THE DIVERSITY OF HETEROTROPHIC BACTERIAL MICROFLORA IN SOIL POLLUTED WITH CRUDE OIL AND DIFFERENT TREATMENTS

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ABSTRACT
Petroleum hydrocarbon pollution is one of the main environmental problems, not only by the important amounts released but also because of their toxicity. It is known that the main microorganisms consuming petroleum hydrocarbons are bacteria. The present research work reports the diversity of microrganis from crude oil polluted soil been treated with a natural biodegradable product and bacterial inoculum. The bacterial inoculum was used to enrich indigenous microbes to enhance biodegradation rate. 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.

INTRODUCTION

The bioremediation methods depend on having the right microbes in the right place with the right environmental factors for degradation to occur. The right microorganisms are bacteria or fungi, which have the physiological and metabolic capabilities to degrade the pollutants.

Furthermore, soil structure and biology can be dramatically disturbed or even destroyed making the land useless for agricultural purposes (Lee and Levy, 1989). Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills is receiving worldwide attention. The interest in environmental pollution has increased for the entire population of the globe.

Various institutions and organizations, some multidisciplinary other specialized publications, focused solely on pollution issues. There is no life without soil (Alexander, M., 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).

There are many genera of known oil-degrading microorganisms, including bacteria such as *Achromobacter, Acinetobacter, Actinomyces, Bacillus, Microbacterium, Pseudomonas, Streptomyces*. Under natural conditions, these microorganisms in most areas comprise very few, compared with the total number of identified microorganisms. However, at petroleum hydrocarbon polluted soils, these populations may grow and increase because they use petroleum hydrocarbon as a carbon source (Alexander, 1997).

MATERIAL AND METHOD

The main objective of this research is testing the natural hydrocarbon absorbent named ECOSOL. To achieve data concerning the bioremediation of polluted soil with petroleum hydrocarbons was achieved a greenhouse experiment. The soil used for this experiment was a cambic chernozems. The plant used in the experiment was maize.

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 + 1 kg ECOSOL/m^2 polluted soil; V_5, polluted soil with 5% crude oil + 1 kg ECOSOL/m^2 polluted soil + bacterial inoculum; V_6, polluted soil with 5% crude oil + 2 kg ECOSOL/m^2 polluted soil; V_7, polluted soil with 5% crude oil + 2 kg ECOSOL/m^2 polluted soil + bacterial inoculum; V_8, polluted soil with 10% crude oil + 2 kg ECOSOL/m^2 polluted soil; V_9, polluted soil with 10% crude oil + 2 kg ECOSOL/m^2 polluted soil + bacterial inoculum; V_{10}, polluted soil with 10% crude oil + 4 kg ECOSOL/m^2 polluted soil; V_{11}, polluted soil with 10% crude oil + 4 kg ECOSOL/m^2 polluted soil + bacterial inoculum.

RESULTS AND DISCUSSIONS

The communities of heterotrophic bacteria in the first 3 stages after soil pollution with crude oil and before applying inoculation, were composed of species belonging to the genera *Pseudomonas, Arthrobacter, Bacillus, Mycobacterium and Streptomyces*.

Effects of the bacterial inoculum was observed in the evaluation of data for bacterial communities from measurements at 45 days from the start of the experiment and approx. 10 days after inoculation, the bacterial variants that have been selected, the number of species identified was the highest from all experiment.

30 days from pollution, after applying inoculation, the bacterial communities of experimental variants are dominated by species of the genera *Pseudomonas*, *Bacillus* and *Arthrobacter*. At the determination effectuated at 60 days, the bacterial diversity of the experimental variants is remarkably high in the soil polluted with 10% and conditioned with 0.5% and 1% Ecosol with or without bacterial inoculation being identified by 8 and 9 bacterial species of which more than half (4-5) degrading of petroleum hydrocarbons.

After 90 days of the start of the experiment, was registered a decrease of bacterial diversity in selecting the most biodegrading species in experimental variants with petroleum hydrocarbons.

The determination made at 300 days was noted a new moment to increase the diversity of bacterial identifying up to 8 species in solution polluted with 10% oil conditioned with 1% Ecosol and inoculated, but the number of species degrading to remained constant or even decreased.

By the end of the research, diversity of bacterial communities was moderate, and the differences between experimental variants were dimmed in the presence and number of users of petroleum hydrocarbons.

Table 1. Diversity of heterotrophic bacterial microflora

No	Experimental variants	7 days	14 days	21 days	30 days	45 days
1	V_1 – sol nepoluat	*Arthrobacter globiformis, Bacillus cereus, Pseudomonas sp., Streptomyces sp.*	*Bacillus megaterium, B. cereus, B. polymyxa, Arthrobacter globiformis, Pseudomonas sp.*	*Arthrobacter globiformis, Bacillus cereus, B. megaterium, B. circulans, B. polymyxa.*	*Arthrobacter globiformis, Bacillus circulans Pseudomonas sp., Bacillus megaterium, B. cereus, Streptomyces sp.*	*Bacillus circulans, Arthrobacter globiformis, Bacillus polymyxa, B. megaterium, B. cereus.*
2	V_2 – sol poluat cu 5% ţiţei	*Pseudomonas sp., Arthrobacter globiformis, Bacillus sphaericus.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans.*	*Pseudomonas sp., Bacillus circulans, Arthrobacter globiformis.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans, B. polymyxa, Arthrobacter*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus polymyxa, B. circulans, Arthrobacter simplex.*

					simplex	
4	V_4 – sol poluat cu 5% ţiţei + 50 g ECOSOL	*Arthrobacter citreus, Streptomyces sp.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus polymyxa.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans.*	*Bacillus circulans, Arthrobacter globiformis.*	*Pseudomonas sp., Bacillus circulans, Arthrobacter globiformis, Bacillus megaterium, B. polymyxa, B. sphaericus, Arhtrobacter citreus.*
5	V_5 – sol poluat cu 5% ţiţei+ 50 g ECOSOL + inocul	*Pseudomonas sp., Arthrobacter globiformis, Bacillus megaterium.*	*Arthrobacter globiformis, Pseudomonas sp., Bacillus circulans, B. megaterium.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus polymyxa, B. circulans.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans, B. megaterium,* ***Micrococcus.***	*Pseudomonas sp., Bacillus circulans, Arthrobacter globiformis, Mycobacterium roseum, Bacillus polymyxa, B. megaterium, B. sphaericus, Enterobacter.*
6	V_6 – sol poluat cu 5% ţiţei + 100 g ECOSOL	*Pseudomonas sp., Bacillus cereus, Arthrobacter globiformis, Bacillus megaterium.*	*Pseudomonas sp., Bacillus cereus, Arthrobacter globiformis, Bacillus megaterium, B. polymyxa.*	*Pseudomonas sp., Bacillus cereus, Arthrobacter globiformis, Bacillus megaterium Bacillus circulans.*	*Pseudomonas sp., Bacillus circulans, B. polymyxa, B. megaterium.*	*Pseudomonas sp., Mycobacterium phley, Arthrobacter simplex, Bacillus polymyxa, B. circulans, B. sphaericus, B. megaterium.*
7	V7 – sol poluat cu 5% ţiţei + 100 g ECOSOL + inocul	*Pseudomonas sp., Arthrobacter citreus, Streptomyces sp.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans, Streptomyces sp.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus polymyxa.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans Mycobacterium phley, Bacillus polymyxa.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus polymyxa, B. circulans, Mycobacterium roseum, Bacillus megaterium, B. sphaericus, B. macerans.*
3	V3 – sol poluat cu 10% ţiţei	*Pseudomonas sp., Arthrobacter globiformis, Streptomyces sp.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus megaterium.*	*Arthrobacter globiformis, Pseudomonas sp., Bacillus megaterium.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans, Streptomyces sp.*	*Pseudomonas sp., Arthrobacter globiformis, Mycobacterium sp., Bacillus megaterium, B. circulans, Arthrobacter simplex, Bacillus sphaericus.*
8	V_8 – sol poluat cu 10% ţiţei + 100 g ECOSOL	*Arthrobacter globiformis, Pseudomonas sp., Streptomyces sp.*	*Arthrobacter globiformis, Bacillus circulans, B. cereus, Mycobacterium sp.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus megaterium, Streptomyces sp.*	*Bacillus circulans, Mycobacterium sp., Bacillus megaterium.*	*Bacillus cereus var. mycoides, Pseudomonas sp., Arthrobacter globiformis, Mycobacterium phley, Bacillus megaterium.*
9	V_9 – sol poluat cu 10% ţiţei + 100 g ECOSOL + inocul	*Pseudomonas sp., Mycobacterium roseum.*	*Pseudomonas sp., Mycobacterium sp., Bacillus circulans.*	*Mycobacterium phley, Pseudomonas sp., Bacillus circulans.*	*Pseudomonas sp., Arthrobacter simplex, Bacillus circulans, B. polymyxa.*	*Pseudomonas sp., Bacilus circulans,* ***Micrococcus****, Bacillus polymyxa, Arthrobacter globiformis, Mycobacterium phley, Bacillus megaterium, Arthrobacter citreus.*
10	V_{10} – sol poluat cu 10% ţiţei + 200 g	*Pseudomonas sp., Arthrobacter globiformis.*	*Pseudomonas sp., Arthrobacter globiformis.*	*Pseudomonas sp., Arthrobacter globiformis,*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans,*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus sphaericus,*

	ECOSOL			Mycobacterium sp.	B. polymyxa.	B. circulans, Mycobacterium sp., Bacillus megaterium, Arthrobacter simplex, Bacillus polymyxa.
11	V_{11} – sol poluat cu 10% ţiţei + 200 g ECOSOL + inocul	Pseudomonas sp., Mycobacterium phley.	Pseudomonas sp., Mycobacterium sp., Arthrobacter globiformis.	Pseudomonas sp., Mycobacterium roseum, Arthrobacter globiformis.	Pseudomonas sp., Arthrobacter globiformis, Mycobacterium sp., Bacillus megaterium, B. circulans.	Pseudomonas sp., Mycobacterium phley, Bacillus circulans, Arthrobacter globiformis, Bacillus megaterium, B. polymyxa, Arthrobacter citreus, Bacillus macerans.

-further table-

No	Experimental variants	60 days	90 days	300 days	330 days
1	V_1 – sol nepoluat	Arthrobacter globiformis, Bacillus circulans, B. polymyxa, Pseudomonas sp.	Bacillus megaterium, B. circulans, B. polymyxa, B. cereus, Streptomyces sp.	Bacillus cereus var. mycoides, Pseudomonas sp., Arthrobacter globiformis, Bacillus megaterium.	Arthrobacter globiformis, Bacillus circulans, Pseudomonas sp., Bacillus polymyxa, Mycobacterium phley.
2	V_2 – sol poluat cu 5% ţiţei	Pseudomonas sp., Arthrobacter globiformis, Mycobacterium roseum, Bacillus circulans, B. megaterium.	Arthrobacter globiformis, Bacillus circulans, B. polymyxa, Pseudomonas sp., Mycobacterium phley, Streptomyces sp.	Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans, Streptomyces sp.	Pseudomonas sp., Arthrobacter globiformis, A. citreus, Mycobacterium roseum, Bacillus circulans, B. megaterium.
4	V_4 – sol poluat cu 5% ţiţei + 50 g ECOSOL	Pseudomonas sp., Arthrobacter globiformis, Bacillus polymyxa, B. circulans, Mycobacterium sp., Bacillus cereus, B. megaterium,, B. macerans, Arhtrobacter simplex, Bacillus sphaericus.	Pseudomonas sp., Arthrobacter simplex, Bacillus circulans, B. megaterium, Mycobacterium phley, Streptomyces sp.	Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans, Arthrobacter citreus, Bacillus megaterium, Streptomyces sp.	Pseudomonas sp., Mycobacterium phley, M. roseum, Bacillus circulans, Arthrobacter globiformis, A. citreus, Bacillus polymyxa, Arthrobacter simplex.
5	V_5 – sol poluat cu 5% ţiţei+ 50 g ECOSOL + inocul	Pseudomonas sp., Arthrobacter simplex, Bacillus circulans, Mycobacterium sp., Bacillus sphaericus, B. megaterium, Arthrobacter globiformis.	Pseudomonas sp., Arthrobacter globiformis, Mycobacterium roseum, Bacillus circulans, Streptomyces sp.	Pseudomonas sp., Bacillus megaterium, Arthrobacter globiformis, Bacillus circulans, B. polymyxa, B. macerans.	Pseudomonas sp., Mycobacterium roseum, Arthrobacter globiformis, Bacillus circulans, B. polymyxa, Arthrobacter citreus.
6	V_6 – sol poluat cu 5% ţiţei + 100 g ECOSOL	Pseudomonas sp., Bacillus circulans, Arthrobacter simplex, Bacillus polymyxa.	Bacillus cereus var. mycoides, Pseudomonas sp., Bacillus circulans, Arthrobacter globiformis, Mycobacterium roseum.	Pseudomonas sp., Arthrobacter globiformis, Mycobacterium roseum, Bacillus megaterium, Streptomyces sp.	Mycobacterium roseum, Pseudomonas sp., Arthrobacter citreus, Bacillus megaterium, B. circulans, Mycobacterium roseum.
7	V7 – sol poluat cu 5% ţiţei + 100 g ECOSOL + inocul	Pseudomonas sp., Arthrobacter globiformis, Bacillus polymyxa, B. circulans, B. sphaericus, B. megaterium,	Arthrobacter globiformis, Pseudomonas sp., Bacillus circulans, B. macerans.	Bacillus circulans, Arthrobacter globiformis, Bacillus megaterium, Streptomyces sp.	Pseudomonas sp., Mycobacterium roseum, Bacillus circulans, B. polymyxa, Arthrobacter globiformis, Bacillus cereus var. mycoides.

		Arthrobacter simplex, Mycobacterium phley, ***Flovobacterium****.*			
3	V3 – sol poluat cu 10% ţiţei	*Pseudomonas sp., Arthrobacter simplex, Bacillus circulans, B. polymyxa, Arthrobacter globiformis, Bacillus megaterium, B. cereus, B. macerans.*	*Pseudomonas sp., Arthrobacter citreus, Bacillus circulans,* ***Enterobacter****, Streptomyces sp.*	*Pseudomonas sp., Bacillus circulans, Arthrobacter citreus, A. globiformis, Bacillus megaterium, Streptomyces sp.*	*Bacillus circulans, Pseudomonas sp., Arthrobacter globiformis, A. citreus, Bacillus circulans, B. polymyxa, Mycobacterium roseum.*
8	V_8 – sol poluat cu 10% ţiţei + 100 g ECOSOL	*Pseudomonas sp., Bacillus circulans, Arthrobacter globiformis, Bacillus polymyxa, Mycobacterium roseum, Bacillus megaterium.*	*Arthrobacter globiformis, Pseudomonas sp., Bacillus polymyxa, Bacillus circulans.*	*Pseudomonas sp., Arthrobacter citreus, Bacillus megaterium, B. polymyxa, Arthrobacter simplex, Bacillus macerans, Streptomyces sp.*	*Pseudomonas sp., Arthrobacter globiformis, Mycobacterium roseum, Arthrobacter simplex, Bacillus ciruclans, B. megaterium, B. polymyxa, B. macerans, Mycobacterium phley.*
9	V_9 – sol poluat cu 10% ţiţei + 100 g ECOSOL + inocul	*Pseudomonas sp., Arthrobacter simplex, Bacilus polymyxa, B. circulans, Arthrobacter citreus, Mycobacterium phley, Bacillus megaterium, B. sphaericus, B. cereus.*	*Pseudomonas sp., Mycobacterium phley, Arthrobacter globiformis, Bacilus circulans, Arthrobacter citreus, Bacillus megaterium.*	*Pseudomonas sp., Bacillus circulans, Arthrobacter globiformis, Bacillus polymyxa, Streptomyces sp.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans, B. polymyxa, B. megaterium, B. macerans, Arthrobacter citreus.*
10	V_{10} – sol poluat cu 10% ţiţei + 200 g ECOSOL	*Pseudomonas sp., Arthrobacter citreus, Bacillus circulans, B. polymyxa, Arthrobacter globiformis, Bacillus megaterium, Mycobacterium roseum, Bacillus sphaericus, Arthrobacter simplex.*	*Pseudomonas sp., Arthrobacter globiformis, Mycobacterium roseum, Bacillus circulans.*	*Pseudomonas sp., Bacillus circulans, Arthrobacter globiformis, Bacillus polymyxa, Bacillus megaterium,* ***Enterobacter****, Arthrobacter citreus, Streptomyces sp.*	*Pseudomonas sp., Mycobacterium roseum, Arthrobacter simplex, Bacillus circulans, Arthrobacter globiformis, Bacillus polymyxa, B. megaterium, Mycobacterium phley.*
11	V_{11} – sol poluat cu 10% ţiţei + 200 g ECOSOL + inocul	*Pseudomonas sp., Mycobacterium phley, Bacillus circulans, Arthrobacter globiformis, Bacillus polymyxa,* ***Flavobacterium****, Bacillus sphaericus, B. megaterium.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus polymyxa, B. circulans, Mycobacterium phley, Bacillus megaterium, B. macerans, Streptomyces sp.*	*Pseudomonas sp., Arthrobacter globiformis, Bacillus circulans, Mycobacterium roseum, Bacillus macerans.*

CONCLUSIONS

Frequent bacterial isolated from experimental variants, in most determination stages were: *Pseudomonas, Arthrobacter, Mycobacterium*. Isolates of *Pseudomonas*, *Arthrobacter* and *Mycobacterium* led to the establishment of inoculum applied to the soil, and very high frequency showed a better survival rate and their adaptability to environmental conditions. Bacterial isolated as *Pseudomonas* and *Arthrobacter* dominated the heterotrophic bacteria population in the majority of experimental variants, occasionally accompanied by species belonging to the genera: *Micrococcus*, *Enterobacter* and *Flavobacterium*. Bacterial genera *Pseudomonas and Arthrobacter* overwhelmingly dominated population heterotrophic bacteria in most experimental versions, accompanied sporadically species belonging to the genera *Micrococcus, Enterobacter and Flavobacterium*.

Dynamics of microorganisms inoculated showed a slight adaptability in soil polluted with 5% crude oil, but complete inhibition in the first 30 days of experiment at 10% crude oil. After the acclimatization period by 30 days, the excessive concentration of pollutants has been massive multiplication of bacteria in inoculated variants, especially in the conditioned variant with Ecosol maximum dose. At 45 days, there was a real explosion in variants of bacteria inoculated with NTB values double, triple compared with uninoculated variants. At 60 days, bacterial populations have begun to reduce their size, as the value of soil polluted with 10% crude oil and untreated. This demonstrates that the application reduces the time needed to adapt to Ecosol substrate for microorganisms involved in petroleum hydrocarbons biodegradation.

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