

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,400

Open access books available

118,000

International authors and editors

130M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Petroleum Hydrocarbon Biodegradability in Soil – Implications for Bioremediation

Snežana Maletić, Božo Dalmacija and Srđan Rončević

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50108>

1. Introduction

The development of human civilization throughout history has led to growing disruption of the natural balance and the occurrence of different types of pollution. The world depends on oil, and the use of oil as fuel has led to intensive economic development worldwide. The great need for this energy source has led to the gradual exhaustion of natural oil reserves. However, mankind will witness the results of oil consumption for centuries after its cessation. Environmental pollution with petroleum and petrochemical products has been recognized as a significant and serious problem (Alexander, 1995, 2000). Most components of oil are toxic to humans and wildlife in general, as it is easy to incorporate into the food chain. This fact has increased scientific interest in examining the distribution, fate and behaviour of oil and its derivatives in the environment (Alexander, 1995, 2000; Semple et al., 2001, 2003; Stroud et al., 2007, 2009). Oil spills in the environment cause long-term damage to aquatic and soil ecosystems, human health and natural resources.

Petroleum oil spills tend to be associated with offshore oil rigs and tankers in marine-related accidents. In contrast, land oil spills often go unnoticed by everyone except environmentalists, yet land oil spills contribute to the pollution of our water supply and soil. Typical sources of land oil spills include accidents as well as oil from vehicles on the road.

Characterization of spilled oil and its derivatives is very important in order to predict the behaviour of oil and its long-term effects on the environment, and in order to select the proper cleaning methods. The potential danger which petroleum hydrocarbons pose to humans and the environment makes testing and characterization of the biodegradation and biotransformation processes of hydrocarbons in contaminated soil necessary in order to develop bioremediation techniques for cleaning such soils to levels that ensures its safe disposal or reuse. Biodegradation is the metabolic ability of microorganisms to transform or mineralize organic contaminants into less harmful, non-hazardous substances, which are

then integrated into natural biogeochemical cycles. Petroleum hydrocarbon biodegradability in soil is influenced by complex arrays of factors, such as nutrients, oxygen, pH value, composition, concentration and bioavailability of the contaminants, and the soil's chemical and physical characteristics.

Bioremediation is considered a non-destructive, cost-effective, and sometimes logistically favourable cleanup technology, which attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions. In order to choose the appropriate bioremediation strategy it is extremely important to investigate and understand all factors which affect biodegradation efficiency. In order to better explain those factors, 4 examples of bioremediation studies (conducted 1 year, 5 years and 8 years after contamination) on soil which was directly contaminated with various petroleum products and their combustion products, are described along with their similarities and differences.

2. Bioremediation

Bioremediation can be briefly defined as the use of biological agents, such as bacteria, fungi, or green plants (phytoremediation), to remove or neutralize hazardous substances in polluted soil or water. Bacteria and fungi generally work by breaking down contaminants such as petroleum into less harmful substances. Plants can be used to aerate polluted soil and stimulate microbial action. They can also absorb contaminants such as salts and metals into their tissues, which are then harvested and disposed of. Bioremediation is a complex process, with biological degradation taking place in the cells of microorganisms which absorb pollutants, where if they have specific enzymes, the degradation of pollutants and their corresponding metabolites will take place. Hydrocarbons from oil are used as a source of nutrients and energy for microorganism growth, and at the same time, microorganisms decompose them to naphthenic acids, alcohols, phenols, hydroperoxides, carbonyl compounds, esters, and eventually to carbon dioxide and water (Eglinnton, 1975; Marković et al., 1996).

Bioremediation is considered a non-destructive, cost- and treatment-effective and sometimes logistically favourable cleanup technology, which attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site (Alexander, 1995). It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate (Maletić et al., 2009; Rončević et al., 2005).

Bioremediation can be divided into two basic types: (1) natural attenuation, which can be applied when the natural conditions are suitable for the performance of bioremediation without human intervention, and (2) engineered bioremediation, which is used when is

necessary to add substances that stimulate microorganisms. The first one is more attractive because of its low cost, minimum of maintenance and minimal environmental impact. Still, this technology is applicable only in cases when the natural level of biodegradation is higher than the degree of pollution migration. Nevertheless, this technology is more often used as a supplement to the other technologies, or after finished engineered bioremediation in order to prevent migration of pollution from the treated area. Engineered bioremediation is faster than natural attenuation because it includes microbial degradation stimulation, by controlling the concentrations of pollution, oxygen, nutrients, moisture, pH, temperature, etc. (Rahman et al., 2003; Yerushalmi et al., 2003). Engineered bioremediation is applied when it is essential to carry out cleaning in a short time or when the pollution is very rapidly expanding. Its application reduces the costs due to the shorter treatment of land and lower number of sampling and analysis, and it is important for political and psychological needs when the community is exposed to pollution. Engineered bioremediation can be divided in two main groups (1) in situ and (2) ex situ bioremediation techniques, with the most applicable of these and their main characteristics given in Tables 1 and 2. In situ techniques are generally the most desirable options due to lower cost and fewer disturbances since they provide treatment in place, avoiding excavation and transport of contaminants (Vidali, 2001). In situ techniques are limited by the depth of the soil that can be effectively treated. In contrast, ex situ techniques involve the excavation or removal of contaminated soil from the ground.

3. Hydrocarbon biodegradation mechanisms and products

Biodegradation is the process by which microorganisms transform or mineralize organic contaminants, through metabolic or enzymatic processes, into less harmful, non-hazardous substances, which are then integrated into natural biogeochemical cycles. Organic material can be degraded by two biodegradation mechanisms: (1) aerobically, with oxygen, or (2) anaerobically, without oxygen.

Anaerobic processes are conducted by anaerobic microorganisms and this pathway of biodegradation is very slow. Originally thought to contribute marginally to overall biodegradation, anaerobic biodegradation mechanisms have been gaining more attention in recent years due to increased information regarding contaminant site conditions and rapid oxygen depletion (Burland & Edwards, 1999). Anaerobic biodegradation follows different biochemical pathways dependent on the electron acceptor utilized by the microorganism. Petroleum-based contaminants have been shown to degrade under various anaerobic conditions, including nitrate reduction, sulphate reduction, ferric iron reduction, manganese reduction and methanogenic conditions. The metabolic pathways behind anaerobic alkane biodegradation are not well understood. Most of the reports related to the anaerobic mineralization of aliphatic hydrocarbons are studies with pure cultures or enrichment cultures in laboratory scale experiments. Hence, the significance of these results in the environment e.g. in contaminated soils and sediments, is not yet known and evidence for the anaerobic degradation of alkanes in environmental samples has been reported in only a few cases (Salminen, 2004).

The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. The initial intracellular attack of organic pollutants is an oxidative process, and the activation and the incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases. Degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism, for example, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate. Sugars required for various biosyntheses and growth are synthesized by gluconeogenesis. The degradation of petroleum hydrocarbons can be mediated by specific enzyme systems. Other mechanisms involved are (1) attachment of microbial cells to the substrates and (2) production of biosurfactants. The uptake mechanism linked to the attachment of cell to oil droplet is still unknown but the production of biosurfactants has been well studied (Nilanjana & Chandran, 2011).

Technique / Definition	Advantages	Disadvantages	Applicability
Biosparging - Involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria (Baker & Moor, 2000; Khan et al., 2004).	Equipment is readily available and easy to install, little disturbance to site operations, treatment times from 6 months to 2 years, low injection rates reduce the need for vapour capture and treatment.	Can only be used in areas where air sparging is suitable, complex chemical, physical and biological processes are not well understood potential for the migration of contaminants.	Most types of petroleum contaminated sites, but it is least effective on heavy petroleum because of the length of time required.
Bioventing - injection of air into the contaminated media at a rate designed to maximize in situ biodegradation and minimize or eliminate the off-gassing of volatilized contaminants to the atmosphere (Khan et al., 2004).	Equipment is readily available and easy to install, short treatment times, from 6 months to 2 years. easy to combine with other technologies, may not require off-gas treatment.	High concentrations of contaminants may be toxic to organisms. cannot always reach low cleanup limits. is effective only in unsaturated soils; other methods are needed for the saturated zone.	Mid-weight petroleum products like diesel.
Phytoremediation - application of green plants to remove pollutants and other harmful components from the environment (Joner et al., 2006).	Cost-effective for large areas, no impact on the environment, formation of secondary waste is minimal, post-treatment soil can remain in the treated area and can be used in agriculture, uses solar energy no formation of toxic compounds.	Longer period required than one growing season, climate and hydro-logical conditions such may limit plant growth and the plant species that can be used, pollutants can enter the food chain, requires special disposal of plants.	Heavy metals, radionuclides, chlorinated solvents, petroleum hydrocarbons, insecticides, explosives and surfactants.

Table 1. The most applicable *in situ* techniques and their main characteristics

Technique / Definition	Advantages	Disadvantages	Applicability
Landfarming - spreading of contaminated soils in a thin layer on the ground surface of a treatment site and stimulating aerobic microbial activity within the soils through aeration and addition of nutrients, minerals, and water (Hejazi et al., 2003; Khan et al., 2004).	The most cost effective, takes less time and money to remediate, leads to complete destruction of pollutants, suitable for treating large volumes of contaminated soil.	Large amount of land required, VOC must be pre-treated not efficient for the heavy components of petroleum, possibility of contamination migration into the environment, difficult to expect a reduction in the concentration of pollutants greater than 95%.	Volatile organic compounds, gasoline, heating and lubricating oil, diesel oil, PAH etc.
Biopile A hybrid of landfarming and composting - engineered cells are constructed as aerated composted piles (Jorgensen et al., 2000).	May be constructed to suit a variety of terrain conditions, the treatment time - 6 months to 2 years, advantages over landfarming: takes up less space, possibility of aeration, VOC control is possible.	Not efficient for the heavy components of petroleum, possibility of contamination migration into the environment, difficult to expect a pollutants concentration reduction > 95%.	Petroleum products, non-halogenated and halogenated VOC and SVOC, PAH.
Composting - combining contaminated soil with non-hazardous organic materials which support the development of a rich microbial population and elevated temperature for composting (Semple et al., 2001).	Cost effective, takes less time and money to remediate leads to complete destruction of pollutants, suitable for treating large volumes of contaminated soil.	VOC must be pre-treated possibility of contamination migration into the environment, composting/compost processes to "lock up" pollutants, the long-term stability of such "stabilized" matrices is uncertain.	Petroleum products, non-halogenated and halogenated VOC and SVOC, PAH, PCB and explosives, pesticides.
Bioslurry systems - the soil is treated in a controlled bioreactor where the slurry is mixed to keep the solids suspended and microorganisms in contact with the contaminants (Nano & Rota, 2003).	Control of temperature, moisture, pH, oxygen, nutrients, VOC emission, addition of surfactants, addition of microorganisms, monitoring of reaction conditions.	Non-homogeneous and clayey soils can handling problems, free product removal is necessary, expensive soil dewatering after treatment, disposal method is needed for wastewater, extensive site and contaminant investigation.	Petroleum products, non-halogenated and halogenated VOC and SVOC, PAH, PCB and explosives.

Table 2. The most applicable *ex situ* techniques and their main characteristics

4. Bioremediation process kinetics

Bioremediation processes are time consuming and as a consequence, many studies have addressed the determination of bioremediation process kinetics. The kinetics for modelling the bioremediation of contaminated soils can be extremely complicated. This is largely due

to the fact that the primary function of microbial metabolism is not for the remediation of environmental contaminants. Instead the primary metabolic function, whether bacterial or fungal in nature, is to grow and sustain more of the microorganism. Because of the involvement of adverse factors and the complexity of the process, it is not possible to predict the duration of bioremediation. Therefore, the formulation of a kinetic model must start with the active biomass and factors, such as supplemental nutrients and oxygen source that are necessary for subsequent biomass growth (Maletić et al., 2009; Rončević et al., 2005).

Studies of the kinetics of the bioremediation process proceed in two directions: (1) the first is concerned with factors influencing the amount of transformed compounds with time, and (2) the other approach seeks the types of curves describing the transformation and determines which of them fits the degradation of the given compounds by the microbiologic culture in the laboratory microcosm and sometimes, in the field.

Determinations based on the literature data for values of the degradation degree are useful but less exact, because they do not take into account all the specific characteristics of the soil such as temperature, moisture, and—most often—the adaptation of bacteria to the specific contaminants. A literature survey has shown that studies of biodegradation kinetics in the natural environment are often empiric, reflecting only a basic level of knowledge about the microbiologic population and its activity in a given environment. One such example of the empirical approach is the simple model:

$$\frac{dC}{dt} = kC^n \quad (1)$$

where C is the concentration of the substrate, t is time, and k is the degradation rate constant of the compound and n is a fitting parameter (most often taken to be unity) (Wethasinghe et al., 2006). Using this model, one can fit the curve of substrate removal by varying n and k until a satisfactory fit is obtained. It is evident from this equation that the rate is proportional to the exponent of substrate concentration. Researchers involved in kinetic studies do not always report whether the model they used was based on theory or experience and whether the constants in the equation have a physical meaning or if they just serve as fitting parameters (Maletić et al., 2009; Rončević et al., 2005).

With the complex array of factors that influence the biodegradation of hydrocarbons noted previously, it is not realistic to expect a simple kinetic model to provide precise and accurate descriptions of concentrations during different seasons and in different environments. The results of short-term degradation experiments are sometimes presented with the implicit assumption of zero-order kinetics (i.e., degradation in mass per unit time or in turnover time). However, short-term degradation experiments may not be adequate to discern the appropriate kinetics. In experiments with a number of samples taken during a length of time sufficient for considerable biodegradation to take place, the concentration of hydrocarbons with time is better described by first-order kinetics, eq. 2 (Collina et al., 2005; Grossi et al., 2002; Hohener et al., 2003; Pala et al., 2006; Rončević et al., 2005).

First order kinetics, such as the well known Michaelis-Menton kinetic model, is the most often used equation for the representation of degradation kinetics (Collina et al., 2005; Grossi

et al., 2002; Hohener et al., 2003; Pala et al., 2006; Pollard et al., 2008). First order kinetics enables the prediction of hydrocarbon concentrations at any time from biodegradation half-times. If the optimal conditions are established, remediation time depends on the biodegradation half-time, initial hydrocarbon concentration in the polluted soil, and the end point concentration which needs to be achieved. Many researchers assume first order kinetics because of the easier presentation and data analysis, simplicity of graphical presentation, and the easier prediction of concentration once half-life has been determined [26, 28]. This approach is least reliable at very high and very low levels of contaminants. Taking the same initial values of concentration, different kinetic models will give significantly different final amounts of unreacted compound (Maletić et al., 2009; Rončević, 2002; Rončević et al., 2005):

$$C = C_0 e^{-kt} \quad (\text{or } \ln C = \ln C_0 - kt) \quad (2)$$

where C - concentration of hydrocarbons (g kg^{-1}), t - time of removal (day), C_0 - initial concentration of hydrocarbons (g kg^{-1}), and k - rate constant of the change in the hydrocarbon content of the soil (day^{-1}).

In the simple model, depending on the nature of the substrate and experimental conditions, various investigators obtain different values for the rate constant of substrate degradation: for n-alkanes, 0.14 to 0.61 day^{-1} ; for crude oil, 0.0051 to 0.0074 day^{-1} ; and for PAHs, 0.01 to 0.14 day^{-1} (Rončević et al., 2005). Reported rates for the degradation of hydrocarbon compounds under field or field-simulated conditions differ by up to two orders of magnitude. The selection of appropriate kinetics and rate constants is essential for accurate predictions or reconstructions of the concentrations of hydrocarbons with time in soil after a spill.

A more reliable prediction of pollution biodegradation can be obtained from more complex models such as the BIOPLUME II model (BIOPLUME is a two-dimensional computer model that simulates the transport of dissolved hydrocarbons under the influence of oxygen-limited biodegradation). Additionally, in recent years, the state of the art in modelling technology allows for even more reliable prediction using the 3D software MODFLOW, which is available in several versions: MODFLOW, MODPATH, MT3D, RT3D and MODFLOW-SURFACT).

For the ex-situ treatment of soil, remediation time generally does not depend on the transport of nutrients and oxygen and can be roughly determined from the degree of degradation, determined in laboratory tests of samples taken from the field. The following factors often interfere with a simple extrapolation of the kinetics described above in natural conditions:

1. Different barriers may limit or prevent contact between microbial cells and their organic substrates. Many organic molecules sorb to clay or soil humus or sediment, and the kinetics of the decomposition of sorbed substrate can be completely different from that of the same compound free in solution.
2. The presence of other organic molecules, which can be metabolized by biodegrading species can reduce or increase the consumption of the examined compounds.
3. Application of inorganic nutrients, oxygen, or growth factors, can affect the speed of transformation and then the process will be governed by diffusion of nutrients or the speed of their formation or regeneration of the other residents of the community.

4. Many species can metabolize the same organic compounds simultaneously.
5. Protozoa or possible species that parasitize on the biodegrading population can manage growth, population size or activity responsible for biodegradation.
6. Many synthetic chemicals have insufficient solubility in water, and the kinetics of their transformation can be completely different from compounds in the aqueous phase.
7. Cells of the active population may be sorbed or can develop microcolonies, and kinetics of sorbed or microcolonies is still unresolved.
8. Many organic compounds disappear only after a period of acclimatization, and there is no method that can predict the length of this period or the expected percentage of time between the occurrence of compounds and their total destruction.

4.1. Bioremediation study – Our experiences

In order to close this issue for readers, experience from four different bioremediation treatments of petroleum contaminated soil are given as examples (Fig. 1, Fig. 2). Thus, as a consequence of the accidental oil spill in the Novi Sad Oil Refinery (Serbia) in 1999, soil was directly contaminated with various petroleum products (gasoline, crude oil, kerosene, diesel fuel, black oil, etc.) and products of their combustion from frequent fires. Bioremediation studies on this soil were conducted 1 year after contamination (Rončević, 2002; Rončević et al., 2005), after 5 years (Rončević, 2007) and after 8 years (Maletić et al., 2009; Maletić, 2010; Maletić et al., 2011), and the bioremediation kinetics which were determined are compared here and discussed.

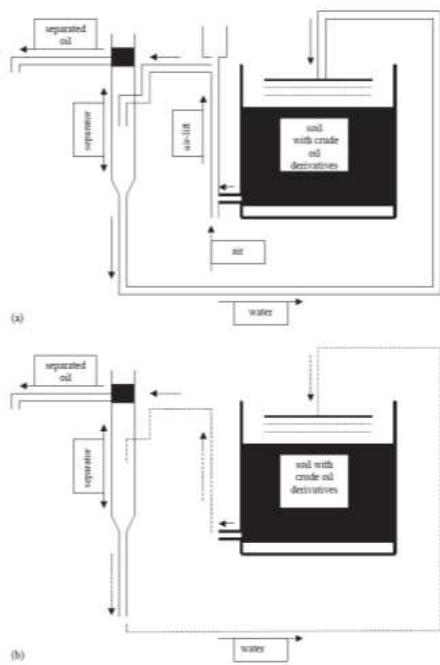
The obtained data from these four studies show changes and differences in the bioremediation kinetic rate, depending on the applied technology and stage of weathering (Fig 3.).

In study 1, bioremediation was carried out on a relatively freshly petroleum contaminated soil (one year after contamination), with a start concentration three times greater than in the other case studies, and the % of removed hydrocarbon is the highest. A slight difference was noticed between the two approaches applied (reactor with continuous and discontinuous flow). Namely, in the reactor with discontinuous flow, the hydrocarbon biodegradation rate in the aerobic part of the reactor was lower, indicating that the aerobic bioremediation conditions are favourable for this type of oil contaminated soil. Generally, satisfactory hydrocarbon degradation and removal rates were established by this technology.

As explained above, in study 2, the initial hydrocarbons concentration is three times lower, due to the different environmental conditions to which this soil was exposed during 5 years of weathering. Three varieties of in situ bioremediation technology were applied (Fig. 1). The first two used in-situ biostimulation feeding with aerated water and magnesium peroxide, and did not provide satisfactory results. The biodegradation kinetic rate constant could not be calculated, since no removal of hydrocarbons was observed during the bioremediation.

With the third variation, which used in situ biostimulation with ex situ biologically treated groundwater, the situation was changed drastically. Hydrocarbon content decreased rapidly, by about 60% in 232 days. Even so, the biodegradation kinetic rate constant is twice as low as the rate constant in study 1. This is probably because the degradation of the easily removable hydrocarbon fraction from the soil already occurred during the weathering process. Thus, only the heavier and less degradable fractions remained in the soil.

Bioremediation study 1 – Laboratory trial bioremediation



- Two samples of 170 and 180 kg were introduced into separate reactors which were filled to a height of 20 cm with water sampled from the piezometers from the refinery area. Experiment duration was 325 days.
- Each reactor was reinoculated daily by replacing 250 ml of the water phase with 250 ml of a suspension of adapted bacteria.
- One reactor (a) had continuous circulation of the water phase, with the aid of an air lift, at a flow rate of approximately 7 l day⁻¹.
- In the second (b), circulation of the water phase was carried out over a short period once a day to give a flow rate of 0.5 l day⁻¹.
- After percolating through the soil, the water phase was passed through a separator in which water insoluble components (free crude oil plus oil derivatives) were separated out by gravity. The separated-oily layer was removed periodically and fed into a third bioreactor that was used to prepare the adapted microbial suspension.

Bioremediation study 2 – Simulation of in situ bioremediation in a laboratory bioreactor

- Cylinder reactor, length 3.2 m and 0.8 m in diameter, with 4 piezometers placed in the soil.
- A layer of sand 10-15 cm thick was first placed in the reactor, then a layer of soil polluted with oil derivatives (thickness of 45-50 cm, 1150 kg of soil). 1 m³ of groundwater from the site was added.
- 3 versions of the technical bioremediation were performed:
 - I - in-situ biostimulation feeding with aerated water - 2.7 dm³ water was discharged into the aerator, where it was saturated with the maximum amount of oxygen, and poured over the surface of the soil at the beginning of the reactor. 306 days, changeable water flow 1.8-22 x10⁻⁷ m/s.
 - II - in-situ biostimulation with magnesium peroxide, the fourth piezometer was filled with magnesium peroxide, whose decomposition provides oxygen in the soil layer. 147 days, water flow 22 x10⁻⁷ m/s
 - III - in-situ biostimulation with ex situ biologically treated groundwater - water from the reactor was drained to a system consisting of three separators of the oil-free phase, a bioreactor, settler and sludge conditioner, and then recirculated over the surface of the soil at the beginning of the reactor. 232 days, changeable water flows 2.5-16 x10⁻⁷ m/s.

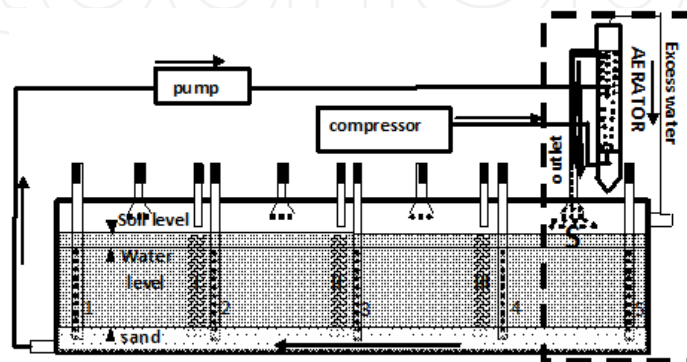
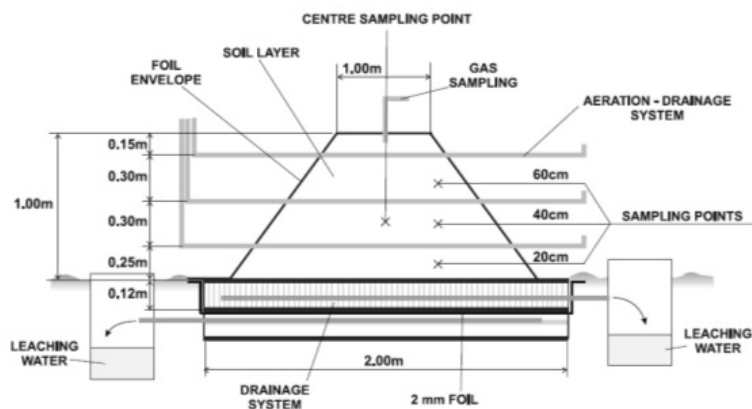


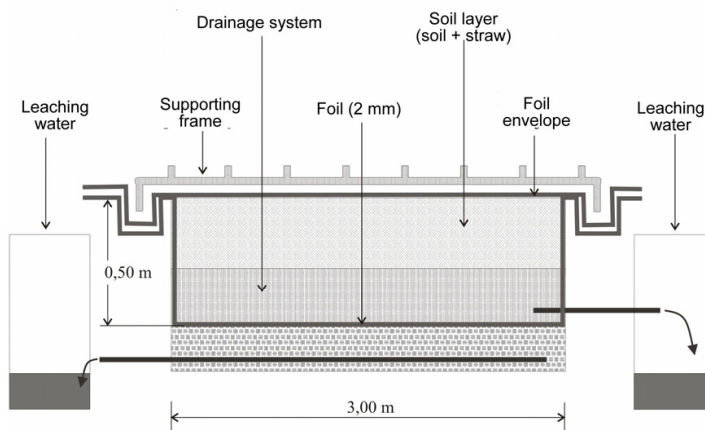
Figure 1. Experimental conditions for Bioremediation studies 1 and 2

Bioremediation study 3 – Biopile bioremediation



- The contaminated soil (2.7 m^3) was placed in a 2.2 m prismatic hole dug to a depth of 0.4 m , and covered with resistant polypropylene foil to prevent contamination spreading from the biopile.
- The layer of contaminated soil above the drainage system was in the form of a 1 m tall truncated pyramid composted with straw, and had a total volume of 2.7 m^3 .
- To facilitate oxygen and water transport through the soil, the contaminated soil was composted with straw.
- At three different heights on the pyramid structure, perforated PVC aeration tubes were placed.
- To accelerate microbiological activity air was additionally piped through the biopile once a week.
- As well as stimulation of native microflora by soil aeration and irrigation, bioaugmentation was also carried out with microorganisms separated from the contaminated soil and cultivated in a laboratory bioreactor.
- The biopile was watered twice a week, and moisture was maintained at approximately 50-80% water holding capacity during the experiment. Leaching water from the biopile was collected in a separate reservoir and used for watering the biopile. Experiment duration 710 days.

Bioremediation study 4 – Landfarming bioremediation



- The contaminated soil (2.7 m^3) was placed in a $3 \times 3 \text{ m}$ wide and 0.4 m deep prismatic hole, and covered with resistant polypropylene foil to prevent contamination spreading from the landfarm. Experiment duration 710 days.
- To facilitate oxygen and water transport through the soil, the soil was composted with straw.
- The landfarm was turned twice a month and watered twice a week; moisture was maintained at approximately 50-80% water holding capacity during the experiment.
- In addition to the stimulation of native microflora by soil aeration and irrigation, bioaugmentation was also carried out with microorganisms separated from the contaminated soil and cultivated in a laboratory bioreactor.
- Approximately 25 dm^3 of the inoculated water from the bioreactor was used together with leaching water for weathering the landfarm.

Figure 2. Experimental conditions for Bioremediation studies 3 and 4

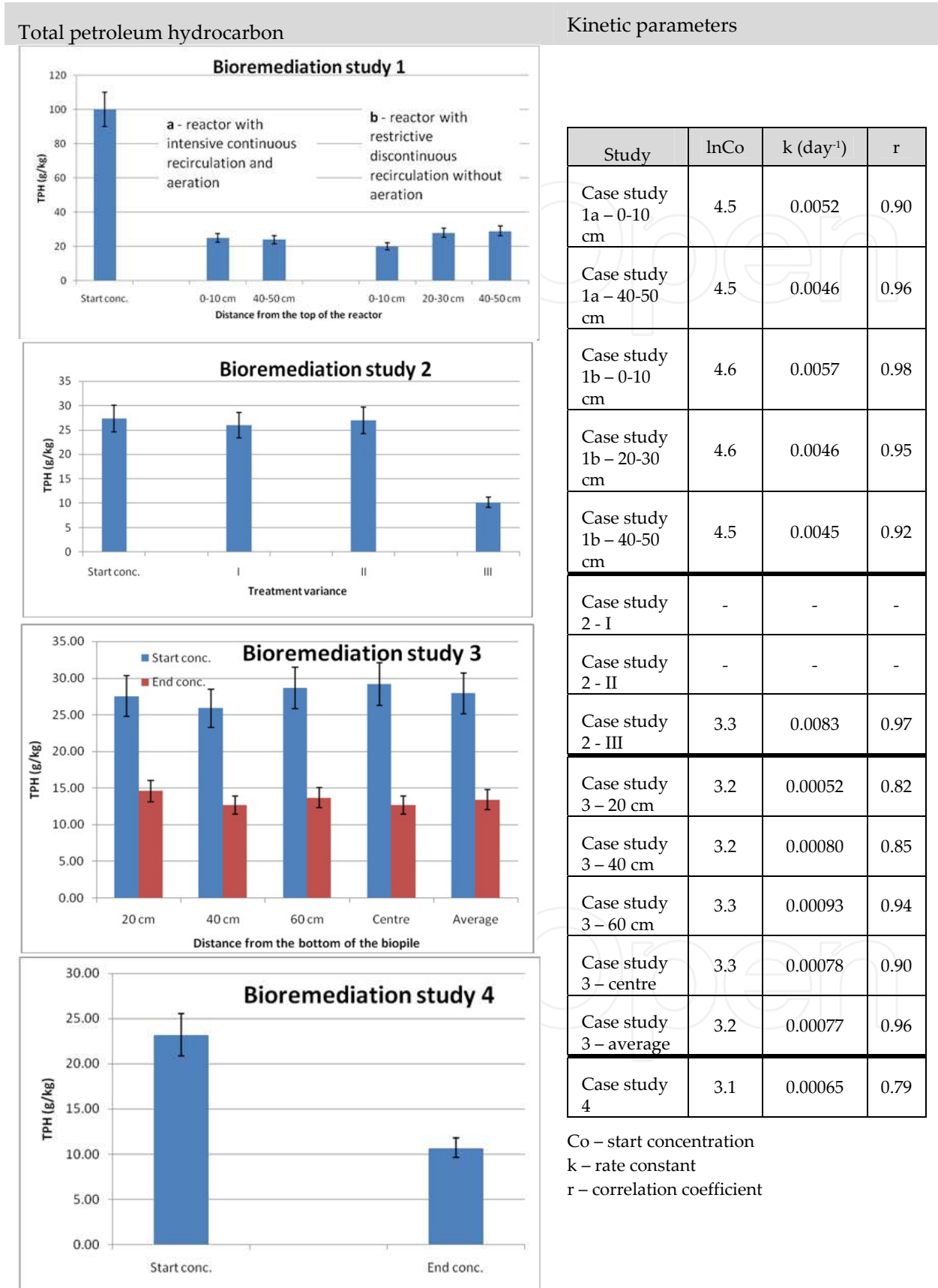


Figure 3. Experimental results from the bioremediation studies 1-4

Co – start concentration
 k – rate constant
 r – correlation coefficient

Studies 3 and 4 had similar hydrocarbon concentrations at the beginning of the experiment as study 2; even so, the biodegradation constant rate for both case studies is one order of magnitude lower than in study 2. The reason for this could be hydrocarbon complexation with the soil organic material and also its sorption and sequestration in the soil nanopores with further weathering of the oil contaminated soil (8 years). In this manner the hydrocarbons become recalcitrant and resistant to biodegradation. In study 3 (biopile) the hydrocarbon biodegradation removals were also monitored at different heights in the biopile.

Similarly to study 1, the lowest biodegradation rate constant was obtained for the lowest layer of the biopile, where the oxygen concentration is limited and anaerobic conditions developed. This confirms the facts from study 1 that aerobic degradation of hydrocarbons is the favourable degradation pathway. It is worth mentioning that in general, greater rate constants were obtained in the biopile than in the landfarming, indicating that the biopile is a better technology choice for bioremediation of this type of soil contamination.

5. Factors affecting oil hydrocarbon biodegradation processes

Successful implementation of bioremediation technologies on contaminated areas depends on the characteristics of the contaminated site and a complex system of many factors that affect the petroleum hydrocarbons biodegradation processes (Jain et al., 2011). The main factors which limit the overall biodegradation rate can be grouped as: soil characteristics, contaminant characteristics, bioavailability, microorganisms number and catabolism evolution (Alexander, 1995). In order to adopt and implement some bioremediation strategy it is extremely important to consider and understand those limiting factors.

5.1. Soil characteristics

Soil characteristics are especially important for successful hydrocarbon biodegradation, some of the main limiting factors are: soil texture, permeability, pH, water holding capacity, soil temperature, nutrient content and oxygen content. Soil texture affects permeability, water content and the bulk density of soil. Soil with low permeability (such as clays) hinders transportation and the distribution of water, nutrients and oxygen. To enable the bioremediation of such soil, it should be mixed with amendments or bulking materials (straw, sawdust etc.), as the bioremediation processes rely on microbial activity, and microorganisms require oxygen inorganic nutrients, water and optimal temperature and pH to support cell growth and sustain biodegradation (Alexander, 1995; Jain et al., 2011). The optimal conditions for microbial growth and hydrocarbon biodegradation are given in table 3.

Parameter	Microbial growth	HC biodegradation
Water holding capacity	25 -28	40-80
pH	5.5-8.8	6.5-8.0
Temperature (°C)	10-45	20-30
Oxygen (air-filled pore space)	10%	10-40%
C:N:P	100:10:1(0.5)	100:10:1(0.5)
Contaminants	Not too toxic	HC 5-10% of dry weight of soil
Heavy metals	<2000 ppm	<700 ppm

Table 3. Optimal conditions for microbial growth and hydrocarbon biodegradation

5.2. Contaminant characteristics

Petroleum hydrocarbons contain a complex mixture of compounds; all the components of petroleum do not degrade at the same rate. The rate by which microorganisms degrade hydrocarbons depends upon their chemical structure and concentration. Petroleum hydrocarbons can be categorized into four fractions: saturates, aromatics, resins and asphaltene. Of the various petroleum fractions, n-alkanes of intermediate length (C₁₀-C₂₅) are the preferred substrates for microorganisms and tend to be the most readily degradable, whereas shorter chain compounds are rather more toxic. Longer chain alkanes (C₂₅-C₄₀) are hydrophobic solids and consequently are difficult to degrade due to their poor water solubility and bioavailability, and branched chain alkanes and cycloalkanes are also degraded more slowly than the corresponding normal alkanes. Highly condensed aromatic and cycloparaffinic structures, tars, bitumen and asphaltic materials, have the highest boiling points and exhibit the greatest resistance to biodegradation. It has been suggested that the residual material from oil degradation is analogous to, and can even be regarded as, humic material (Balba et al., 1998; Loehner et al., 2001; Ivančev-Tumbas et al., 2004; Brassington et al., 2007; Stroud et al., 2007).

5.3. Bioavailability

Even if the optimal conditions for hydrocarbon biodegradation are provided at the field, it has been shown that a residual fraction of hydrocarbon remains undegraded. Namely, after its arrival in the soil, an organic contaminant may be lost by biodegradation, leaching or volatilization, or it may accumulate within the soil biota or be sequestered and complex within the soil's mineral and organic matter fractions. The rate at which hydrocarbon-degrading microorganisms can convert chemicals depends on the rate of transfer to the cell and the rate of uptake and metabolism by the microorganisms. It is controlled by a number of physical-chemical processes such as sorption/desorption, diffusion, and dissolution. (Brassington et al., 2007; Cuypers et al., 2002; Maletić et al., 2011; Semple et al., 2003). The mass transfer of a contaminant determines microbial bioavailability. The term "bioavailability" refers to the fraction of chemicals in soil that can be utilized or transformed by living organisms. The bioavailability of a compound is defined as the ratio of mass transfer and soil biota intrinsic activities. Most soil contaminants show biphasic behaviour, whereby in the initial phase of hydrocarbon biodegradation, the rate of removal is high and removal is primarily limited by microbial degradation kinetics. In the second phase, the rate of hydrocarbon removal is low and removal is generally limited by slow desorption. Altogether, the poorly bioavailable fraction of hydrocarbon contamination is formed by hydrocarbons which desorb slowly in the second phase of bioremediation (Loehner et al., 2001). The biodegradation of an oil-contaminated soil can also be seriously affected by the contamination time, due to weathering processes, which decrease the bioavailability of pollutants to microorganisms. Weathering refers to the results of biological, chemical and physical processes that can affect the type of hydrocarbons that remain in a soil (Maletić et al., 2011; Loehner et al., 2001; Semple et al., 2005). Those processes enhance the sorption of hydrophobic organic contaminants to the soil matrix, decreasing

the rate and extent of biodegradation. Moreover, a weathered oil-contaminated soil normally contains a recalcitrant fraction of compounds composed basically of high molecular weight hydrocarbons, which cannot be degraded by indigenous microorganisms (Balba et al., 1998; Maletić et al., 2011; Loeher et al., 2001). In contrast, a recently oil-contaminated soil contains a higher amount of saturated and aliphatic compounds, which are the most susceptible to microbial degradation. However, the pollutant compounds in a recently contaminated soil are potentially more toxic to the native microorganisms, leading to a longer adaptation time (lag phase) before degradation of the pollutant and even to an inhibition of the biodegradation process (Margesin et al., 2000; Loeher et al., 2001; Petrović et al., 2008).

As was mentioned above, sequestration and weathering of organic contaminants in the soil reduces the bioavailability of organic compounds and results in non-degraded residues in the soil. Contaminants that have been weathered and sequestered in soil are not available for biodegradation in soil, even though freshly added compounds are still biodegradable (Alexander, 1995). Sorption is a major factor preventing the complete bioremediation of hydrocarbons in soil. Slow sorption leads to the hydrocarbon fraction becoming resistant to desorption and increases its persistence within the soil organic matrix. The following hypotheses have been proposed as a explanation for weathering: (1) weathering results in a slow diffusion of the hydrocarbon fraction in the solid fraction of the organic matter in the soil; (2) the contaminant slowly diffuses through the soil and becomes sorbed and trapped in the soil nano- and micropores (Semple et al., 2003; Trinidade et al., 2005).

5.4. Microorganisms number and catabolism evolution

The ability of the soil's microbial community to degrade hydrocarbons depends on the microbes number and its catabolic activity. Microorganisms can be isolated from almost all environmental conditions. Soil microflora contain numbers of different microorganisms including bacteria, algae, fungi, protozoa and actinomycetes, which have a diverse capacity for attacking hydrocarbons. The main factors which affect the rate of microbial decomposition of hydrocarbons are: the availability of the contaminants to the microorganisms that have the catabolic ability to degrade them; the numbers of degrading microorganisms present in the soil; the activity of degrading microorganisms, and the molecular structure of the contaminant (Semple et al., 2003). The soil microorganisms number is usually in the range 10^4 to 10^7 CFU, for successful biodegradation this number should not be lower than 10^3 per gram of soil. Microorganism numbers lower than 10^3 CFU per gram of soil indicate the presence of toxic concentrations of organic or inorganic contaminants (Margesin et al., 2000; Petrović et al., 2008). The activity of soil microflora can be controlled by the factors discussed above - pH, temperature, nutrients, oxygen etc. For successful biodegradation, it is also necessary that the microorganisms can develop catabolic activity, by the following activities: induction of specific enzymes, development of new metabolic capabilities through genetic changes, and selective enrichment of organisms able to transform the target contaminant (Margesin et al., 2000, Semple et al., 2003).

5.5. Bioremediation study – Our experiences

With the aim of better understanding the factors which affect hydrocarbon biodegradation, results from the bioremediation studies described above are also given here, along with a comparison and discussion of changes in hydrocarbon composition and bioavailability over the years (Ivančev-Tumbas et al., 2004; Maletić, 2010; Maletić et al., 2011; Rončević, 2002; Rončević, 2007). The compounds detected by GC-MS analysis of extracts of the various soil samples taken at the start and end of bioremediation studies are given in Fig. 1, with only the main compounds from the hit lists of the probability-based matching (PBM $\geq 60\%$) search given.

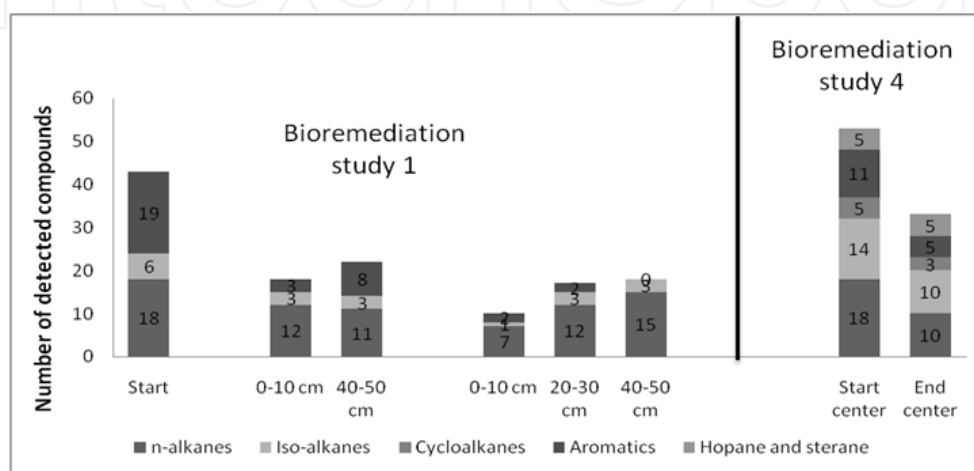


Figure 4. GC-MS SCAN qualitative analysis of soil samples

The data reflect the fact that the soil used in this investigation was sampled from the dumping area of a refinery where the initial pollutants were of very diverse composition, i.e. a mixture of crude oil, mazut, diesel, middle distillates, heavy distillates, kerosene, etc. The untreated soil samples contained a large variety of straight-chain hydrocarbons and their methyl derivatives (those with both even and odd numbers of C atoms), many of which persisted during the treatment. However, if we compare the untreated soil samples at the start of study 1 (1 year after contamination), and study 4 (8 years after contamination) the difference is significant. Namely, in study 1, the soil mostly contains n-alkanes and derivatives of aromatic hydrocarbons, and few compounds of iso-alkanes, whereas the soil in study 4 contains mostly n-alkanes and iso-alkanes, with only a few aromatics derivatives detected, with PBM < 50%, and few cycloalkanes. The fact that mainly substituted polycyclic aromatic hydrocarbons were not detected in the weathered soil samples (study 4), shows their lower persistence than alkanes. Additionally, the greater number of iso-alkanes in weathered soil indicates their persistence. The cycloalkanes detected represent one of the main hydrocarbon residual fractions in weathered contaminated soil.

In both studies, at the end of the experiment, the number of detected compounds is significantly reduced. In study 1, the aromatic hydrocarbons were almost completely removed in both reactors, while the number of n-alkanes detected was reduced, but they are still present in significant numbers in the soil at the end. This is because the aromatics have lower persistence than n-alkanes, but is also due to the higher n-alkanes concentration at the beginning. It is worth mentioning that in the reactor with continuous flow (aerobic), the number of removed n-

alkanes is almost the same, while in the reactor with discontinuous flows (partially anaerobic), the number of removed n-alkanes progressively reduced with depth, as a consequence of the lack of oxygen for microbial degradation, indicating that for this type of hydrocarbon, aerobic conditions are favourable. No such observation was noticed for aromatics. In study 4, only 3 n-alkanes compounds were detected at the end, also the number of poorly degradable iso-alkanes was also significantly reduced; this could be consequence of the lack of more degradable substrate which was probably removed during the weathering process.

Although the number of detected compounds (Fig. 4) and TPH concentration (Fig. 3) at the end was significant, the bioremediation rate was too slow to suggest that further bioremediation was possible. With the aim of investigating whether the lack of further hydrocarbon biodegradation was a consequence of the absence of the bioavailable hydrocarbon fraction for microbial degradation, the accumulation of toxic hydrocarbon degradation by-products, or the high concentration of hydrocarbons, a laboratory trial on the soil from study 4 was conducted (Maletić, 2010; Maletić et al., 2011). Study 4 was carried out for almost 2 years, however, after about one year, the biodegradation process slowed down significantly; at that point, some of the soil from study 4 was taken for the laboratory trial. The laboratory trials aimed in two directions: (1) bioavailability and (2) biodegradability investigation. Additionally, in order to test the impact of concentration, chemical composition and weathering on the biodegradation processes, the same tests were conducted on soil freshly contaminated by crude oil and diesel oil [36]. The bioavailability test was done by extraction of hydrocarbon contaminated soil with Tween 80. Table 4 shows the main results obtained from this test. To test whether high concentration or the accumulation of toxic by-products was the reason for the lack of biodegradation, the same soil sample was diluted with clean soil and then subjected to biodegradation under laboratory conditions (48 days). To ensure the process was not limited by other factors, the optimal conditions was provided, with respect to pH, temperature, water holding capacity, nutrients and oxygen content. The biodegradation process was monitored by measuring daily CO₂ production and TPH concentrations at the beginning and at the end of the experiment (Table 5).

The obtained results show that only 33% of the total amount of TPH is bioavailable in the weathered oil contaminated soil (soil taken from study 4). In the freshly contaminated soil, the bioavailable TPH fraction was three times larger, clearly indicating that in the weathered contaminated soil, the hydrocarbon is highly sequestered in the soil pores and complexed with soil organic matter. As a result of these processes, petroleum hydrocarbons become resistant and unavailable for biodegradation.

Parameter	Type of the soil contaminant		
	Weathered oil	Crude oil	Diesel oil
TPH g/kg at the beginning	12 (± 1.2)	26 (± 2.6)	28 (± 2.8)
TPH g/kg residual after Tween extraction	8 (± 0.8)	3.6 (± 0.4)	1.2 (± 0.1)
%removed by Tween extraction	33	86	96

Table 4. Laboratory bioavailability trial results

The biodegradation study showed there was little difference between the respiration of the original and diluted samples of weathered oil contaminated soil (Table 5.). The evolved CO₂ from those samples could originate from basal microbial respiration and from the very slow degradation of poorly biodegradable hydrocarbon compounds. This is confirmed by the removed amount of TPH in the samples. In contrast, in the freshly contaminated soil, respiration and the amount of TPH removed both strongly depended on the TPH concentration and origin. Thus, the highest quantity of evolved CO₂ was produced by the soil contaminated with diesel oil (16 mg TPH/g), with the sample contaminated with crude oil (13 mg TPH/g) producing a slightly lower cumulative quantity of evolved CO₂. The sample which contained the highest TPH concentration in the soils contaminated with diesel or crude oil had a lower respiration, which is a consequence of the high level of soluble hydrocarbons and the possible generation of toxic biodegradation products which can be toxic to the microorganisms present. Likewise, the sample with soil contaminated with the highest amount of diesel oil produced the second smallest amount of CO₂ in the range of diesel contaminated soils. Thus, the diesel oil contains mostly midrange alkanes which have varying solubility and can cause toxic effects. The smallest amount of evolved CO₂ was obtained for the samples with the lowest TPH concentrations of diesel and crude oil, where the biodegradable fraction was readily degraded. The amounts of TPH removed were in general agreement with the respiration rate, but less TPH was removed from the samples with crude oil contaminated soil. This could be due to the higher amounts of polar hydrocarbons (which are not included in the TPH fraction) in crude oil which can be degraded faster than the TPH.

From comparing the end TPH concentration in the biodegradation sample on the original weathered oil contaminated soil (Table 5), and the predicted bioavailable fraction (Table 4), it can be concluded that a small amount of bioavailable substrate remained at the end of the treatment. Nevertheless, it should be borne in mind that the bioavailability test was conducted at the beginning of the experiment, and that as well as the biodegradation processes during the experiment, the sorption and sequestration of hydrocarbons also took place. These processes reduced the bioavailable hydrocarbon fraction during the treatment. Additionally, it is worth mentioning that during the 2 years of bioremediation study 4, the TPH concentration was reduced by 53% (21% in the first year and 32% in the second year), indicating that all of the biodegradable TPH fraction was removed during the treatment.

From the above discussion it can be concluded that the lack of hydrocarbon biodegradation was due to highly sorbed and sequestered hydrocarbons in the soil pores and soil organic matter as a consequence of weathering, and not due to high hydrocarbon concentrations or accumulation of toxic products in the soil. This soil is therefore not suitable for further bioremediation, and if further removal of hydrocarbons is required, other technologies must be applied.

Contaminated soil	TPH g/kg after dilution	Evolved CO ₂ mg/g	g/kg removed TPH
Weathered oil contaminated oil	12 (±1.2) (original soil)	6.1 (±0.9)	2.2 (±0.2)
	4.9 (±0.5)	6.8 (±1.0)	1.3 (±0.1)
	3.8 (±0.4)	5.2 (±0.8)	1.5 (±0.2)
	2.3 (±0.2)	4.6 (±0.7)	0.76 (±0.1)
Crude oil contaminated soil	26 (±2.6)	15 (±2.2)	16 (±1.6)
	13 (±1.3)	20 (±3.0)	11 (±1.1)
	7.5 (±0.8)	11 (±1.7)	6.5 (±0.7)
	5.5 (±0.6)	5.3 (±0.8)	4.4 (±0.4)
Diesel oil contaminated soil	28 (±2.8)	14 (±2.2)	11 (±1.1)
	16 (±1.6)	23 (±3.4)	11 (±1.1)
	9.2 (±0.9)	17 (±2.6)	6.8 (±0.7)
	7.0 (±0.7)	7.9 (±1.2)	5.1 (±0.5)

Table 5. Laboratory biodegradability results

6. Conclusion

The cleaning up of petroleum hydrocarbons in the soil environment is a real world problem. Better understanding of the mechanisms and factors which affect biodegradation is of great ecological significance, since the choice of bioremediation strategy depends on it. Microbial degradation processes aid the elimination of spilled oil from the environment, together with various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy. Even if the optimal conditions for microbial degradation are provided, the extent of hydrocarbon removal is strongly affected by its bioavailability and stages of weathering. As a consequence, some fractions of hydrocarbons remain undegraded. This residual fraction of hydrocarbon in soil can represent an acceptable end point for bioremediation if (1) hydrocarbon biodegradation is too slow to allow further bioremediation, in which case other technologies must be applied; (2) those concentrations are unable to release from the soil and pose adverse effects to the environment and human health, like those presented in the given case studies. Such residual material from oil degradation is analogous to, and could even be regarded as, humic material. Its inert characteristics, insolubility and similarity to humic materials mean it is unlikely to be environmentally hazardous.

Author details

Snežana Maletić, Božo Dalmacija and Srđan Rončević
*University of Novi Sad Faculty of Sciences, Department of Chemistry,
 Biochemistry and Environmental Protection,
 Republic of Serbia*

Acknowledgement

This research was financed by the Ministry of Education and Science (Projects No. III43005 and TR37004) and the Novi Sad Oil Refinery.

7. References

- Alexander, M. (1995). How toxic are toxic chemicals in soil? *Environmental Science and Technology*, Vol. 29, No. 11, pp. 2713–2717, ISSN 0013-936X.
- Alexander, M. (2000). Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environmental Science and Technology*, Vol. 34, No. 20, pp. 4259–4265, ISSN 0013-936X.
- Baker, R.S. & Moore, A.T. (2000). Optimizing the effectiveness of in situ bioventing. *Pollution Engineering*, Vol. 32, No. 7, pp. 44–47, ISSN 0032-3640.
- Balba, M.T.; Al-Awadhi, N. & Al-Daher, R. (1998). Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation. *Journal of Microbiological Methods*, Vol. 32, No. 2, pp. 155–164, ISSN 0167-7012.
- Brassington, K.J.; Hough, R.L.; Paton, G.I.; Semple, K.T.; Risdon, G.C.; Crossley, J.; Hay, I.; Askari, K. & Pollard, S.J.T. (2007). Weathered Hydrocarbon Wastes: A Risk Management Primer. *Critical Reviews in Environmental Science and Technology*, Vol. 37, No. 3, pp. 199-232, ISSN 1064-3389.
- Burland, S.M. & Edwards, E.A. (1999). Anaerobic benzene biodegradation linked to nitrate reduction. *Applied and Environmental Microbiology*, Vol. 65, No. 2, pp. 529-533, ISSN 0099-2240.
- Collina, E.; Bestetti, G.; Di Gennaro, P.; Franzetti, A.; Gugliersi, F.; Lasagni, M. & Pitea, D. (2005). Naphthalene biodegradation kinetics in an aerobic slurry-phase bioreactor. *Environment International*, Vol. 31, No. 2, pp. 167– 171, ISSN 0160-4120.
- Cuyppers, C.; Pancras, T.; Grotenhuis, T. & Rulkens, W. (2002). The estimation of PAH bioavailability in contaminated sediments using hydroxypropyl- β -cyclodextrin and triton X-100 extraction techniques. *Chemosphere*, Vol. 46, No. 8, pp. 1235–1245, ISSN 0045-6535.
- Eglinnton, G. (1975). *Environmental chemistry*, Vol. 1, Specialist periodical reports. The Chemical Society, Burlington House, ISBN 0851867553, London.
- Grossi, V.; Massias, D.; Stora, G. & Bertrand, J.C. (2002). Exportation and degradation of acyclic petroleum hydrocarbons following simulated oil spill in bioturbated Mediterranean coastal sediments. *Chemosphere*, Vol. 48, No. 9, pp. 947–954, ISSN 0045-6535.
- Hejazi, R.; Husain, T. & Khan, F.I. (2003). Landfarming operation in arid region-human health risk assessment. *Journal of Hazardous Materials*, Vol. 99, No. 3, pp. 287–302, ISSN 0304-3894.
- Hohener, P.; Duwig, C.; Pasteris, G.; Kaufmann, K.; Dakhel, N. & Harms, H. (2003). Biodegradation of petroleum hydrocarbon vapours: Laboratory studies on rates and

- kinetics in unsaturated alluvial sand. *Journal of Contaminant Hydrology*, Vol. 66, No. 1-2, pp. 93–115, ISSN 0169-7722.
- Ivančev-Tumbas, I.; Tričković, J.; Karlović, E.; Tamaš, Z.; Rončević, S.; Dalmacija, B.; Petrović, O. & Klašnja, M. (2004). GC/MS-SCAN to follow the fate of crude oil components in bioreactors set to remediate contaminated soil. *International Biodeterioration and Biodegradation*, Vol. 54, No. 4, pp. 311-318, ISSN 0964-8305.
- Jain, P.K.; Gupta, V.K.; Guar, R.K.; Lowry, M.; Jaroli, D.P. & Chauhan, U.K. (2011). Bioremediation of petroleum contaminated soil and water. *Research Journal of Environmental Toxicology*, Vol. 5, No. 1, pp. 1-26, ISSN 1819-3420.
- Joner, E.J.; Leyval, C. & Colpaert, V.J. (2006). Ectomycorrhizas impede phytoremediation of polycyclic aromatic hydrocarbons (PAHs) both within and beyond the rhizosphere. *Environmental Pollution*, Vol. 142, No. 1, pp. 34-38, ISSN 0269-7491.
- Jorgensen, K.S.; Puustinen, J. & Suortti, A.M. (2000). Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. *Environmental Pollution*, Vol. 107, No. 2, pp. 245–254, ISSN 0269-7491.
- Khan, I.F.; Husain, T. & Hejazi, R. (2004). An overview and analysis of site remediation technologies. *Journal of Environmental Management*, Vol. 71, No. 2, pp. 95-122, ISSN 0301-4797.
- Loeher, R.C.; Mc Millen, S.J. & Webster, M.T. (2001). Predictions of biotreatability and actual results: soils with petroleum hydrocarbons. *Practice periodical of hazardous, toxic, and radioactive waste management*, Vol. 5, No. 2, pp. 78–87, ISSN 1090-025X.
- Maletić, S. (2010) Characterisation of the biodegradability of petroleum hydrocarbons in soil and the bioremediation processes during treatment by biopiles and landfarming, *PhD Thesis*.
- Maletić, S.; Dalmacija, B.; Rončević, S.; Agbaba, J. & Ugarčina Perović, S. (2011). Impact of hydrocarbon type, concentration and weathering on its biodegradability in soil. *Journal of Environmental Science and Health, Part A*, Vol. 46, No. 10, pp. 1042-1049, ISSN 1093-4529.
- Maletić, S.; Dalmacija, B.; Rončević, S.; Agbaba, J. & Petrović, O. (2009). Degradation Kinetics of an Aged Hydrocarbon-Contaminated Soil. *Water Air Soil Pollution*, Vol. 202, No. 1-4, pp. 149-159, ISSN 0049-6979.
- Margesin, R.; Zimmerbauer, A. & Schinner, F. (2000). Monitoring of bioremediation by soil biological activities. *Chemosphere*, Vol. 40, No. 4, pp. 339–346, ISSN 0045-6535.
- Marković, D.A.; Đarmati, Š.A.; Gržetić, I.A. & Veselinović, D.S. (1996). *Fizičko-hemijski osnovi zaštite životne sredine, Izvori zagađivanja, posledice i zaštita*. ISBN 86-81019-27-9 Univerzitet u Beogradu, Beograd.
- Namkoonga, W.; Hwangb, E.Y.; Parka, J.S. & Choic, J.Y. (2002). Bioremediation of diesel-contaminated soil with composting. *Environmental Pollution*, Vol. 119, No. 1, pp. 23–31, ISSN 0269-7491.
- Nano, A.G. & Rota, R. (2003). Combined slurry and solid –phase bioremediation of diesel contaminated soils. *Journal of Hazardous Materials*, Vol. 100, No. 1-3, pp. 79–94, ISSN 0304-3894.

- Nilanjana, D. & Chandran, P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research International*, Vol. 2011, pp. 1-13, ISSN 2090-3138.
- Pala, D.M.; de Carvalho, D.D.; Pinto, J.C. & Sant'Anna, Jr G.L. (2006). A suitable model to describe bioremediation of a petroleum-contaminated soil. *International Biodeterioration and Biodegradation*, Vol. 58, No. 3-4, pp. 254-260, ISSN 0964-8305.
- Petrović, O.; Knežević, P.; Marković, J. & Rončević, S. (2008). Screening method for detection of hydrocarbon-oxidizing bacteria in oil-contaminated water and soil specimens. *Journal of Microbiological Methods*, Vol. 74, No. 2-3, pp. 110–113, ISSN 0167-7012.
- Pollard, S.J.T.; Hough, R.L.; Kim, K.; Bellarby, J.; Paton, G.I.; Semple, K.T. & Coulon, F. (2008). Fugacity modelling to predict the distribution of organic contaminants in the soil:oil matrix of constructed biopiles. *Chemosphere*, Vol. 71, No. 8, pp. 1432–1439, ISSN 0045-6535.
- Rahman, K.S.M.; Rahman, T.J. Kourkoutas, Y.; Petsas, I.; Marchant, R. & Banat, I.M. (2003). Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients, *Bioresource Technology*, Vol. 90, No. 2, pp. 159–168, ISSN 0960-8524.
- Rončević, S. (2002). Kinetics of bioremediation processes in soil contaminated by oil and oil derivatives, *MSc Thesis*.
- Rončević, S. (2007). Characterisation of the bioremediation processes in soil and groundwater contaminated by oil and oil derivatives at the site of Ratno Ostrvo, *PhD Thesis*.
- Rončević, S.; Dalmacija, B.; Ivančev-Tumbas, I.; Petrović, O.; Klašnja, M. & Agbaba, J. (2005). Kinetics of Degradation of Hydrocarbons in the Contaminated Soil Layer. *Archives of Environmental Contamination and Toxicology*, Vol. 49, No. 1, pp. 27-36, ISSN 0090-4341.
- Salminen, J.M.; Tuomi, P.M.; Suortti, A. & Jørgensen, K.S. (2004). Potential for Aerobic and Anaerobic Biodegradation of Petroleum Hydrocarbons in Boreal Subsurface. *Biodegradation*, Vol. 15, No. 1, pp. 29-39, ISSN 0923-9820.
- Semple, K.T.; Morris, A.W.J. & Paton, G.I. (2003). Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. *European Journal of Soil Science*, Vol. 54, pp. 809-818, ISSN 0022-4588.
- Semple, K.T.; Reid, B.J. & Fermor, T.R. (2001). Impact of composting strategies on the treatment of soils contaminated with organic pollutants. *Environmental Pollution*, Vol. 112, No. 2, pp. 269-283, ISSN 0269-7491.
- Stroud, J.L.; Paton, G.I. & Semple, K.T. (2007). Microbe–aliphatic hydrocarbon interactions in soil: implications for biodegradation and bioremediation. *Journal of Applied Microbiology*, Vol. 102, No. 5, pp. 1239–1253, ISSN 1364-5072.
- Stroud, J.L.; Paton, G.I. & Semple, K.T. (2009). Predicting the biodegradation of target hydrocarbons in the presence of mixed contaminants in soil. *Chemosphere*, Vol. 74, No. 4, pp. 563–567, ISSN 0045-6535.
- Trinidade, P.V.O.; Sobral, A.C.L.; Rizzo, S.G.F. & Leite Soriano, A.U. (2005). Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: a comparasion study. *Chemosphere*, Vol. 58, No. 4, pp. 515-522, ISSN 0045-6535.

- Vidali, M. (2001). Bioremediation. An overview. *Pure and Applied Chemistry*, Vol. 73, No. 7, pp. 1163–1172, ISSN 1365-3075.
- Wethasinghe, C.; Yuen, S.T.S.; Kaluarachchi, J.J. & Hughes, R. (2006). Uncertainty in biokinetic parameters on bioremediation: Health risks and economic implications. *Environment International*, Vol. 32, No. 3, pp. 312 – 323, ISSN 0160-4120.
- Yerushalmi, L. Rocheleau, S.; Cimpoaia, R.; Sarrazin, M.; Sunahara, G.; Peisajovich, A; Leclair, G. & Guiot, S.R. (2003). Enhanced Biodegradation of Petroleum Hydrocarbons in Contaminated Soil. *Bioremediation Journal*, Vol. 7, No. 1, pp. 37–51, ISSN 1088-9868.

IntechOpen