THE EVOLUTION OF TOTAL NUMBER OF FUNGI IN SOIL POLLUTED WITH CRUDE OIL

Mariana MARINESCU, M. DUMITRU, Anca LACATUSU, D.M. MOTELICA, M. MARINESCU

National Research Development Institute for Soil Science, Agrochemistry and Environmental Protection, 61 Marasti Avenue, Bucharest, Romania
Corresponding author: Mariana Marinescu, e-mail:maramarinescu2003@yahoo.com

Abstract: *For bioremediation to be successful, the bioremediation methods depend on having the right microbes in the right place with the right environmental factors for degradation to occur. The right microbes are bacteria or fungi, which have the physiological and metabolic capabilities to degrade the pollutants. Recent studies have reported several bacteria and filamentous fungi species with the capacity to mineralize or to degrade petroleum hydrocarbons. Bioremediation can be done on site, is often less expensive and site disruption is minimal, it eliminates waste permanently, eliminates long-term liability, and has greater public acceptance, with regulatory encouragement, and it can be coupled with other physical or chemical treatment methods. Chemical pollution of the soil environment has become a major source of concern. Crude oil bioremediation of soils is limited by the bacteria activity in degrading the spills hydrocarbons. In this paper are presented the results obtained in a bioremediation laboratory experiment. The aim of this study is to enhance the bioremediation of soils polluted with crude oil by adding the natural biodegradable product and bacterial inoculum. A natural biodegradable product and bacterial inoculum was used for total petroleum hydrocarbon (TPH) removal from an artificial polluted soil. Soil polluted with 50000 mg/kg of TPH was treated with 0.25%, respective 0.5% and/or bacterial inoculum to increase the biodegradability rate. Also, the soil contaminated with 100000 mg/kg of TPH was treated with 0.5%, respective 1% and/or bacterial inoculum. The main objective of this work is to accelerate the biodegradation processes. The enhancement of petroleum hydrocarbons degradation was achieved under natural product treatment and bacterial inoculum. The bacterial inoculum was used to enrich indigenous microbes to enhance biodegradation rate in the green house experiment. In soil excessively polluted with crude oil, bacterial population size in conditioned variant with Ecosol maximum dose (1%) presented values comparable to those of inoculated variants, demonstrating the protective and stimulation effect of soil bacteria, including those involved in the degradation of petroleum hydrocarbons exercised by organic compound applied Ecosol. At each phase of the study, the natural biodegradable product was found to significantly enhance the biodegradation of petroleum hydrocarbons.*

Key words: *total number of fungi, crude oil, pollution, cambic chernozem.*

INTRODUCTION

In-situ bioremediation is based on the activity of microorganisms to use hydrocarbons oil as a source of carbon and energy. This method is considered to be the most effective because it does not have irreversible negative effects on the characteristics of soil and low cost (Mancera-López et al., 2008). Microorganisms such as bacteria, fungi and yeasts decompose these hazardous chemicals in non-toxic or less toxic compounds (Dart and Strestton, 1994; April et al. 2000).

Recent research has shown that a number of filamentous fungi play an incorporated role into the biodegradable in-situ, being able to degrade the aliphatic hydrocarbon fractions C12-C26 in the oil (Foght et al., 1999; D'Annibale et al., 2006). Moreover, by cultivating on media as the single existing carbon energy source petroleum hydrocarbons has been proven the capacity to biodegrade crude oil of the genera strains *Aspergillus*, *Penicillium* and *Fusarium Paecilimyces*, *Cladosporium*, *Tricodarma* (Lemos et al., 2002; Potin et al., 2004).

Once isolated and purified, fungi were tested for their capacity to degrade hydrocarbons from crude oil. By removing delicate conidia and mycelium of each isolates, they were inoculated in the medium in which the mineral oil was added crude oil as the sole source of carbon and energy. Nine filamentous fungi were selected because of the capacity to increase and to use petroleum hydrocarbons. Experiment conclusions were that filamentous fungi actively involved in biodegradation of crude oil soil are: *Aspergillus niveus*, *A. niger*, *A. versicolor*, *A. terreus*, *A. fumigatus*, *Penicillinium corylophilium*, *Paecilomyces variotti*, *P. niveus* and *Fusarrium* sp. The active species was *Aspergillus versicolor* with the highest pollutant removal efficiency (10.8%) (Chávez-Gómez et al., 2003; Voiculescu et al., 2005).

MATERIALS AND METHOD

The main objective of this research is testing the natural hydrocarbon absorbent named ECOSOL. It is tested the capacity to increase the biodegradation of petroleum hydrocarbons by stimulating the bacteria. To achieve data concerning the bioremediation of polluted soil with petroleum hydrocarbons was realized a greenhouse experiment. The soil used for this experiment (calcic chernozems) was reaped from arable layer 0-20 cm (Teleorman). This type of soil was chosen because of its currency in our country, also, for its physical, chemical and biological properties favorable to plant growth.

The study focused on the application of the two major technologies known in bioremediation method such as: soil biostimulation based on environmental conditions improvement for microorganisms multiplication and activity to degrade petroleum hydrocarbons, and bioaugmentation based on enriching the soil with specific biodegrading hydrocarbons microorganisms.

Biostimulation - the first technological link included a innovation element based on 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 analysing a series of organic compounds suitable for conditioning soil contaminated with organic pollutants, especially because of its biodegradability properties.

Bioaugmentation - the second technology link was achieved by soil inoculation with bacterial bioproducts made from specific bacteria selected and tested in the laboratory for their ability to degrade petroleum hydrocarbons.

ECOSOL is an absorbent natural product, meant to facilitate quick and efficient biodegradation of hydrocarbons from contaminated soils. Accelerates biostimulation and favors the development of existing bacteria from the soil, with strong effects in crude oil biodegradation. This natural biodegradable product 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 experimental variants are:

- ✓ V_1, control (unpolluted soil);
- ✓ V_2, polluted soil with 5% crude oil;
- ✓ V_3, polluted soil with 10% crude oil;
- ✓ V_4, polluted soil with 5% crude oil + 50 g ECOSOL;
- ✓ V_5, polluted soil with 5% crude oil + 50 g ECOSOL + bacterial inoculum;

- ✓ V_6, polluted soil with 5% crude oil + 100 g ECOSOL;
- ✓ V_7, polluted soil with 5% crude oil + 100 g ECOSOL + bacterial inoculum;
- ✓ V_8, polluted soil with 10% crude oil + 100 g ECOSOL;
- ✓ V_9, polluted soil with 10% crude oil + 100 g ECOSOL + bacterial inoculum;
- ✓ V_{10}, polluted soil with 10% crude oil + 200 g ECOSOL;
- ✓ V_{11}, polluted soil with 10% crude oil + 200 g ECOSOL + bacterial inoculum.

Fungi were identified macroscopically and by microscopic observation.

The values obtained by analyzing soil and plant samples were processed using more specific methods of mathematical statistics. Analysis of variance for establishing Fischer and Tukey tests determined for $\alpha = 0.05$, which shows the changes produced on soil and plant characteristics, the effects of treatments applied. ANOVA method provides information allowing the calculation of limit differences used in multiple comparison methods and the mean average for each graduation of studied factor. By correlation method was determined the linear correlation coefficient or the correlation ratio (index), for assessing the intensity of the relationship between variables. For the estimation of a link between the two characteristics studied, stochastic experiments were conducted by achieving regression equations.

RESULTS AND DISCUSSION

The native fungi were able to grow in a complex solid mixture of hydrocarbons of high molecular weight, after previous acclimatization in liquid culture.

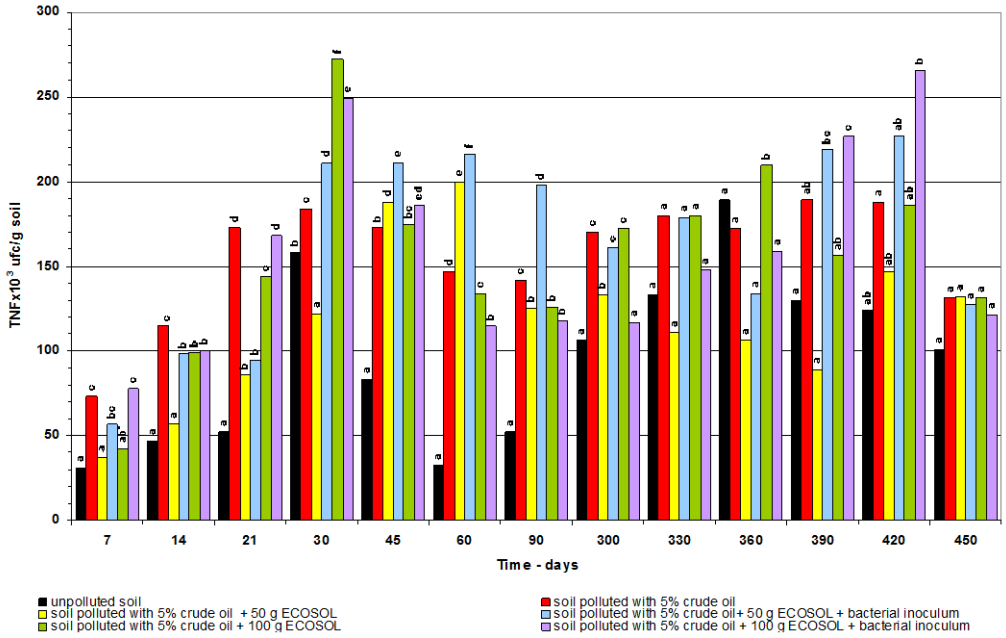
In the experimental variant with soil polluted with 5% crude oil, from the first stage of measurements, at 7 days class, the values of total number fungi in the polluted soil were higher than that of the control (Figure 1).

The proliferation of filamentous fungi in polluted soil continues until reaching a peak at 30 days from the beginning of the experiment with very high especially in the variants inoculated with selected bacteria and those treated with 0.5% Ecosol.

Filamentous fungi known for metabolic opportunism makes them very competitive in the use of readily degradable petroleum hydrocarbons, even better than heterotrophic bacteria hydrocarbon degrading.

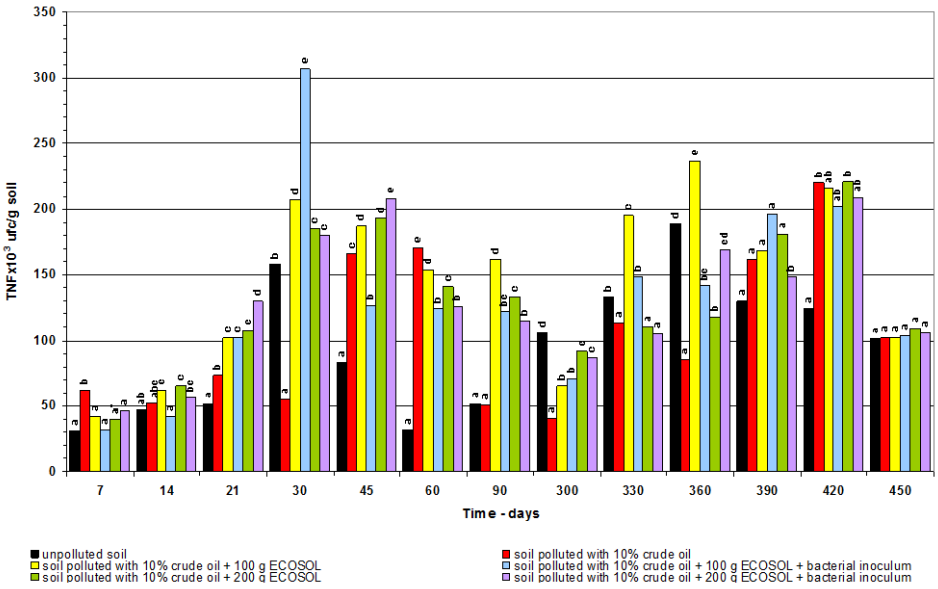
In the early stages of the experiment carried out at 300 days until the end of the research, the size of fungal populations in polluted variants remained high compared with the unpolluted one. Values significantly greater of total number of fungi in polluted soil compared to the control, and to the 390 to 420 days from the start of the experiment may be explained by the selection of those species of fungal communities equipped with capable degrading enzyme of complex organic molecules such as polyaromatic hydrocarbons and asphaltene.

The experimental variants of soil polluted with 10% oil, excessive concentration of pollutant is manifested by inhibition of microfungi communities in the first two steps of determining, in contrast to the described above in soil polluted with only 5% oil (Figure 2).



***for each moment of TNF deteremination the experimental variants were marked with the same letter (a, b, ...) do not differ significant (test Tukey - α = 0,05)**

Figure 1. The evolution of total number of fungi (TNF) in soil polluted with 5% crude oil in different experimental variants – analysis of variance



***for each moment of TNF deteremination the experimental variants were marked with the same letter (a, b, ...) do not differ significant (test Tukey - α = 0,05)**

Figure 2 The evolution of total number of fungi (TNF) in soil polluted with 10% crude oil in different experimental variants – analysis of varianc

The inhibitory effect of the pollutant in excess disappears after 21 days, the total number of fungi in all experimental variants were significantly higher compared with unpolluted soil. At 30 days and 45 days after the beggining of the experiment, the polluted experimental variants with the conditioned Ecosol both doses, can be observed an explosion increasing of fungal population showed by values of total number of fungi, much higher than control. In the case of crude oil pollution of soil by 10%, the superiority of fungi communities size from bioremediation experimental variants were maintained higher until the end of experiment. Only after 450 days, the values of the total number of fungi in polluted variants were equalized with the value determined in unpolluted soil.

CONCLUSIONS

Explosive multiplication of filamentous fungi in soil polluted with crude oil compared to unpolluted soil clearly show the presence of an alternative nutrient substrate can be represented only by petroleum hydrocarbons, known metabolic opportunism of filamentous fungi making them very powerful competitors in the use of easily degradable petroleum hydrocarbons, heterotrophic bacteria even at the expense of degrading hydrocarbons.

Quantitative analytical data of filamentous fungi in chernozem polluted with crude oil shows that this group of organisms demonstrates at least very high tolerance to the pollutant presence in the soil and continue to build further research and the microorganisms role in the petroleum hydrocarbons degradation, is, safety required.

BIBLIOGRAPHY

1. APRIL T.M., FOGHT J.M., CURRAH R.S., *Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada*, Canadian Journal of Microbiology 46, p. 38–49, 2000.
2. CHÁVEZ-GÓMEZ B., QUINTERO R., ESPARZA-GARCÍA F., MESTA-HOWARD A., de la Serna F.J.Z.D., Hernández-Rodríguez C., Gillen T., Poggi-Varaldo H., Barrera-Cortés J., Rodríguez-Vázquez R., *Removal of phenanthrene from soil by co-cultures of bacteria and fungi pregrown on sugarcane bagasse pit*, Bioresource Technology 89, p. 177–183, 2003.
3. D'ANNIBALE A., ROSETTO F., LEONARDI V., FEDERICI F., PETRUCCIOLI M., *Role of autochthonous filamentous fungi in bioremediation of a soil historically contaminated with aromatic hydrocarbons*, Applied and Environmental Microbiology 72, p. 28–36, 2006.
4. DART R.K., STRETTON R.J., *Microbiological Aspects of Pollution Control*, 2nd edition, Elsevier, 265 p., ISBN 0444415890, 1994.
5. FOGHT J., SEMPLE K., GAUTHIER C., WESTLAKE D., BLENKINSOPP S., SERGY G., WANG Z., FINGAS M., *Effect of nitrogen source on biodegradation of crude oil by a defined bacterial consortium incubated under cold, marine conditions*, Environmental Technology 20, p. 839-849, 1999.
6. LEMOS J.L., RIZZO A.C., MILLIOLI V.S., SORIANO A.U., de MOURA SARQUIS M.I., Santos R., *Petroleum degradation by filamentous fungi*, http//ipec.utulsa.edu/lpec/conf2002/lemos_santos_7.pdf, 2002.
7. MANCERA-LÓPEZ M.E., ESPARZA-GARCÍA F., CHÁVEZ-GÓMEZ B., VÁSQUEZ R., SAUCEDO-CASTAÑEDA G., BARRERA-COTÉS J., *Bioremediation of an aged hydrocarboncontaminated soil by a combined system of biostimulation-bioaugmentation with filamentous fungi*, International Biodeterioration and Biodegradation 61, p. 151-160, 2008.
8. POTIN O., RAFIN C., VEIGNIE E., *Bioremediation of an agedpolycyclic aromatic hydrocarbons (PAHs)-contaminated soil by filamentous fungi isolated from the soil*, International Biodeterioration and Biodegradation 54, p. 45–52, 2004.
9. VOICULESCU Anca-Rovena, DUMITRU M., TOTI M., *Decontaminarea Solurilor Poluate cu Compuşi Organici*, Ed. SITECH, Craiova, ISBN 973-657-939-5, 2003.