Abstract: Biodegradation is the process by which organic substances are broken down by the enzymes produced by living organisms. The term is often used in relation to ecology, waste management and environmental remediation (bioremediation). Some microorganisms have the capacity to degrade, transform or accumulate petroleum hydrocarbons. Bioremediation of petroleum hydrocarbon contaminated soils has been recognized as an efficient, economic, versatile and environmentally good treatment. Inadequate bioavailability of the hydrocarbons to microorganisms due to low solubility is a limiting step in biodegradation. So, it must be found a method to increase the microorganisms activity. This research is based on the microorganisms activity increase by adding natural absorbent biodegradable and bacterial inoculums. The elimination of petroleum hydrocarbons from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. In this paper are presented the results obtained in greenhouse experiment concerning the bioremediation of artificial polluted soil using a natural hydrocarbon absorbent product and bacterial inoculums to enhance the biodegradation of petroleum hydrocarbons. The plant use in the experiment was maize. These are some preliminary results, therefore the experimental research will continue in Green House on the same polluted soil.

Key words: biodegradation, artificial polluted soil, petroleum hydrocarbons

INTRODUCTION

Crude oil contamination/pollution is a frequent and very serious problem all over the world, also in Romania, mainly at the transformer stations. Biodegradation of petroleum hydrocarbons in the environment may be limited by a large number of factors. An important limiting factor in the bioremediation of contaminated/polluted soils is often the low
bioavailability and solubility of the hydrocarbon (MOLNÁR et al., 2005).

The hydrocarbon degrading microorganisms occur in most environments, where hydrocarbons may serve as organic carbon sources. Bioremediation, is based on the use of microorganisms or microbial processes to degrade environmental contaminants, and offers several advantages over the conventional chemical and physical technologies. It can be a cost effective, environmental friendly technology. Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds (ALEXANDER, 1994; PEPPER, 1996).

Bioaugmentation is frequently used for the enhancement of the biodegradative capacities of soils contaminated with poorly degradable crude oil hydrocarbon compounds (HWANG and CUTRIGAHT 2002).

Augmentation is the reinoculation of indigenous microorganisms directly isolated from the contaminated soil (MARGESIN et al. 2000) or the inoculation of a specific consortium. A fast degradation rate can be observed after bioaugmentation with microorganisms adapted to the contaminant (VOGEL 1996).

MATERIAL AND METHODS

The aim of this research was carried out to prove the efficiency of a biodegradable product of the existing microorganisms and the inoculation of microbial consortium on the bioremediation process in crude oil contaminated soil.

To achieve data concerning the bioremediation of polluted soil with petroleum hydrocarbons was realized a greenhouse experiment.

The soil used in experiment was s cambic chernozem from Teleorman. The plant used in experiment was maize.

The experiment had 11 experimental variants with soil polluted 5% and 10% crude oil, treated with 50g, 100g and 200g ECOSOL, inoculated and no-inoculated with bacteria selected according to the experimental scheme presented in table 1.

<table>
<thead>
<tr>
<th>Experimental variant</th>
<th>The name of experimental variant</th>
<th>Crude oil concentration (%)</th>
<th>ECOSOL Treatment (g)</th>
<th>Bacterial inoculums</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V2</td>
<td>Polluted soil</td>
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<td>-</td>
</tr>
<tr>
<td>V3</td>
<td>Polluted soil</td>
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<td>50</td>
<td>Yes</td>
</tr>
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<td>5</td>
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<td>-</td>
</tr>
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</tr>
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<td>200</td>
<td>-</td>
</tr>
<tr>
<td>V11</td>
<td>Polluted soil</td>
<td>10</td>
<td>200</td>
<td>Yes</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSIONS

The results obtained in the second experimental year shows that the biodegradation processes take time according to the existing literature data. A decrease was recorded in time and this agrees with the observation existing in scientific literature.

This study was necessary to observe the influence of the treatment with ECOSOL and bacterial inoculums on biodegradation rate of petroleum hydrocarbons. It was registered an increase of biodegradation in time. Soil samples were sampled and analyzed in the beginning of the experiment, after 1 month, 2 months, 3 months, 4 months and 5 months.
The maize plants grew up in the experimental variants with unpolluted soil and soil polluted with 5% crude oil. In the experimental variants with soil polluted with 5% crude oil the maize harvest was two times lower than control. The high concentration of crude oil determined the unappearance of maize plant in the experimental variant with soil polluted 10% crude oil. The phytotoxicity was to higher in these plots.

![Figure 1: Biodegradation of petroleum hydrocarbons in time in unpolluted soil, polluted soil with 5% crude oil and polluted soil with 10% crude oil (V1, V2, V3)](image)

The biodegradation of petroleum hydrocarbons with time in experimental variant V1 - unpolluted soil, V2 - polluted soil with 5% crude oil and V3 - polluted soil with 10% crude oil is presented in figure 1. The figure shows that biodegradation values were higher in experimental variants with polluted soils compared to the control suggesting the biodegradation of crude oil.

The biodegradation increases in time, reaching a value by 19%, respectively 22% in the polluted soil with 5%, respectively 10% crude oil.

The biodegradation of petroleum hydrocarbons in the polluted soil with 5% crude oil conditioned with 50 g ECOSOL is presented in figure 2. As it can be observed, the biodegradation rate increases in time with 21% in the case of V4 experimental variant comparatively with the inoculated variant V5 in which the increase was by 39%.

The biodegradation of petroleum hydrocarbons in the polluted soil with 5% crude oil conditioned with 100 g ECOSOL is presented in figure 3. The biodegradation rate increases in time reaching a value by 30% in both experimental variants.

As it can be observed, in the experimental variants polluted with 5% crude oil, conditioned with 50 g ECOSOL, respectively 100 g ECOSOL, the increase of biodegradation were by 21%, respectively 30%. In the experimental variants polluted with 5% crude oil, inoculated with bacteria, conditioned with 50 g ECOSOL, respectively 100 g ECOSOL, the increase were by 39%, respectively 30%.
The treatment recommended to remediate a polluted soil with 5% crude oil could be the treatment with 1050 g ECOSOL/m² polluted soil, 0-40 cm depth, and bacterial inoculums.

The biodegradation of petroleum hydrocarbons in the polluted soil with 10% crude oil conditioned with 100 g ECOSOL is presented in figure 4. As it can be observed, the biodegradation rate increases in time reaching a value by 13% in the case of V₈ experimental variant comparatively with the inoculated variant V₉ in which the increase was by 20%.

The biodegradation of petroleum hydrocarbons in the polluted soil with 10% crude oil conditioned with 200 g ECOSOL is presented in figure 5. The biodegradation of petroleum hydrocarbons increases with 13% in V₁₀ experimental variant and with 18% in the inoculated variant V₁₁.
In the experimental variants polluted with 10% crude oil, conditioned with 100 g ECOSOL, respectively 200 g ECOSOL, the increase of biodegradation rate were by 13%, in both experimental variants. In the experimental variants polluted with 10% crude oil, inoculated with bacteria, conditioned with 100 g ECOSOL, respectively 200 g ECOSOL, the increase were by 20%, respectively 18%.

The treatment recommended to remediate a polluted soil with 10% crude oil could be the treatment with 2100 g ECOSOL/m² polluted soil, 0-40 cm depth, and bacterial inoculum.
CONCLUSIONS

The maize plants grew up in the experimental variants with unpolluted soil and soil polluted with 5% crude oil. In the experimental variants with soil polluted with 5% crude oil the maize harvest was two times lower than control. The high concentration of crude oil determined the non-appearance of maize plant in the experimental variant with soil polluted 10% crude oil. The phytotoxicity was to higher in these plots.

The treatment recommended to remediate a polluted soil with 5% crude oil could be the treatment with 1050 g ECOSOL/m² polluted soil, 0–40 cm depth, and bacterial inoculum.

The treatment recommended to remediate a polluted soil with 10% crude oil could be the treatment with 2100 g ECOSOL/m² polluted soil, 0–40 cm depth, and bacterial inoculum.

The research will continue in Green House to obtain more data concerning this important environmental problem.

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