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BIODEGRADATION OF PETROLEUM HYDROCARBONS IN A POLLUTED SOIL AS MEDIATED BY A NATURAL BIODEGRADABLE PRODUCT

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Abstract

The major part of soil petroleum hydrocarbon pollution is derived from the spillages related to the use and transportation of petroleum products. It is known that the main microorganisms consuming petroleum hydrocarbons are bacteria, so in this paper are presented the results obtained in a bioremediation laboratory experiment. 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 50 g (0.25%), respective 100 g ECOSOL/20 kg polluted soil (0.5%) and/or bacterial inoculum to increase the biodegradability rate. Also, the soil contaminated with 100000 mg/kg of TPH was treated with 100 g (0.5%), respective 200 g ECOSOL/20 kg polluted soil (1%) and/or bacterial inoculum. The highest TPH removal was obtained with a treatment consisting in 100 g ECOSOL/20 kg polluted soil (0.5%) and bacterial inoculum in case of 5% TPH and 200 g ECOSOL/20 kg polluted soil (1%) and bacterial inoculum in case of 10% TPH, corresponding with the highest rates of microbial respiration and the greatest increases in bacterial counts. At each phase of the study, the natural biodegradable product was found to significantly enhance the biodegradation of petroleum hydrocarbons.

Key words**:** *biodegradation, petroleum hydrocarbons, polluted soil, a natural biodegradable product.*

INTRODUCTION

In recent years, bioremediation of soils polluted with petroleum hydrocarbons is a challenge in research [12]. Research has shown that the bioremediation is a superior method of soil remediation, effective and cheaper compared with physicochemical methods. Bioremediation is based on microorganisms activity to use of petroleum hydrocarbons as carbon and energy source. This method is considered to be most effective because no irreversible effects can on pedogenetical soil characteristics and low cost. Microorganisms such as bacteria, fungi and yeasts decompose these hazardous chemicals in non-toxic or less toxic compounds by the enzymatic complex [6].

Crude oil contains hydrocarbons whose molecular mass varies from 16 (methane) to about 1800. Is a complex mixture of gaseous or solids hydrocarbons dissolved in liquid hydrocarbons, and therefore likely to appear in the liquid state. Crude oil also contains organic compounds with oxygen (phenols, naphthenic acids), sulfur (thiophen, mercaptan), nitrogen (quinoline). Acyclic hydrocarbons are saturated hydrocarbons (alkanes or paraffins), cyclic saturated hydrocarbons (cycloparaffins or naphthenes) and aromatic hydrocarbons. These three classes of hydrocarbons are found in varying ratios in all types of oil. In crude oil are not unsaturated hydrocarbons [1, 10].

Accidental oil pollution has become nowadays a common phenomenon that can cause environmental and social disasters [3, 4].

Main sources of crude oil pollution are anthropogenic, but there are also some natural sources. In this category are leakages due to the crude oil deposits and organic matter degradation. There are some data that some organisms, such as high plants are able to synthesize hydrocarbons and can penetrate the soil. These sources have a low potential and do not cause excessive soil contamination [13, 14].

Biodegradation is the process by which organisms that are already commonly present in soil, degrade organic contaminants such as crude oil. The process occurs without

intervention. However, degradation rates can be accelerated by plowing to mix with the soil contaminants, by adding chemical fertilizers to provide nutrients deficient, so the addition of organic matter such as manure to stimulate microbial action by aeration of the soil [8, 9]. Crude oils in warm soils, wetlands degrade, in a completely natural, half from the initial batch concentration during the first year and the residue decreases again, with half in each successive year [7].

Biodegradation of hydrocarbons can be converted partially or wholly by a number of microorganisms [5, 11]. Mostly hydrocarbons are not totally degraded by microorganism-mediated processes [2].

MATERIAL AND METHOD

It has been achived an experiment in Green House. The soil used in the experiment was cambic chernozem.

The chemical characteristics of the soil are presented in table 1.

Table 1 Chemical characteristics of the soil

Soil type	pH	C (%)	Nt (%)	C/N Ratio	P_{AL} mg kg^{-1}	K_{AL} mg kg^{-1}
Calcic chernozem	8.09	2.99	0.279	12.5	50	215

The chemical characteristics of the natural biodegradable product used in the experiment are presented in table 2. 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.

Table 2 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

The experiment has 11 experimental variants with soil polluted 5% and 10% crude oil, treated with 50g, 100g and 200g ECOSOL/20 kg polluted soil, inoculated and uninoculated with bacteria selected according to the following experimental scheme:

- ✓ 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/20 kg polluted soil (0.25%);
- ✓ V_5, polluted soil with 5% crude oil + 50 g ECOSOL/20 kg polluted soil (0.25%) + bacterial inoculum;
- ✓ V_6, polluted soil with 5% crude oil + 100 g ECOSOL/20 kg polluted soil (0.5%);
- ✓ V_7, polluted soil with 5% crude oil + 100 g ECOSOL/20 kg polluted soil (0.5%) + bacterial inoculum;
- ✓ V_8, polluted soil with 10% crude oil + 100 g ECOSOL/20 kg polluted soil (0.5%);
- ✓ V_9, polluted soil with 10% crude oil + 100 g ECOSOL/20 kg polluted soil (0.5%) + bacterial inoculum;
- ✓ V_{10}, polluted soil with 10% crude oil + 200 g ECOSOL/20 kg polluted soil (1%);
- ✓ V_{11}, polluted soil with 10% crude oil + 200 g ECOSOL/20 kg polluted soil (1%) + bacterial inoculum.

The experiment was set up by artificial pollution of a cambic chernozem with crude oil and treatment with different quantities of ECOSOL. 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*.

In this research were used two technological methods, biostimulation and bioaugmentation, to improve the biodegradation process. In the first experimental year was observed an increase of biodegradability rate in accordance with the applied treatment, so the experiment continued. The beneficial effects of the treatments applied were observed in the development of maize plants grown in the second experimental year, that could develop only in the polluted variants with 5% crude oil. In pots polluted with excessive concentrations of 10% crude oil had a very severe phytotoxic effect, preventing total germination. In the second experimental year with plant, maize

plants have sprung up in pots polluted with crude oil at both concentrations. Were observed very visible difference between plants affected by crude oil pollution and those developed on clean soil. Inoculation of soil polluted with bacteria selected in combination with soil treatments by 0.5% and 1% ECOSOL had a beneficial effect on maize plant vigor.

Analyses of variances (ANOVAs) were performed to statistically select the treatment with the high efficiency. Results (TPH concentration) were tested by using two-way ANOVA ($p < 0.05$). All calculations were performed with Prism 3.03

RESULTS AND DISCUSSIONS

The soil polluted with 5% and 10% crude oil, treated with different quantities of natural product, inoculated and uninoculated with bacterial inoculum determined a decrease of total petroleum hydrocarbons concentration in accordance with the applied treatment.

The results obtained shows that the biodegradation processes take time according to the existing literature data.

The evolution of total petroleum hydrocarbons concentration with time in experimental variant V_1

- unpolluted soil, V_2 - polluted soil with 5% crude oil and V_3 - polluted soil with 10% crude oil are used as control for treatments. The total petroleum hydrocarbons values were higher on experimental variants V_2 and V_3 suggesting the presence of crude oil.

The total petroleum hydrocarbons concentration decreases in time with 63.8%, respectively 65% in the polluted soil with 5%, respectively 10% crude oil. The evolution of total petroleum hydrocarbons concentration in the polluted soil with 5% crude oil treated with 50 g ECOSOL/20 kg polluted soil is presented in figure 1. The polluted soil with 5% crude oil treated with 50 g ECOSOL/20 kg polluted soil presented a total petroleum hydrocarbons decrease in time with 82.2% in the case of V_4 experimental variant and with 86.6% in case of inoculated variant V_5 (Fig. 3).

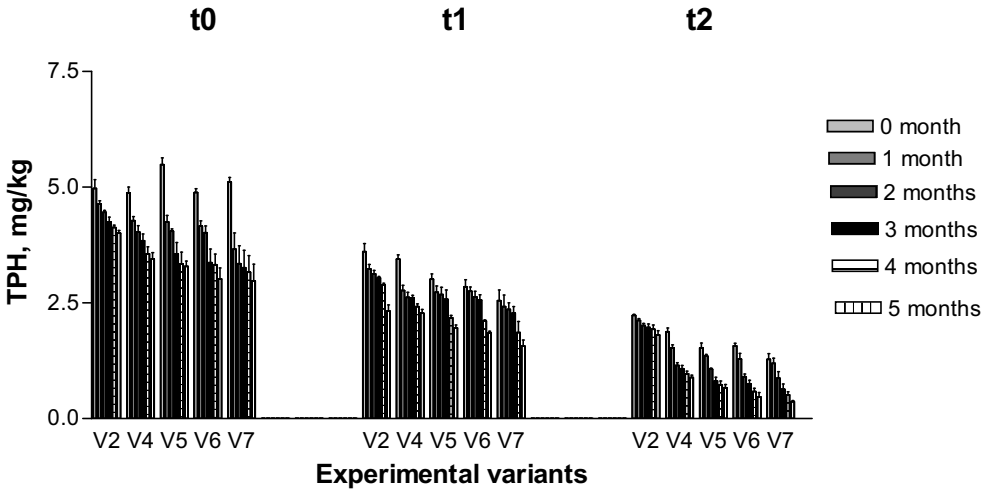

Fig. 1. The evolution of total petroleum hydrocarbons in soils polluted with 5% crude oil

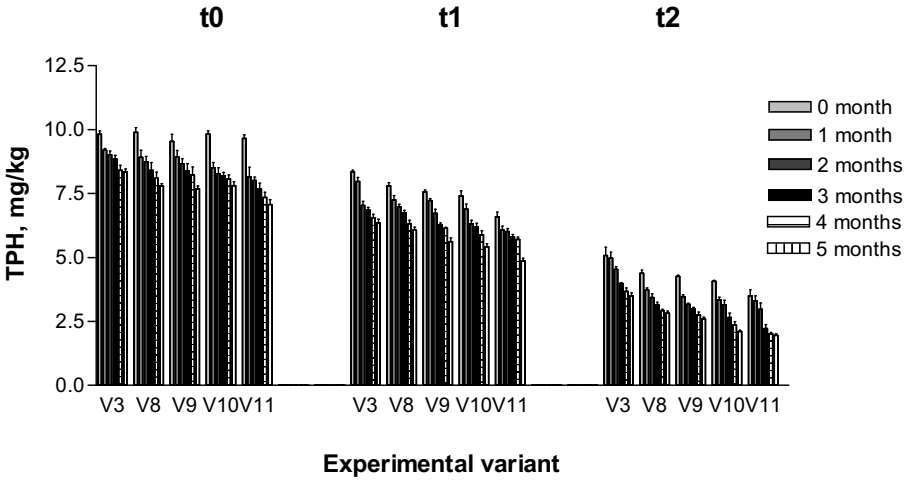

Fig. 2. The evolution of total petroleum hydrocarbons in soils polluted with 10% crude oil

The total petroleum hydrocarbons concentration decreases with 90.6% in V_6 experimental variant and with 92.6% in the inoculated variant V_7.

To remediate a soil polluted with 5% crude oil it is recommended the treatement with 0.5% ECOSOL and bacterial inoculum.

The evolution of total petroleum hydrocarbons concentration in the polluted soil with 10% crude oil treated with 100 g ECOSOL/20 kg polluted soil is presented in figure 2.

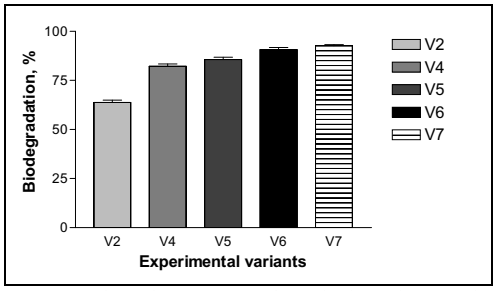

Fig. 3. Biodegradation of petroleum hydrocarbons in the polluted soil with 5% crude oil

As it can be observed, the polluted soil with 10% crude oil treated with 100 g ECOSOL/20 kg polluted soil presented a total petroleum hydrocarbons decrease in time with 71.8% in the case of V_8 experimental variant and with 74.1% in case of inoculated variant V_9.

The total petroleum hydrocarbons concentration decreases with 79% in V_{10} experimental variant and with 80.5% in the inoculated variant V_{11}.

In the experimental variants polluted with 10% crude oil, treated with 0.5% ECOSOL, respectively 1% ECOSOL, the decrease were by 71.8%, respectively 79%. In the experimental variants polluted with 10% crude oil, inoculated with bacteria, treated with 0.5% ECOSOL, respectively 1% ECOSOL, the decrease were by 74.1%, respectively 80.5% (Fig. 4).

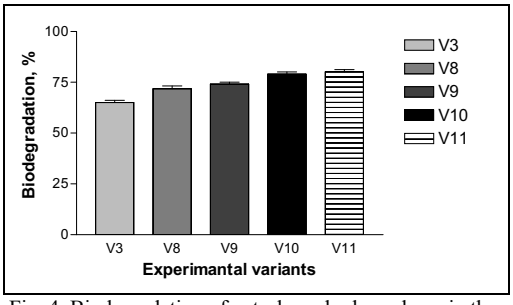

Fig. 4. Biodegradation of petroleum hydrocarbons in the polluted soil with 10% crude oil

The experimental variant recommended to remediate a polluted soil with 10% crude oil could be the one treated with 1% ECOSOL polluted soil and bacterial inoculum to increase the biodegradability rate.

CONCLUSIONS

According to the study achieved, for bioremediation in both groups of experimental variants proved that the soil treated with maximum dose of ECOSOL (0.5% for soil polluted with crude oil 5% and 1% for soil polluted with 10% oil) and inoculated with selected bacteria, the two links of bioremediation technology acting synergistically to remove the pollutant from the soil.

The results obtained leads to the conclusion that the treatment of the crude oil polluted soil with natural biodegradable product and bacterial inoculum determined a decrease of total petroleum concentration in time. The reduction of pollution degree in experimental variants is due to the intensification of biodegradation processes and the establishment of a favorable equilibrium from microbiological point of view.

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