THE INFLUENCE OF A NATURAL BIODEGRADABLE PRODUCT ON THE BIODEGRADATION OF CRUDE OIL POLLUTED SOIL

Mariana MARINESCU [1], Mihai DUMITRU [1], Anca LĂCĂTUŞU [1], Georgiana PLOPEANU [1], Mihai MARINESCU [1]

[1] National Research Development Institute for Soil Science, Agrochemistry and Environmental Protection, Bucharest

Abstract

Crude oil pollution is a world-wide threat to environment and the remediation of crude oil polluted soil is a challenge for environmental protection research. Bioremediation is a useful method for soil remediation without second effect on soil quality and it is not an expensive technology from economical point of view. Biodegradation is the process by which organic substances are broken down by the enzymes produced by living organisms. Although biodegradation of petroleum hydrocarbons may be successfully achieved under controlled conditions, the bioremediation of polluted soils still remains a challenge. The main objective of this study is to investigate the influence of a natural biodegradable product on petroleum hydrocarbons degradation in a crude oil polluted soil. It is a laboratory experiment achieved during two years in Green House. Soil was artificially polluted with 5% and 10% crude oil and treatment applied consisted in a natural biodegradable product and bacterial inoculum. Soil polluted with 5% crude oil was treated with 50 g, respective 100 g ECOSOL/20 kg polluted soil and/or bacterial inoculum to increase the biodegradability rate and the soil polluted with 10% crude oil was treated with 100 g, respective 200 g ECOSOL/20 kg polluted soil and/or bacterial inoculum. The plant used in experiment was maize. The parameters followed during experiment were total petroleum hydrocarbons, soil reaction, total carbon, nitrogen, C/N ratio. At each phase of the study, the natural biodegradable product was found to significantly enhance the biodegradation process and detoxification of the crude oil polluted soils employed by increasing the bioavailability of the pollutants and the activity of indigenous microorganisms. During the experiment, the biodegradation rate reached higher values according with treatment.

Key words: biodegradation, crude oil, polluted soil, a natural biodegradable product

Oil-contamination is one of the most common types of pollution, endangering aquatic and terrestrial ecosystems. Typical sources of soil pollution are fuel oils, diesel, carburetor oils (petrol) and lubricating oils, but, mainly due to war impacts, also heavy oils (e.g. crude oil, fuel oil and storage oil) have been spilled into the ground and require extensive remediation (Pepper, 1999).

In recent years, bioremediation of soils polluted with petroleum hydrocarbons is a research challenge (Rahman et al., 2003). Research has shown that the bioremediation is a superior method of remediating soil, effective and much cheaper compared with physicochemical methods. Bioremediation can be: in situ and ex situ. Bioremediation based on micro-business use of petroleum hydrocarbons as carbon and energy source. This method is considered to be most effective because no irreversible effects on soil characteristics affected pedogenetical and low cost (Snape et al., 2001).

Microorganisms such as bacteria, fungi and yeasts decompose these hazardous chemicals non-toxic or less toxic compounds by the enzyme complex (Leahy and Colwell, 1990).

Interest in environmental pollution has increased dramatically for everyone in the world, various institutions and organizations, some multipurpose other specialized nature, focused solely on pollution issues. There is no life without soil (Walworth, 1997).

To survive, microorganisms, like any body need nutrients, carbon and energy source. Microorganisms transform a series of organic compounds found naturally in food and energy for its own ecosystem. For example, many species of soil bacteria can use petroleum hydrocarbons as energy and food source. This natural process converts hydrocarbons from oil in less hazardous substances such as carbon dioxide, water and fatty acids. Biodegradation of organic wastes has become a great way for treatment of polluted soils (Atlas, 1992). The advantage of this method is cheap equipment that has no negative environmental effects (Nadeau et al., 1993). A disadvantage of this problem is the speed of the process than degradation.

Although the remediation is an option to be implemented widely, even in Antarctic and sub-Antarctic soils is not fully known process of biodegradation and remediation performance in cold areas (Margesin and, Schinner, 2001). The temperature at which the degradation process has maximum efficiency in that area is 10°C (Coulon et al., 2005).

All these risks are related, first, the mobility of the pollutant. Petroleum products once they reach the ground, where conditions are met both aerobic and anaerobic suffer significant chemical transformations. Most authors consider that the rate of degradation of petroleum products in soil is a function of oxygen concentration in the soil or, in other words, the degree of aeration of polluted soil. The basement can be found different oxygen concentrations leading to different rates of biodegradation of petroleum products.

Biodegradation is accelerated in the presence of substances called nutrients (compounds of phosphorus, potassium, nitrogen), moisture and temperature relatively constant, factors leading to a rapid increase in populations of bacteria. Also, the degradation intermediates may result in increased water solubility or high volatility leading to increased pollution as high speed (Fava and Ciccotosto, 2002).

MATERIAL AND METHOD

The purpose of the present study was to investigate possible methods to enhance the rate of biodegradation of hydrocarbons from crude oil. Enhancement of biodegradation was achieved through the treatment with ECOSOL and bacterial inoculation. The objective was to simulate conditions of a major spill.

To achieve data concerning the bioremediation of polluted soil with petroleum hydrocarbons was realized a greenhouse experiment. It was used for this experiment a cambic chernozem. At the beginning of the experiment, the soil was contaminated / polluted with crude oil and treated with the natural hydrocarbon absorbent (ECOSOL). After 21 days from pollution, the soil was inoculated with bacteria.

ECOSOL is an absorbent natural product, meant to facilitate quick and efficient biodegradation of hydrocarbons from contaminated soils. Accelerates bio-stimulation and favors the development of existing bacteria from the soil, with strong effects in crude oil degradation. It is obtained from vegetal fibers from celluloid waste, all treated and with additives, being used in order to bring soils back to normal fertility levels.

The chemical characteristics of the natural biodegradable product are:

- Total nitrogen: 0.935%;
- Organic carbon: 23.72%;
- Phosphorous: 0.39%
- Potassium: 3.32%;
- Sodium: 4.97%.

The inoculum was developed from microorganisms that occur naturally in the soil with the addition of crude oil. The microorganisms use the petroleum hydrocarbons as a source of carbon. It was stimulated the growth of the microbial population by adding the fibers provided from celluloid wastes. The bacterial inoculum applied in this experiment contained bacterial stem isolated, purified and tested in laboratory for their capacity to degrade the petroleum hydrocarbons, as: *Pseudomonas*, *Mycobacterium* (*M. roseum* şi *M. phley*), *Arthrobacter* (*A. globiformis, A. citreus*), *Bacillus megaterium*, and *Streptomyces griseus*.

The polluted soil with petroleum hydrocarbons was treated with different quantities of the natural hydrocarbon absorbent and bacterial inoculum determined variation of the chemical characteristics of the soil. It was analysed the following chemical characteristics using standard methods: soil reaction (pH), organic carbon (Walkley and Black method), total azote (Kjeldahl method), C/N ratio by calculating.

The total petroleum hydrocarbons were quantified by a gravimetric method, following previous solid–liquid extraction in a Soxhlet system. The extraction was carried out with methylene chloride in 1-2 g soil samples, which had been previously dried and grounded.

RESULTS AND DISCUSSIONS

The technology proposed for remediation has to most appropiate to the natural processes and in the same time will become an available technology on market.

Chemical characteristics of the soil from experimental variants during the experiment such as, pH, total organic carbon, nitrogen, C/N ratios were determined using characteristic methods.

In *table 1* and *2* are presented the results obtained for two experimental years as a mean of three repetitions with its standard deviation.

In *table 1* are presented some chemical characteristics of the soil used in all the experimental variants, like soil reaction, organic carbon content, total nitrogen content, C/N ratio during the first experimental year, in the beginning and in the end of the year.

In the soil polluted with crude oil, the organic carbon contents were higher than the control at all concentrations of contamination and at all treatments applied. As it can be observed in the table, the organic carbon content increases with crude oil concentration in the experimental variants where the soil was polluted with 5% crude oil, respectively 10% crude oil, comparatively with the control.

Table 1

Chemical characteristics of the soil from experimental variants during the first experimental year

Perimental variant	pH		Organic C (%)		Total N (%)		C/N ratio	
	In the beginning	In the end	In the beginning	In the end	In the beginning	In the end	In the beginning	In the end
V_1, control (unpolluted soil)	8.08±0.041	8.02±0.058	3.27±0.223	3.22±0.219	0.324±0.023	0.340±0.064	11.86±0.877	11.53±1.330
V_2, polluted soil with 5% crude oil	8.12±0.018	8.13±0.071	6.78±0.396	7.23±0.095	0.301±0.017	0.290±0.028	26.36±0.984	29.67±3.212
V_3, polluted soil with 10% crude oil	8.11±0.023	8.13±0.162	9.36±0.996	7.77±0.557	0.283±0.020	0.326±0.020	39.44±6.244	27.78±0.447
V_4, polluted soil with 5% crude oil + 50 g ECOSOL	8.19±0.020	8.26±0.072	8.45±0.944	7.56±0.791	0.308±0.025	0.284±0.015	32.93±6.110	31.10±2.941
V_5, polluted soil with 5% crude oil + 50 g ECOSOL + bacterial inoculum	8.24±0.022	8.24±0.067	7.99±0.580	7.69±0.040	0.311±0.012	0.298±0.018	30.18±3.027	30.33±1.821
V_6, polluted soil with 5% crude oil + 100 g ECOSOL	8.28±0.050	8.33±0.061	7.42±0.749	7.57±0.156	0.334±0.005	0.324±0.034	25.89±2.240	27.95±3.336
V_7, polluted soil with 5% crude oil + 100 g ECOSOL + bacterial inoculum	8.34±0.035	8.35±0.079	8.17±0.383	7.81±0.126	0.328±0.027	0.369±0.046	29.35±1.963	25.60±3.532
V_8, polluted soil with 10% crude oil + 100 g ECOSOL	8.30±0.032	8.40±0.092	8.68±1.123	10.57±1.559	0.305±0.004	0.357±0.050	33.19±4.064	34.79±3.480
V_9, polluted soil with 10% crude oil + 100 g ECOSOL + bacterial inoculum	8.33±0.065	8.42±0.084	10.46±1.837	7.99±0.362	0.289±0.009	0.304±0.016	42.68±8.632	30.72±0.694
V_{10}, polluted soil with 10% crude oil + 200 g ECOSOL	8.49±0.117	8.53±0.123	9.39±0.876	8.29±0.217	0.298±0.016	0.314±0.031	36.94±3.841	31.43±3.524
V_{11}, polluted soil with 10% crude oil + 200 g ECOSOL + bacterial inoculum	8.44±0.079	8.56±0.137	9.17±0.829	8.00±0.245	0.283±0.004	0.343±0.043	37.84±3.851	27.87±2.494

Table 2

Chemical characteristics of the soil from experimental variants during the second experimental year

Experimental variant	pH		Organic C (%)		Total N (%)		C/N ratio	
	In the beginning	In the end	In the beginning	In the end	In the beginning	In the end	In the beginning	In the end
V_1, control (unpolluted soil)	8.09±0.012	8.03±0.025	2.95±0.046	3.28±0.087	0.308±0.006	0.331±0.011	11.17±0.308	11.61±0.644
V_2, polluted soil with 5% crude oil	8.14±0.018	8.17±0.027	6.23±0.024	5.62±0.196	0.347±0.005	0.305±0.003	20.96±0.291	21.49±0.585
V_3, polluted soil with 10% crude oil	8.10±0.015	7.82±0.044	12.24±1.023	8.44±0.910	0.342±0.026	0.310±0.010	42.49±6.187	31.63±2.721
V_4, polluted soil with 5% crude oil + 50 g ECOSOL	8.15±0.006	8.17±0.021	6.51±0.202	6.43±0.393	0.306±0.006	0.298±0.010	24.83±0.389	25.29±2.330
V_5, polluted soil with 5% crude oil + 50 g ECOSOL + bacterial inoculum	8.21±0.021	8.17±0.019	6.24±0.100	5.73±0.157	0.337±0.002	0.333±0.025	21.63±0.294	20.24±1.247
V_6, polluted soil with 5% crude oil + 100 g ECOSOL	8.18±0.015	8.26±0.026	6.29±0.073	5.84±0.143	0.330±0.021	0.314±0.022	22.41±1.508	21.83±1.344
V_7, polluted soil with 5% crude oil + 100 g ECOSOL + bacterial inoculum	8.30±0.009	8.21±0.012	6.04±0.107	5.71±0.038	0.374±0.012	0.314±0.007	20.05±1.010	21.22±0.363
V_8, polluted soil with 10% crude oil + 100 g ECOSOL	8.24±0.015	7.93±0.058	10.28±0.834	8.89±0.428	0.346±0.014	0.340±0.011	34.92±3.970	30.64±2.064
V_9, polluted soil with 10% crude oil + 100 g ECOSOL + bacterial inoculum	8.23±0.012	8.07±0.023	10.34±0.230	8.70±0.410	0.311±0.009	0.331±0.012	38.84±0.545	30.79±2.365
V_{10}, polluted soil with 10% crude oil + 200 g ECOSOL	8.27±0.006	7.98±0.026	10.47±0.141	8.60±0.214	0.330±0.014	0.324±0.025	32.10±4.529	31.48±2.993
V_{11}, polluted soil with 10% crude oil + 200 g ECOSOL + bacterial inoculum	8.29±0.006	7.99±0.060	10.67±0.406	8.46±0.131	0.387±0.050	0.327±0.011	33.29±4.494	30.26±0.737

The total nitrogen contents fluctuate in the experimental variants contaminated with crude oil, treated with ECOSOL and bacterial inoculum.

In the soil contaminated with crude oil, the C/N ratios were higher than the control at all concentrations of contamination and at all treatments applied. As it can be observed in the table, the C/N ratio increases with crude oil concentration in the experimental variants where the soil was polluted with 5% crude oil, respectively 10% crude oil, comparatively with the control. In the experimental variants V_4, V_5, V_6 and V_7, the C/N ratios increase comparatively with V_2, even it is the same concentration of petroleum hydrocarbons, because of the treatment with different quantities of ECOSOL.

In *table 2* are presented some chemical characteristics of the soil used in all the experimental variants, like soil reaction, organic carbon content, total nitrogen content, C/N ratio during the second experimental year, in the beginning and in the end of the year. The same fluctuation of the chemical parameters can be observed during the second experimental year.

In *figure 1* and *2* are presented the evolution of total petroleum hydrocarbons in the soil polluted with crude oil (5% and 10%) during the two experimental years, in all variants and repetitions.

The obtained results show that the biodegradation takes time. A decrease was recorded in time and this agrees with the observation existing in scientific literature.

The obtained results revealed that the total petroleum hydrocarbons concentrations were higher on contaminated soils compared to the control suggesting the presence of more petroleum hydrocarbons.

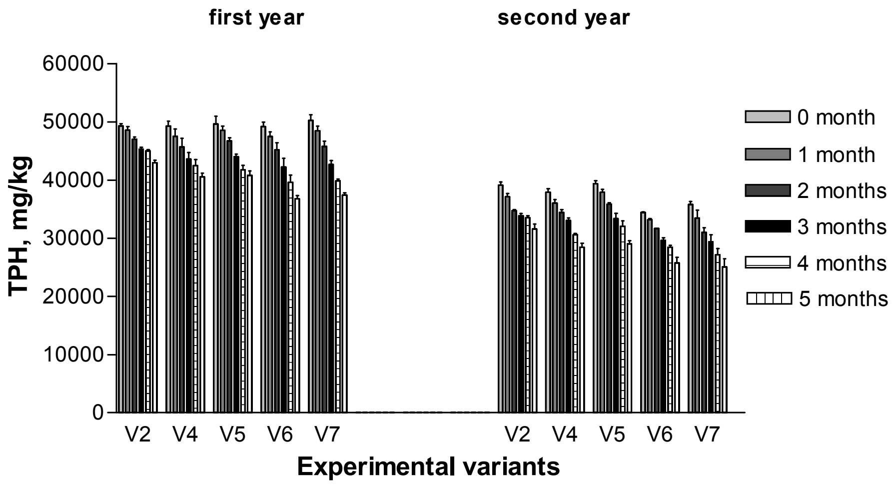

Figure 1 **The evolution of total petroleum hydrocarbons in the soil pollutd with 5% crude oil during two experimental years**

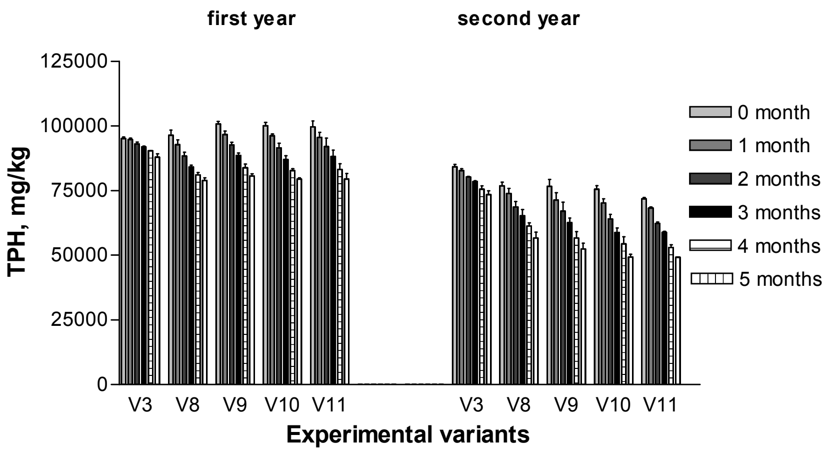

Figure 2 **The evolution of total petroleum hydrocarbons in the soil pollutd with 10% crude oil during two experimental years**

CONCLUSIONS

The treatment with the biodegradable product can achieve the degradation of petroleum hydrocarbons in agricultural soils. Also is necessary to supply the microorganisms existing in soil by bacterial inoculum.

The crude oil represented a mineralisable carbon source, as demonstrated by the decrease of TPH concentrations. The natural biodegradable product enhanced TPH degradation by stimulating microbial activity and growth via excretion of readily degradable carbon.

The experiment will continue in Green House to evidence the effectiveness of the biodegradable product and bacterial inoculum.

BIBLIOGRAPHY

Atlas, R.M., Bartha, R., 1992 - *Hydrocarbon biodegradation and oil spill, Bioremediation.In: Advances in Microbial Ecology*, Marshall K.C (editor), Vol 12, Plenum. New York,.USA, p. 287-338.

Coulon, F., Pelletier, E., Gourhant, L., Delille, D., 2005 - *Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soils*, Chemosphere 58(10), p. 1439-1448.

Fava, F., Ciccotosto, V.F., 2002 - *Effects of Randomly Methylated-b-Cyclodextrins (RAMEB) on the bioavailability and aerobic biodegradation of polychlorinated biphenyls in three pristine soils spiked with an transformer oil*, Applied Microbiology and Biotechnology 58, p. 393-399.

Leahy, J.G., Colwell, R.R., 1990 - *Microbial degradation of hydrocarbons in the environment*, Microbiol. Rev. 54, p. 305–315.

Margesin, R., Schinner, F., 2001 - *Biodegradation and bioremediation of hydrocarbons in extreme environments*, Applied Microbiotechnology 56, p. 650-663.

Nadeau, R.R, Singhvi, J., Ryabik, Y., Lin, I., Syslo, J., 1993 - *Monitoring bioremediation for bioremediation efficacy: the Marrow Marsh experience*, Proceedings of the sOil Spill Conference. American Petroleum Institute, Washington D.C., p. 477-485.

Pepper, Ian L., Gerba, Charles P., Brusseau, Mark L, 1996 - *Pollution Science*, Academic Press, ISBN 0-12-550660-0.

Rahman, K.S.M., Thahira-Rahman, J., Kourkoutas, Y., Petsas, I., Marchant, R., Banat, I.M., 2003 - *Enhanced bioremediation of n-alkanes in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients*, Bioresource Technology 90, p. 159–168.

Snape, I. Riddle, M.J., Stark, J.S., Cole, C.M., King, C.K., Duquesne, S., 2001 - *Management and remediation of contaminated sites at Casey Station*, Antarctica, Polar record, 37, p. 199-214.

Walworth, J.L.,Woolard, C.R., Braddock, J.F., Reynolds, C.M., 1997 - *Enhancement and inhibition of soil petroleum biodegradation through the use of fertilizer nitrogen: an approach to determining optimum levels*, Journal of Soil Contamination 6 (5), p. 465–480.