EFFECTS OF BIOREMEDIATION TREATMENTS OF A SOIL POLLUTED WITH CRUDE OIL ON SOIL REACTION

Mariana MARINESCU, Anca LACATUSU, Eugenia GAMENT, Georgiana PLOPEANU, M. MARINESCU

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

Abstract. *Physical, chemical and thermal methods have commonly been employed to clean up the crude oil polluted sites. However, these techniques are relatively expensive and also require site restoration. Therefore, it is an urgent need for promotion of environment friendly techniques for reclamation of crude oil polluted sites. Recent studies have been reported on environment friendly bioremediation. These methods are relatively affordable and do not introduce any additional chemicals to the environment. The uses of biological materials like bacteria, fungi, algae with vermicompost, animal and plant compost, have been reported with good bioremediation potential. Bacteria are one of the best candidates for bioremediation because they have biodiversity, variety of catabolic genes and vast potential for degradation of harmful contamination. The best bioremediation technique is intrinsic bioremediation because it has minimum intervention in the environment. In this paper are presented the effect of bioremediation treatments of a soil polluted with crude oil on soil reaction. Experiments included two groups of experimental variants in which the soil was controlled polluted by 5% and 10% crude oil. For each concentrations of crude oil were created 4 experimental variants, achieved by conditioning the soil with Ecosol in two doses, with or without inoculation with bacteria selected, plus a control variant were the soil polluted was subject to natural attenuation. All data were reported at a variant with unpolluted soil achieved and maintained under the same experimental conditions. Soil reaction in unpolluted soil - control, varies around the average value of 8.11 which defines a weakly alkaline soil. The effect of the application of Ecosol product on soil reaction is to increase values of up to 8.78 or 8.83 in the experimental variants with 1% Ecosol. This pH increasing defines soil as a moderated alkali. This could be detrimental to the bioremediation treatments efficiency of soil polluted with crude oil, as microorganisms metabolizing petroleum hydrocarbons have an optimum around a neutral range in the soil reaction. During the three experimental years it can be observed an uniformity of soil reaction. Ecosol product caused a slight alkalizing immediately after application in soil, but with a limited duration, not the activity of bacteria exerted negative influences on the optimal environment for development which is located around neutral.*

Keywords*: bioremediation treatments, soil polluted, crude oil, soil reaction*

INTRODUCTION

Bioremediation is cost-effective (COUTO ET AL., 2010) and an environmental friendly method (SINGH ET AL., 2009) to remove pollutants using microbes (HARITASH AND KAUSHIK, 2009). Bioremediation has been applied for the treatment of hydrocarbon-contaminated sites (COLOMBO ET AL., 2011), but biological-based remediation technologies may be time-consuming. Soil conditions such as aging of crude oil-contaminated soil (Jho et al., 2014), differences in soil matrix type (SUTTON ET AL., 2013), and the nature of the contaminants may limit the efficiency of biodegradation (TANG ET AL., 2012).

Various institutions and organizations, some multidisciplinary other specialized publications, focused solely on pollution issues. There is no life without soil (ALEXANDER, 1997, PEPPER ET AL., 1996). Crude oil is a complex mixture of hydrocarbons. It includes a saturate fraction, an aromatic fraction, asphaltenes, and resins (ATLAS AND BARTHA, 1992; OKOH AND TREJO-HERNANDEZ, 2006).

Due to this complexity, petroleum hydrocarbons cannot be fully degraded by a single strain of microorganisms but its decomposition is achieved by microbial consortia and their broad enzymatic capacity (NORRIS AND MATTHEWS, 1994).

MATERIAL AND METHODS

In this paper are presented the effect of bioremediation treatments of a soil polluted with crude oil on soil reaction. Experiments included two groups of experimental variants in which the soil was controlled polluted by 5% and 10% crude oil. For each concentrations of crude oil were created 4 experimental variants, achieved by conditioning the soil with Ecosol in two doses, with or without inoculation with bacteria selected, plus a control variant were the soil polluted was subject to natural attenuation.

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 + 0.25% ECOSOL;
- ✓ V_5, polluted soil with 5% crude oil + 0.25% ECOSOL + bacterial inoculum;
- ✓ V_6, polluted soil with 5% crude oil + 0.5% ECOSOL;
- ✓ V_7, polluted soil with 5% crude oil + 0.5% ECOSOL + bacterial inoculum;
- ✓ V_8, polluted soil with 10% crude oil + 0.5% ECOSOL;
- ✓ V_9, polluted soil with 10% crude oil + 0.5% ECOSOL + bacterial inoculum;
- ✓ V_{10}, polluted soil with 10% crude oil + 1% ECOSOL;
- ✓ V_{11}, polluted soil with 10% crude oil + 1% ECOSOL + bacterial inoculum.

All data were reported at a variant with unpolluted soil achieved and maintained under the same experimental conditions. 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.

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.

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.

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 chemical characteristics of Calcic chernozem from Teleorman area are presented in table 1.

Table 1

Chemical characteristics of Calcic chernozem from Teleorman area (n=3)

Characteristics	U.M.	Mean value
pH_{H2O}	-	8.09
Total nitrogen content	%	0.279
Organic carbon content	%	2.99
C/N Ratio	-	12.5
Mobile phosphorous content	$mg \cdot kg^{-1}$	50
Mobile potassium content	$mg \cdot kg^{-1}$	215

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 chemical characteristics of the natural biodegradable product ECOSOL are presented in table 2.

Table 2

Chemical characteristics of the natural biodegradable product ECOSOL (n=3)

Characteristics	U.M.	Mean value
Total nitrogen content	%	0.935
Organic carbon content	%	23.72
Total phosphorous content	%	0.39
Total potassium content	%	3.32
Total natrium content	%	4.97

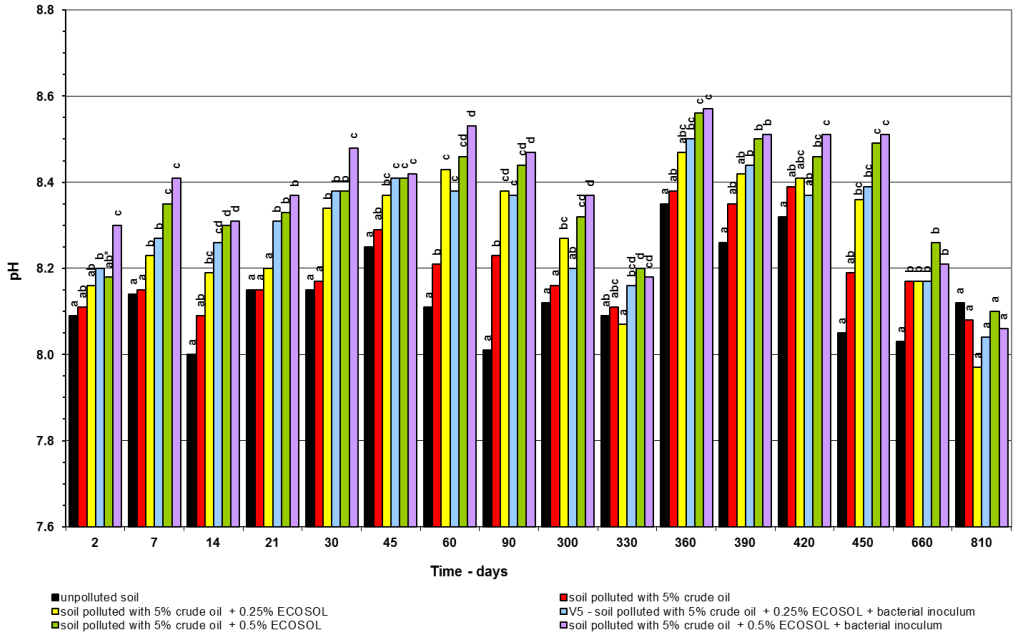
RESULTS AND DISCUSSIONS

Soil reaction in unpolluted soil - control, varies around the average value of 8.11 which defines a weakly alkaline soil. The effect of the application of Ecosol product on soil reaction is to increase values of up to 8.78 or 8.83 in the experimental variants with 1% Ecosol. This pH increasing defines soil as a moderated alkali. This could be detrimental to the bioremediation treatments efficiency of soil polluted with crude oil, as microorganisms metabolizing petroleum hydrocarbons have an optimum around a neutral range in the soil reaction. During the three experimental years it can be observed an uniformity of soil reaction. Ecosol product caused a slight alkalizing immediately after application in soil, but with a limited duration, not the activity of bacteria exerted negative influences on the optimal environment for development which is located around neutral.

The results of the soil reaction determination carried out throughout the experimental period of three years, both experimental variants polluted with 5% and 10% crude oil are shown in Figures 1 and 2.

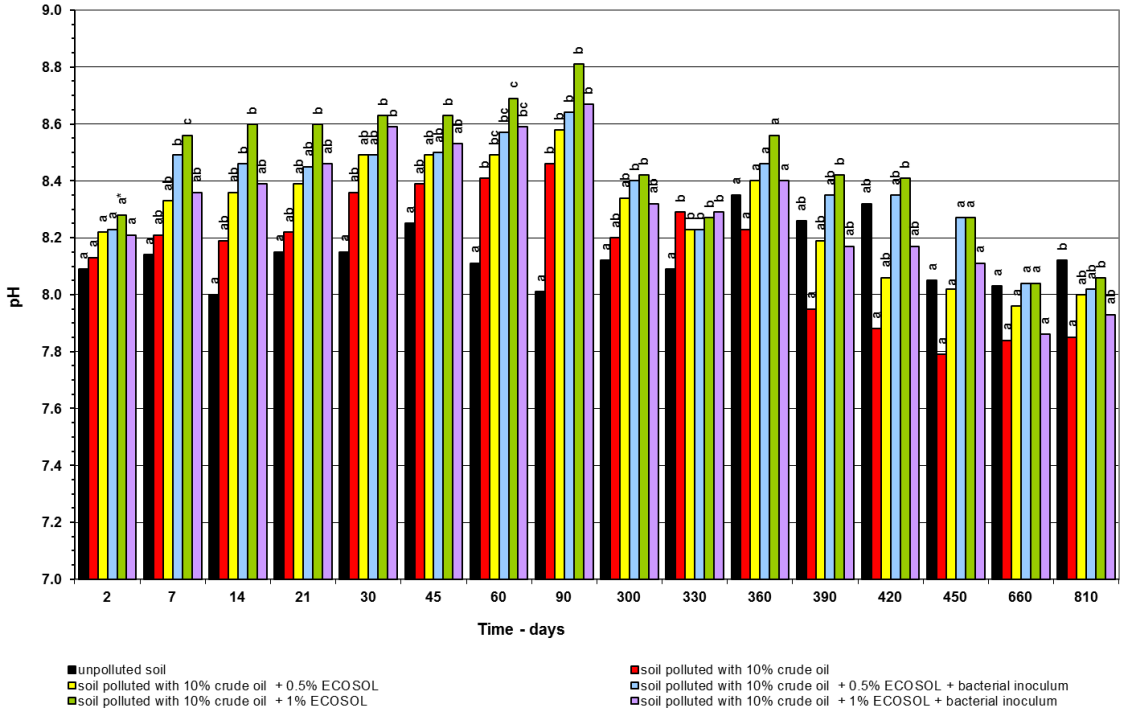
In case of soil polluted with 5% crude oil (Figure 1) is most notable the uniformity in the soil reaction to the end of the experiment, results with a high degree of statistical assurance.

In experimental variants with soil polluted by 10% crude oil, it can be observed the same uniformity as in the case of soil polluted with 5% crude oil, being registred higher pH values in the experimental variants treated with higher Ecosol concentration (Figure 2).



***for each moment of soil reaction 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 soil reaction in soil polluted with 5% crude oil in different experimental variants – analysis of variance



***for each moment of soil reaction 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 soil reaction in soil polluted with 10% crude oil in different experimental variants – analysis of variance

CONCLUSIONS

During the three experimental years it can be observed an uniformity of soil reaction. Ecosol product caused a slight alkalizing immediately after application in soil, but with a limited duration, not the activity of bacteria exerted negative influences on the optimal environment for development which is located around neutral.

This could be detrimental to the bioremediation treatments efficiency of soil polluted with crude oil, as microorganisms metabolizing petroleum hydrocarbons have an optimum around a neutral range in the soil reaction.

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