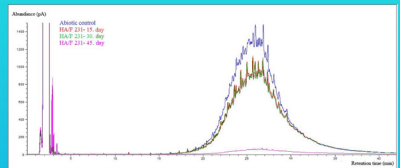


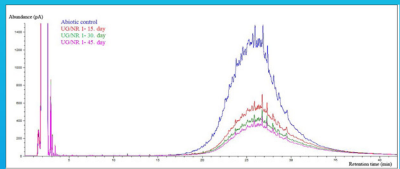Fig 1. Biodegradation of motor oil by *Bacillus* sp. (F 231) after 15, 30 and 45 days.



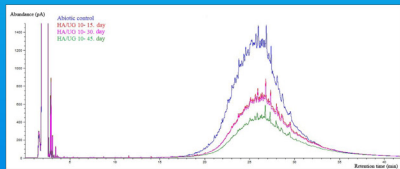Fig 2. Biodegradation of motor oil by *Stenotrophomonas* sp. (NR 1) after 15, 30 and 45 days.



Fig 3. Biodegradation of motor oil by *Rhodococcus* sp. (UG 10) after 15, 30 and 45 days.

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