

## BIODEGRADATION YIELD OF CRUDE OIL IN SOIL POLLUTED AND TREATED IN DIFFERENT EXPERIMENTAL VARIANTS

Mariana MARINESCU, Anca LĂCĂTUȘU, Eugenia GAMENT,  
Georgiana PLOPEANU, Mihai MARINESCU

National Research and Development Institute for Soil Science, Agrochemistry and Environment Protection, 61 Mărăști Blvd., District 1, Bucharest, Romania

Corresponding author email: maramarinescu2003@yahoo.com

### Abstract

*Soils pollution with crude oil is becoming an ever increasing problem, especially in the light of several breakdowns of oil pipelines and wells reported recently. Due to its toxicity, widespread presence and complex nature, this type of pollution is a serious problem, one reason being that as the modern civilisation, urbanisation and mechanisation develop the use of crude oil products grow. The influence of soil pollution with crude oil depended on the type of soil, crude oil concentration in soil and fertilisation. The study has been undertaken to assay the biodegradation yield of soil polluted with crude oil and treated in different experimental variants. In soil polluted with 5% crude oil, doubling fertiliser dose from 0.25% to 0.5% has exercised the most spectacular effect, causing an increase of yield to 45.7% in absence of bacterial inoculation. At low fertiliser dose, inoculation with selected bacteria induced a strong stimulation effect; the bioremediation yield is 24% higher than that determined in uninoculated variant. In variants excessively polluted with 10% crude oil, microorganisms with biodegradation capacity of petroleum hydrocarbons have required more or less period of time to adapt to pollutant presence in excess. Necessary period to adapt to environmental changing, microorganisms was significantly decreased by soil conditioning with Ecosol at 1% concentration.*

**Key words:** biodegradation yield, soil polluted, crude oil, different treatments.

### INTRODUCTION

Nonetheless, major points of soil pollution with petroleum hydrocarbons are crude oil stations (Michalcewicz, 1995). Other areas of concern are mining and distribution of petroleum based products (Song and Barhta, 1990; Amadi et al., 1996; Jørgensen et al., 2000).

Certain negligence when transporting, collecting or storing crude oil together with unsatisfactory care while disposing of old or used crude oil products lead to considerable pollution of the natural environment (Leahy and Colwell, 1990).

Crude oil penetrating soil cause its degradation (Sztompka, 1999). Once they enter an ecosystem, crude oil initiate a series of processes, affecting both its biotic and abiotic elements (Małachowska-Jutysz et al., 1997).

Crude oil is composed of aliphatic, oleic, naphthenic and aromatic hydrocarbons (Chi Yuan and Krishnamurthy, 1995), which modify physical and chemical properties of soil and its structure. These compounds are largely responsible for changed in soil fertility (Iwanow et al., 1994).

Moreover, crude oil has a negative effect on the biochemical and physicochemical characteristics of soils (Kucharski and Wyszowska, 2001).

Since soil pollution with crude oil deteriorates its biochemical and physicochemical properties, it also limits the growth and development of plants, whose nutritive and technological value can be low and often questionable.

In this connection, the present study has been undertaken to increase the biodegradation yield of crude oil in soil polluted.

### MATERIALS AND METHODS

The main objective of this research is to increase the biodegradation yield of crude oil by biostimulation and bioaugmentation.

Biostimulation have been achieved by using an organic compound made from cellulose fibers for soil polluted conditioning with additives to optimize its structure, water and air circulation regime in soil, and not least achieving a protective interface between degrading

microorganisms and pollutant. Ecosol compound was chosen for experiment by analyzing a series of organic compounds suitable for conditioning soil polluted with organic pollutants, especially because of its biodegradability properties.

Bioaugmentation have been achieved by soil inoculation with bacterial bioproducts made from specific bacteria selected and tested in the laboratory for their ability to degrade crude oil. The experiment was set up by an artificial soil pollution and treated with different quantities of ECOSOL (Table 1).

Table 1. Chemical characteristics of the natural biodegradable product ECOSOL

Natural biodegradable product	Nt (%)	C (%)	P (%)	K (%)	Na (%)
ECOSOL	0.935	23.72	0.39	3.32	4.97

After 21 days from pollution, the soil was inoculated with bacteria. The bacterial inoculum was developed from microorganisms that occur naturally in the soil like *Pseudomonas*, *Mycobacterium*, *Arthrobacter globiformis* and *Bacillus megaterium* and

which are demonstrated as petroleum degrading microorganisms.

It have been achieved a greenhouse experiment. The soil used for this experiment was a calcic chernozems (Table 2).

Table 2. Chemical characteristics of the soil

Soil type	pH	C (%)	Nt (%)	C/N Ratio	P <sub>AL</sub> mg kg <sup>-1</sup>	K <sub>AL</sub> mg kg <sup>-1</sup>
Calcic chernozem	8.09	2.99	0.279	12.5	50	215

The experimental variants are: V<sub>1</sub>, control (unpolluted soil); V<sub>2</sub>, polluted soil with 5% crude oil; V<sub>3</sub>, polluted soil with 10% crude oil; V<sub>4</sub>, polluted soil with 5% crude oil + 0.25% ECOSOL; V<sub>5</sub>, polluted soil with 5% crude oil + 0.25% ECOSOL + bacterial inoculum; V<sub>6</sub>, polluted soil with 5% crude oil + 0.5% ECOSOL; V<sub>7</sub>, polluted soil with 5% crude oil + 0.5% ECOSOL + bacterial inoculum; V<sub>8</sub>, polluted soil with 10% crude oil + 0.5 ECOSOL; V<sub>9</sub>, polluted soil with 10% crude oil + 0.5% ECOSOL + bacterial inoculum; V<sub>10</sub>, polluted soil with 10% crude oil + 1% ECOSOL; V<sub>11</sub>, polluted soil with 10% crude oil + 1% ECOSOL + bacterial inoculum.

## RESULTS AND DISCUSSIONS

In the case of soil polluted with 5% crude oil (Figure 1) is very obvious difference between experimental variants in terms of reducing both dynamic and final values of total petroleum hydrocarbons concentrations, pointing out the clear superiority of experimental variant treated with 0.5% ECOSOL and inoculated with selected bacteria.

In the beginning of the experiment in variants polluted with 5% crude oil, increased levels of TPH in inoculated variants is caused by bacterial biomass pollutant dead on impact. After 14 days from the pollution, the TPH values begin to decrease in the treated experimental variants compared with the polluted and untreated variant.

After 21 days, began to be evident that in the experimental variant V<sub>7</sub> treated with 0.5% ECOSOL and inoculated, the speed of pollutant disappearance in the soil is much higher compared to the other experimental variants, results with a high insurance degree statistics. In the experimental variants polluted with 10% crude oil only after 21 days can be observed decreases in the TPH concentration higher than in the polluted and untreated variant (Figure 2). Also, at this time of determination, it have been found a higher biodegradation yield of crude oil in the experimental variant treated with the highest dose of 1% ECOSOL and bacterial inoculation compared to other variants. Also, in the variants polluted with 10% crude oil was found relatively slow process of crude oil degradation in the 30-300 days range.

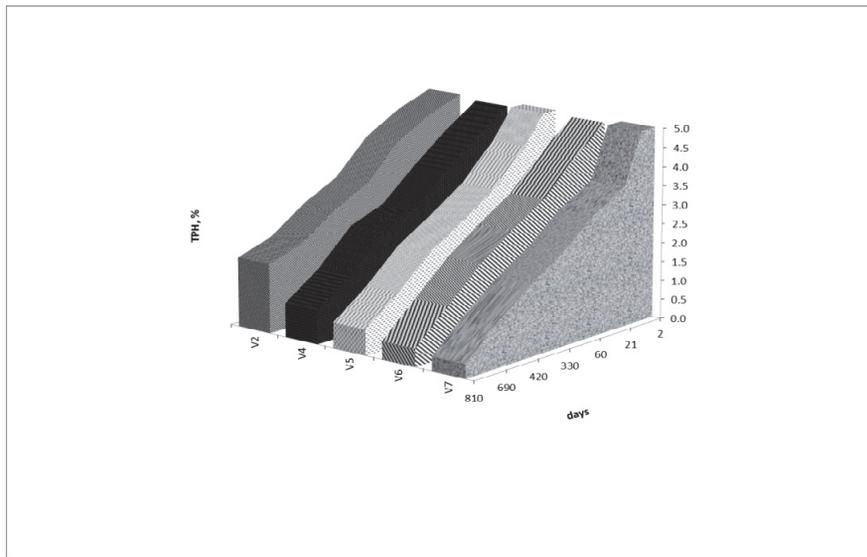


Figure 1. The evolution of total petroleum hydrocarbons (TPH) in soil polluted with 5% crude oil in different experimental variants

After 330 days until the end of the experiment was noted an accelerated biodegradation of crude oil that been manifested till the end of the experiment in all treated variants. This long slowdown processes (bio)degradation of total petroleum hydrocarbons in the case of excessively polluted soil with crude oil can lead to significant difficulty to adapting of biodegrading microorganisms, both its own soil, and those introduced by the addition of bacterial inoculum in case of excessive pollution. This is one of the major reasons for which are not recommended bioremediation processes in soils polluted with petroleum hydrocarbons at concentrations higher than 5% TPH. Though, biodegrading processes,

happens, takes place after an extremely long and unprofitable period of time.

At the end of the experiment, after 810 days of the onset research, TPH concentration in soil polluted with 5% crude oil still present a value of 1.81%. This can be translated that into the soil polluted with 5% crude oil, the process yield of disappearance of the pollutant in the soil without applying any treatments, so only through its own detoxification mechanisms of soil defined as natural attenuation is 63.8%. The yield may seem high for a natural attenuation process if is taking into account the longer period of time - 810 days, which may mean no more than three years to miss land use.

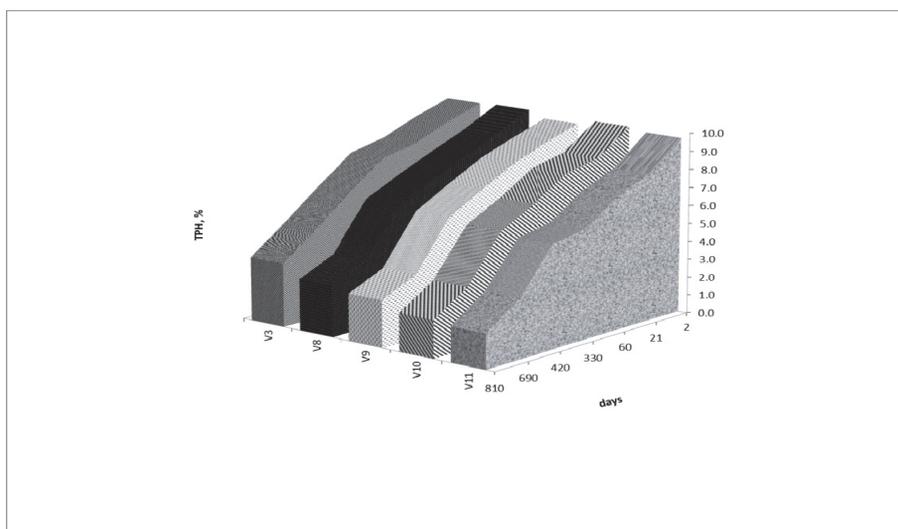


Figure 2. The evolution of total petroleum hydrocarbons (TPH) in soil polluted with 10% crude oil in different experimental variants

In the experimental variant in which the soil was polluted with 5% crude oil and treated with 0.25% ECOSOL, the content of the total petroleum hydrocarbon in the pot determined at the end of the experiment was 0.89% TPH, corresponding to a yield by 82.2%. In the experimental variant polluted with 5% crude oil treated with 0.25% ECOSOL and inoculated with selected bacteria, the total petroleum hydrocarbons content after 810 days was 0.67%, corresponding to a yield by 86.6%. For the experimental variants where the soil was

polluted with 5% crude oil and treated with 0.5% ECOSOL, the petroleum hydrocarbons content at the end of the experiment was 0.47%, representing a yield of 90.6%. And finally, in the variant where the soil was polluted with 5% crude oil treated with 0.5% ECOSOL and inoculated with selected bacteria, the total petroleum hydrocarbons content of the crude oil at the end of the experiment was only 0.37%, representing a yield by 92.6% (Figure 3).

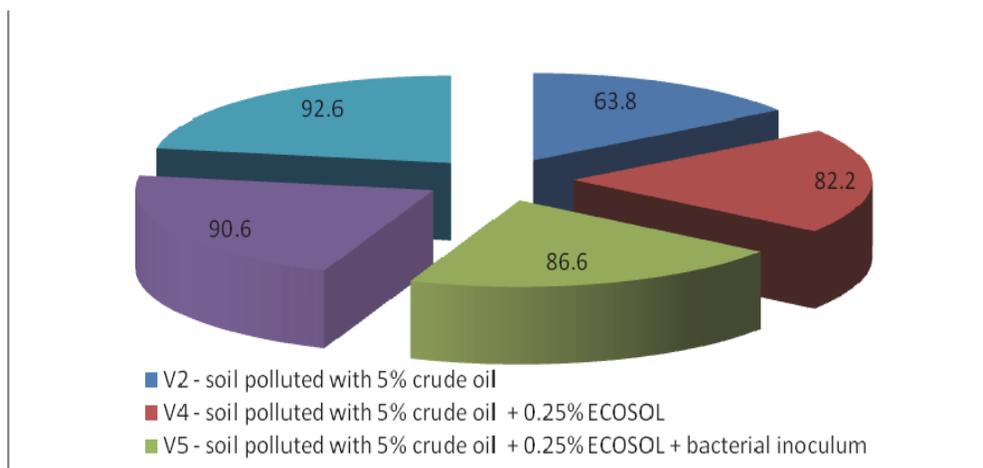


Figure 3. The biodegradation yield in soil polluted with 5% crude oil in different experimental variants

In the case of the experimental variant with 10% crude oil polluted soil in which no treatments were applied to remediate, the total petroleum hydrocarbons concentration determined at the end of the experiment was 3.5% TPH, which is a yield of 65% attributed to the natural attenuation.

In the experimental variant in which the soil was polluted with 10% crude oil and treated with 0.5% ECOSOL, the total petroleum hydrocarbon content determined in the pot at the end of the experiment was 2.82% THP, corresponding to a yield by 71.8%. In the experimental variant where soil was polluted with 10% crude oil treated with 0.5% ECOSOL and inoculated with selected bacteria, the petroleum hydrocarbon content after 810 days was 2.59%, corresponding to a yield by 74.1%. For the experimental variant where the soil was polluted with 10% crude oil and treated with 1% ECOSOL, the total petroleum hydrocarbon content at the end of the experiment was 2.10%, representing a yield of 79%. And

finally, in the experimental variant where the soil was polluted with 10% crude oil treated with 1% ECOSOL and inoculated with selected bacteria, the total petroleum hydrocarbons content at the end of the experiment was only 1.95%, equivalent to a yield by 80.5% (Figure 4).

## CONCLUSIONS

In the variant with soil polluted with 5% crude oil, treated by 0.5% ECOSOL and inoculated with selected bacteria, petroleum hydrocarbon content at the end of the experiment was only 0.37%, representing a yield by 92.6%.

Doubling ECOSOL dose from 0.25% to 0.5% has exercised the most spectacular effect, causing an increase of yield to 45.7% in absence of bacterial inoculation.

At low ECOSOL dose, inoculation with selected bacteria induced a strong stimulation effect; the bioremediation yield is 24% higher than that determined in uninoculated variant.

In soil polluted with 10% crude oil, where no ameliorative treatments were applied, the crude oil concentration determined at the end of experiment was 3.5% TPH, and represents a yield of 65% attributed to natural attenuation process.

The bioremediation process yield increased due to the complexity of treatments applied as follows: in soil treated by 0.5% ECOSOL, petroleum hydrocarbon content determined in the pot at the end of the experiment was 2.82% TPH, corresponding to a yield of 71.8%, in the

variant treated by 0.5% ECOSOL and inoculated with selected bacteria, final petroleum hydrocarbon content was 2.59%, corresponding to a yield of 74.1%, the variant in which the soil was treated with 1% ECOSOL, the final content of petroleum hydrocarbons was 2.10%, representing a yield of 79% and, finally, the variant in which soil was treated with 1% ECOSOL and inoculated with selected bacteria, petroleum hydrocarbon content at the end of the experiment was only 1.95%, representing a yield of 80.5%.

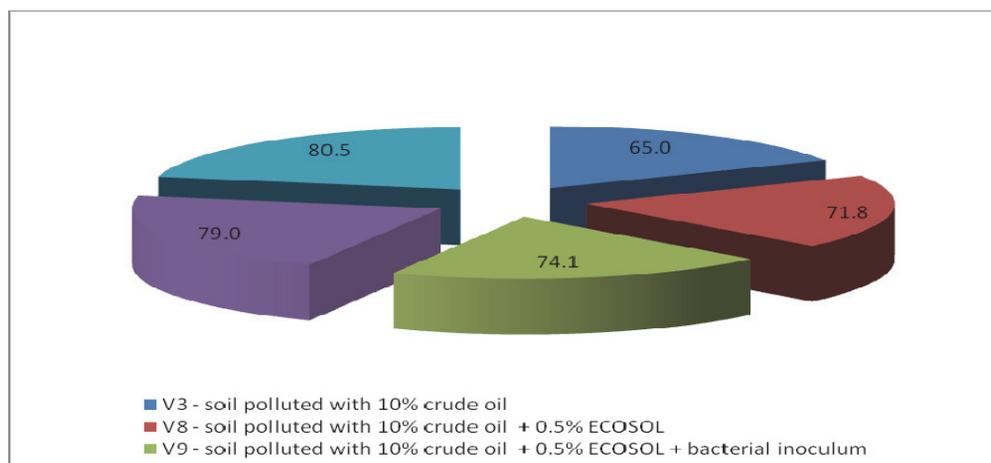


Figure 4. The biodegradation yield in soil polluted with 10% crude oil in different experimental variants

## ACKNOWLEDGEMENTS

The financial support provided by the UEFISCDI - Executive Unit for Financing Education Higher Research Development and Innovative - project no. 91/2014 - Bioremediation innovative technology of ex-situ soils polluted with crude oil (BIORESOL).

## REFERENCES

- Amadi A., Abbey S.D., Nma A., 1996. Chronic effects of oil spill on soil properties and microflora of rainforest ecosystem in Nigeria. *Water Air Soil Pollut.*, 86: 1–11.
- Chi Yuan Fan, Krishnamurthy M. 1995. Enzymes for enhancing bioremediation of petroleum-contaminated soils: A brief review. *Air Waste Manage. Assoc.*, 45: 453–460.
- Iwanow W.N., Dylgierow A.N., Stabnikowa E., 1994. Aktivnost niekatorych ekologo-troficzeskich grup mikroorganizmow pri zagraznieniu cziernoziema obyknowiennowo ygliewodorami niefci / The activity of certain ecological-trophic groups of microorganisms in the presence of cows in the population is unknown. *Mikrobiol. Zurn.*, 6: 59–63.
- Jørgensen K.S., Puustinen J., Suortti A.M., 2000. Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. *Environ. Pollut.*, 107: 245–254.
- Kucharski J., Wyszowska J., 2001. Microbiological properties of soil contaminated with diesel oil. *ActaAgrophis.*, 51: 113–120.
- Leahy J.G., Colwell R.R., 1990. Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* Sept., 54: 305–315.
- Małachowska-Jutysz A., Mrozowska J., Kozielska M., Miksch K., 1997. Aktywność enzymatyczna w glebie skażonej związkami ropopochodnymi w procesie jej detoksykacji /Enzymatic activity in soil contaminated with petroleum compounds in the process of its detoxification. *Biotechnologia*, 36: 79–91.
- Michalciewicz W., 1995. Wpływ oleju napędowego do silników Diesla na liczebność bakterii, grzybów, promieniowców oraz biomasę mikroorganizmów glebowych / Impact of diesel to diesel engines on the abundance of bacteria, fungi, radionews and biomass of soil microorganisms. *Rocz. PZH*, 46: 91–97.
- Song H., Bartha R., 1990. Effects of jet fuel spills on the microbial community of soil. *Appl. Environ. Microb.* Mar., 56: 646–651.
- Sztompka E., 1999. Biodegradation of engine oil in soil. *ActaMicrob. Pol.*, 489: 185–196.