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BIOREMEDIATION OF A CAMBIC CHERNOZEM POLLUTED WITH PETROLEUM HYDROCARBONS

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Abstract: The exploitation of petroleum has generated various sources of pollution in soil. In order to resolve this problem, several techniques have been developed. Physical and chemical methods are the most widely methods used for land treatment of oil polluted soils. These methods are expensive, ineffective and could cause a lot of damages on soil. The biological methods are not expensive and do not cause changes in the soil physical and chemical characteristics. Bioremediation is based on the capacity of microorganisms to degrade organic pollutant compounds, such as hydrocarbons.

This study presents the preliminary results of a bioremediation alternative for soils polluted with crude oil (petroleum hydrocarbons). The bioremediation was achieved by increasing the microbial population by adding the fibers provided from celluloid wastes and bacteria inoculum. The paper presents the effect of a bioremediation treatment applied to a cambic chernozem polluted with 50000 mg kg^{-1} (5% crude oil) and 100000 mg kg^{-1} (10% crude oil) of a complex mixture of total petroleum hydrocarbons (TPH).

INTRODUCTION

Oil pollution is a world-wide prevalent threat to the environment and the remediation of oil contaminated soils, sediments and water is a major challenge for environmental research. On land, crude oil presents a risk for air, groundwater, soil quality, soil fauna and flora. Appropriate disposal and/or clean-up of contaminated sites are a legal requirement in many countries.

Physical and chemical methods are the most widely used methods for land treatment of crude oil, and often, these methods are grossly inadequate and ineffective, and may result in further contamination of the environment. It is important to evaluate less expensive remediation technologies that could clean up the contaminated / polluted soils with petroleum, areas found around industrial areas and automobile repair villages all over the country. The bioremediation is a method of great interest because of the possibility of soil reuse.

Soil remediation has gained increasing interest. The choice of the remediation method depends on the type, mobility and concentration of the pollutant and the future land use. According to the recent research, bioremediation is used when the pollution level is moderate and non-biological methods are not economical.

Since oil degradation is a natural process limited by temperature, pH, and scarcity of nutrients such as N, P, and O_2 (Leahy and Colwell, 1990), bioremediation, a process whereby the natural biodegradation capacities are enhanced by nutrient addition and/or culture microorganisms, is among the most promising technologies currently in use with the

advantages as cost effectiveness and the potential ability to remediate an environment without causing much damage. While biodegradation of petroleum hydrocarbon may be successfully conducted under controlled conditions, the bioremediation of large volume of contaminated soils still present some technical challenges (Pala et al., 2006).

Bioremediation is a useful method for soil remediation, if pollutant concentrations are moderate and non-biological techniques are not economical. The scope of this study was to investigate if the fibers provided from celluloid wastes and bacteria inoculum can enhance the microbial degradation of petroleum hydrocarbons in soil.

These compounds are important soil pollutants because of the high toxicity of the polycyclic aromatic hydrocarbon (PAH) fraction. According to the Environmental Protection Agency (EPA), 16 PAHs have been reported as carcinogenic and mutagenic compounds, so it is necessary to remove them from contaminated sites. Recent studies have reported several bacteria species with the capacity to mineralize or to degrade PAHs.

Schaefer and Juliane (2007) also concluded that bioremediation is a useful method of soil remediation if pollutant concentrations are moderate.

However, it is important to remark that the level of decontamination will depend principally on the hydrocarbon composition and the available microorganisms existing in the soil (Vogel, 1996).

MATERIAL AND METHODS

This study evaluated the applicability of the fibers provided from celluloid wastes and bacteria inoculum as remediation alternative in oil polluted soils. Hydrocarbons were quantified by using the gravimetric method (Moreli et al., 2005), following previous solid–liquid extraction in a Soxhlet system (EPA method 3540c). The extraction was carried out with methylene chloride in 2 g soil samples, which had been previously dried and grounded.

Alexander (1994) have reported that the physicochemical characteristics of polluted soils successfully remediated by biological technologies are very important in the biodegradation process.

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 soil existing bacteria.

To achieve data concerning the bioremediation of polluted soil with petroleum hydrocarbons was realized a greenhouse experiment. The soil used for this experiment (cambic 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 soil used in the experiment are presented in table 1.

Table 1. Chemical characteristics of the soil

Soil type	pH	Organic Carbon (%)	Total Nitrogen (%)	C/N ratio
Calcic chernozem	8,09	2,99	0,279	12,5

The fibers provided from celluloid wastes and bacteria inoculum to contaminated plots was adding nutrients to stimulate biodegradation of the crude oil.

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 are presented in the following table.

Table 2 Chemical characteristics of the natural biodegradable product ECOSOL

Natural biodegradable product	Total nitrogen (%)	Organic carbon (%)	Phosphorous (%)	Potassium (%)	Sodium (%)
ECOSOL	0,935	23,72	0,39	3,32	4,97

There are 11 experimental variants, two degrees of crude oil concentration (5% and 10% crude oil), 3 different quantities of ECOSOL (50 g, 100 g and 200 g ECOSOL) and the absence or the presence of bacterial inoculum. 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.

RESULTS AND DISCUSIONS

The aim of the experiment was to investigate the influence of the fibers provided from celluloid wastes and bacteria inoculum on total petroleum hydrocarbons (TPH) degradation in a crude oil polluted soils.

It is important to note that the observed reduction in crude oil or TPH may not only be due to the biodegradation process induced by nutrient additions, but other processes such as volatilization, adsorption to organic compounds, and other abiotic factors are equally implicated in the reduction process.

Since microorganisms are primary agents for the degradation of organic contaminants in soil, increasing microbial density can accelerate the degradation process of the contaminants.

It was hypothesised that the application of additional organic additives would optimise the microbial activity and this may increase TPH metabolism.

At the beginning of the experiment, the soil was contaminated / polluted with crude oil and conditioned with the natural hydrocarbon absorbent (ECOSOL) according with the experimental scheme.

Each value for total petroleum hydrocarbons (TPH) concentration represents the mean of 3 replicates.

In the figure 1 is presented the gravimetric loss of total petroleum hydrocarbons (TPH) concentration before inoculation. It was prelevated samples after 7, 14 and 21 days and the results of TPH analysis are presented in the following figure.

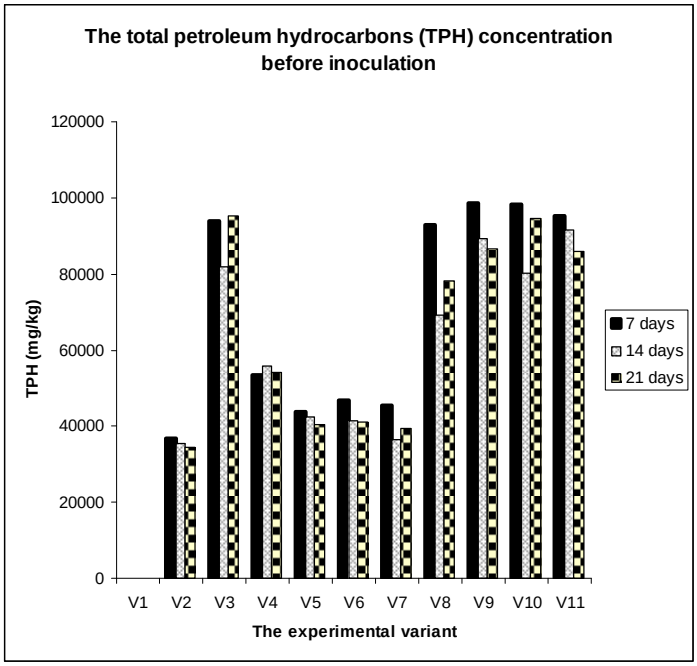

Figure 1. Gravimetric loss of total petroleum hydrocarbons (TPH) in the experimental variants before inoculation

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 the figure 2 is presented the gravimetric loss of total petroleum hydrocarbons (TPH) concentration after inoculation. It was prelevated samples after 25, 45 and 60 days and the results of TPH analysis are presented in the following figure.

In the figure 3 is presented the gravimetric loss of total petroleum hydrocarbons (TPH) concentration at the end of the first experimental year. It was prelevated samples after 90, 120 and 150 days and the results of TPH analysis are presented in the figure 3.

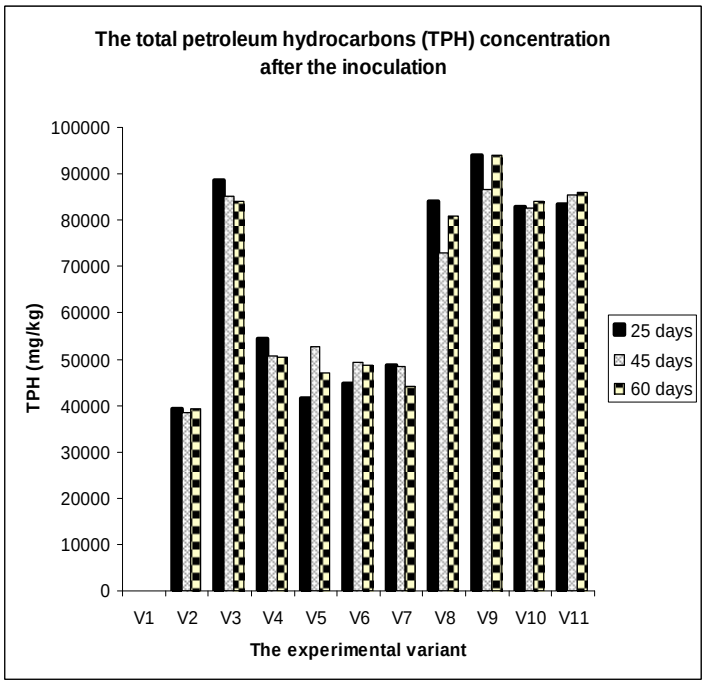

Figure 2. Gravimetric loss of total petroleum hydrocarbons (TPH) in the experimental variants after inoculation

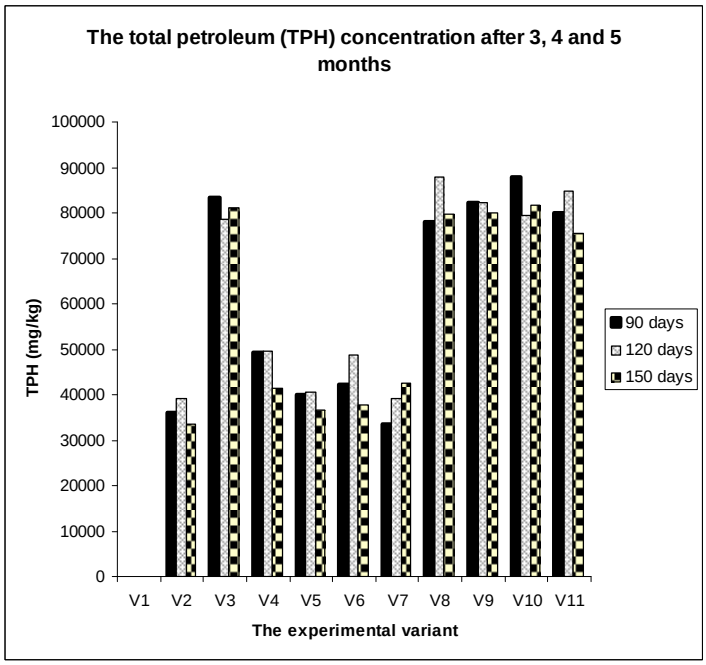

Figure 3. Gravimetric loss of total petroleum hydrocarbons (TPH) in the experimental variants after 3, 4 and 5 months

As it can be observed in the figure 1, 2 and 3 have been recorded a decrease of total petroleum hydrocarbons concentration in five months of experiment with 8%, respectively 12% in the polluted soil with 5%, respectively 10% crude oil comparatively with the control.

Also, the total petroleum hydrocarbons decrease in time with 12% in the experimental variant V_4 comparatively with the inoculated variant V_5 in which the decrease was by 17%. The total petroleum hydrocarbons concentration decreases with 20% in V_6 experimental variant and with 25% in the inoculated variant V_7.

In the experimental variants polluted with 10% crude oil, conditioned with 100 g ECOSOL, respectively 200 g ECOSOL, the decrease were by 15%, respectively 20%. In the experimental variants polluted with 10% crude oil, inoculated with bacteria, conditioned with 100 g ECOSOL, respectively 200 g ECOSOL, the decrease were by 18%, respectively 25%.

CONCLUSIONS

General observation at the end of the first experimental year showed that the bioremediation with the addition of fibers provided from celluloid wastes and bacteria inoculum was effective in stimulating the biodegradation of the crude oil on low (5% crude oil) and high (10% crude oil) concentrations, especially on high additions of ECOSOL and in the experimental variants inoculated.

These are the preliminary results, therefore the experimental research will continue in Green House on the same polluted soil. Maize will be cultivated to follow the growth and behaviour in function with the concentrations of total petroleum hydrocarbons, the treatment with ECOSOL and bacterial inoculum.

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