Bioremediation Potential of Native Hydrocarbons Degrading Bacteria in Crude Oil Polluted Soil

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ABSTRACT

Bioremediation of crude oil contaminated soil is an effective process to clean petroleum pollutants from the environment. Crude oil bioremediation of soils is limited by the bacteria activity in degrading the spills hydrocarbons. Native crude oil degrading bacteria were isolated from different crude oil polluted soils. The isolated bacteria belong to the genera *Pseudomonas*, *Mycobacterium*, *Arthrobacter* and *Bacillus*. A natural biodegradable product and bacterial inoculum were used for total petroleum hydrocarbon (TPH) removal from an artificial polluted soil. For soil polluted with 5% crude oil, the bacterial top, including those placed in the soil by inoculation was 30 days after impact, respectively 7 days after inoculum application, while in soil polluted with 10% crude oil, multiplication top of bacteria was observed in the determination made at 45 days after impact and 21 days after inoculum application, showing once again how necessary is for microorganisms habituation and adaptation to environment being a function of pollutant concentration. The 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.

Keywords: *bioremediation, crude oil, native hydrocarbons degrading bacteria, polluted soil*

INTRODUCTION

Due to the persistence nature of crude oil, it remains in the environment for long time, which affects the soil quality by changing the physical, physiological, biochemical properties and microbial diversity (Margesin *et al.*, 2013). Plants are also susceptible to the crude oil exposure, due to phytotoxic nature of crude oil and immobilization of availability of plant nutrient in soil (Udo, and Fayemi, 1995)]. Physical, chemical and thermal methods have commonly been employed to clean up the crude oil polluted soils (Roy *et al.*, 2016). Techniques are relatively expensive and require site restoration (Lundstedt *et al.*, 2003). Biological methods are relatively affordable and do not introduce any additional chemicals to the environment. These bacterial strains have

potential in recovering the crude oil polluted soils as well as improving plant health (Glick, 2010). The rate of crude oil degradation using beneficial microorganisms had also been augmented in the field by supplying limiting nutrients to the affected sites (Atlas, 1991). Bioaugmentation involves inoculating exogenous degrading microorganisms to the soil (Taccari *et al.*, 2012; Wu *et al.*, 2016). Biostimulation stimulates the degrading capacity of the indigenous community by adding nutrients to avoid metabolic limitations (Abed *et al.*, 2014). Currently, many reports have shown that bioaugmentation and biostimulation enhance the biodegradation of hydrocarbons in crude oil polluted soil (Suja *et al.*, 2014). Because the process of bioremediation is carried out by various microorganisms in soil, understanding how bio-

augmentation and biostimulation influence the populations of hydrocarbon degraders, the diversity and activity of the microbial community, and the ability of bacterial inoculum to adapt to new environmental conditions are very important in ensuring effective bioremediation of crude oil polluted soil (Liu *et al.*, 2012).

MATERIALS AND METHODS

The main objective of this research is to test the capacity to increase the biodegradation of crude oil by stimulating the bacteria. To achieve data concerning the bioremediation of polluted soil with crude oil was realized a greenhouse experiment. The soil used for this experiment was a calcic chernozems. This type of soil was chosen because of its physical, chemical and biological properties favorable to plant growth. 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 crude oil, and bioaugmentation based on enriching the soil with specific biodegrading hydrocarbons microorganisms. Biostimulation - the first technological link included an innovation element based on using an organic compound made from cellulose fibers for soil polluted conditioning with additives to optimize its structure, water and air circulation regime in soil, and not least achieving a protective interface between degrading microorganisms and pollutant.

Ecosol compound was chosen for experiment by analysing a series of organic compounds suitable for conditioning soil polluted with organic pollutants, especially because of its biodegradability properties. Bioaugmentation - the second technology link was achieved by soil inoculation with bacterial bioproducts made from specific bacteria selected and tested in the laboratory for their ability to degrade crude oil.

The experiment was set up by an artificial pollution of soil 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*.

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 + 0.25% 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.

RESULTS AND DISCUSSIONS

Bacterial inoculum application after 21 days from the experiment beginning was reflected in very significant increases in the levels of total heterotrophic bacteria determined in polluted soil. Immediately after artificial soil pollution with crude oil, at impact, bacterial populations have decreased the multiplication rate, and later, after an adaptation and selection process, the resistance component will extensively proliferate.

For soil polluted with 5% crude oil, the bacterial top, including those placed in the soil by inoculation was 30 days after impact, respectively 7 days after inoculum application, while in soil polluted with 10% crude oil, multiplication top of bacteria was observed in the determination made at 45 days after impact and 21 days after inoculum application, showing once again how necessary is for microorganisms habituation and adaptation to environment being a function of pollutant concentration. 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 crude oil exercised by Ecosol organic compound applied.

The quantitative evolution of bacterial communities from soil polluted with crude oil clearly demonstrated that the Ecosol application decreases the time needed for microorganisms involved in crude oil biodegradation to adapt at substrate and conditions of pollution.

Frequent bacterial isolated from experimental variants, in most determination stages were: *Pseudomonas*, *Arthrobacter*, *Mycobacterium*, bacterial of the most reputable in terms of species with high capacities in hydrocarbon biodegradation.

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*. The results are presented in table 1 and 2.

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 maximum dose of Ecosol. At 45 days, there was a real explosion in variants of bacteria inoculated with total heterotrophic bacteria 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.

Bacterial microcosms developed on the surface of nutrient agar media (Topping and CZPA) most abundant were *Arthrobacter* and *Pseudomonas* strains, which were derived from both the inoculum is applied, and its own microflora of the soil (Figs 1-4).

The value of the total heterotrophic bacteria in soil is a response to a large number of factors, including crude oil content, which causes new ecological conditions with a strong impact on microbial evolution. These conditions affect not only the size, but also the diversity of bacterial communities. The soil is mainly colonized by bacterial genera species able to use hydrocarbons from crude oil in its metabolism.

Tab. 1. Diversity of heterotrophic bacterial microflora in the soil polluted with 5% crude oil

Experimental variants	7 days	14 days	21 days	30 days	45 days	60 days	90 days
V ₁ – unpolluted soil	<i>Arthrobacter globiformis</i> , <i>Bacillus cereus</i> , <i>Pseudomonas</i> sp., <i>Streptomyces</i> sp.	<i>Bacillus megaterium</i> , <i>B. cereus</i> , <i>B. polymyxa</i> , <i>Arthrobacter globiformis</i> , <i>Pseudomonas</i> sp.	<i>Arthrobacter globiformis</i> , <i>Bacillus cereus</i> , <i>B. megaterium</i> , <i>B. circulans</i> , <i>B. polymyxa</i> .	<i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>Pseudomonas</i> sp., <i>Bacillus megaterium</i> , <i>B. cereus</i> , <i>Streptomyces</i> sp.	<i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> , <i>B. megaterium</i> , <i>B. cereus</i> .	<i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>B. polymyxa</i> , <i>Pseudomonas</i> sp.	<i>Bacillus megaterium</i> , <i>B. circulans</i> , <i>B. polymyxa</i> , <i>B. cereus</i> , <i>Streptomyces</i> sp.
V ₂ – soil polluted with 5% crude oil	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus sphaericus</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> .	<i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>B. polymyxa</i> , <i>Arthrobacter simplex</i>	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> , <i>B. circulans</i> , <i>Arthrobacter simplex</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Mycobacterium roseum</i> , <i>Bacillus circulans</i> , <i>B. megaterium</i> .	<i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>B. polymyxa</i> , <i>Pseudomonas</i> sp., <i>Mycobacterium phley</i> , <i>Streptomyces</i> sp.
V ₄ – soil polluted with 5% crude oil + 0.25% Ecosol	<i>Arthrobacter citreus</i> , <i>Streptomyces</i> sp.	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> .	<i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> .	<i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> , <i>B. polymyxa</i> , <i>B. sphaericus</i> , <i>Arthrobacter citreus</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> , <i>B. circulans</i> , <i>Mycobacterium</i> sp., <i>Bacillus cereus</i> , <i>B. megaterium</i> , <i>B. macerans</i> , <i>Arthrobacter simplex</i> , <i>Bacillus sphaericus</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter simplex</i> , <i>Bacillus circulans</i> , <i>B. megaterium</i> , <i>Mycobacterium phley</i> , <i>Streptomyces</i> sp.

Tab. 1. Diversity of heterotrophic bacterial microflora in the soil polluted with 5% crude oil – continued

Experimental variants	7 days	14 days	21 days	30 days	45 days	60 days	90 days
V ₅ – soil polluted with 5% crude oil + 0.25% Ecosol + bacterial inoculum	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> .	<i>Arthrobacter globiformis</i> , <i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , <i>B. megaterium</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> , <i>B. circulans</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>B. megaterium</i> , Micrococcus .	<i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> , <i>Mycobacterium roseum</i> , <i>Bacillus polymyxa</i> , <i>B. megaterium</i> , <i>B. sphaericus</i> , Enterobacter .	<i>Pseudomonas</i> sp., <i>Arthrobacter simplex</i> , <i>Bacillus circulans</i> , <i>Mycobacterium</i> sp., <i>Bacillus sphaericus</i> , <i>B. megaterium</i> , <i>Arthrobacter globiformis</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Mycobacterium roseum</i> , <i>Bacillus circulans</i> , <i>Streptomyces</i> sp.
V ₆ – soil polluted with 5% crude oil + 0.5% Ecosol	<i>Pseudomonas</i> sp., <i>Bacillus cereus</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> .	<i>Pseudomonas</i> sp., <i>Bacillus cereus</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> , <i>B. polymyxa</i> .	<i>Pseudomonas</i> sp., <i>Bacillus cereus</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> , <i>Bacillus circulans</i> .	<i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , <i>B. polymyxa</i> , <i>B. megaterium</i> .	<i>Pseudomonas</i> sp., <i>Mycobacterium phley</i> , <i>Arthrobacter simplex</i> , <i>Bacillus polymyxa</i> , <i>B. circulans</i> , <i>B. sphaericus</i> , <i>B. megaterium</i> .	<i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , <i>Arthrobacter simplex</i> , <i>Bacillus polymyxa</i> .	<i>Bacillus cereus</i> var. <i>mycoides</i> , <i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> , <i>Mycobacterium roseum</i> .
V7 – soil polluted with 5% crude oil + 0.5% Ecosol + bacterial inoculum	<i>Pseudomonas</i> sp., <i>Arthrobacter citreus</i> , <i>Streptomyces</i> sp.	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>Streptomyces</i> sp.	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>Mycobacterium phley</i> , <i>Bacillus polymyxa</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> , <i>B. circulans</i> , <i>Mycobacterium roseum</i> , <i>Bacillus megaterium</i> , <i>B. sphaericus</i> , <i>B. macerans</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> , <i>B. circulans</i> , <i>B. sphaericus</i> , <i>B. megaterium</i> , <i>Arthrobacter simplex</i> , <i>Mycobacterium phley</i> , Flovobacterium .	<i>Arthrobacter globiformis</i> , <i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , <i>B. macerans</i> .

Fig. 1. Bacterial microcosm (Topping medium) **Fig. 2.** Macroscopic appearance - *Pseudomonas* sp. colonies on Topping medium (experimental variant V8)

Tab 2. Diversity of heterotrophic bacterial microflora in the soil polluted with 10% crude oil

Experimental variants	7 days	14 days	21 days	30 days	45 days	60 days	90 days
V ₁ – unpolluted soil	<i>Arthrobacter globiformis</i> , <i>Bacillus cereus</i> , <i>Pseudomonas</i> sp., <i>Streptomyces</i> sp.	<i>Bacillus megaterium</i> , <i>B. cereus</i> , <i>B. polymyxa</i> , <i>Arthrobacter globiformis</i> , <i>Pseudomonas</i> sp.	<i>Arthrobacter globiformis</i> , <i>Bacillus cereus</i> , <i>B. megaterium</i> , <i>B. circulans</i> , <i>B. polymyxa</i> .	<i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>Pseudomonas</i> sp., <i>Bacillus megaterium</i> , <i>B. cereus</i> , <i>Streptomyces</i> sp.	<i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> , <i>B. megaterium</i> , <i>B. cereus</i> .	<i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>B. polymyxa</i> , <i>Pseudomonas</i> sp.	<i>Bacillus megaterium</i> , <i>B. circulans</i> , <i>B. polymyxa</i> , <i>B. cereus</i> , <i>Streptomyces</i> sp.
V ₃ – soil polluted with 10% crude oil	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Streptomyces</i> sp.	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> .	<i>Arthrobacter globiformis</i> , <i>Pseudomonas</i> sp., <i>Bacillus megaterium</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>Streptomyces</i> sp.	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Mycobacterium</i> sp., <i>Bacillus megaterium</i> , <i>B. circulans</i> , <i>Arthrobacter simplex</i> , <i>Bacillus sphaericus</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter simplex</i> , <i>Bacillus circulans</i> , <i>B. polymyxa</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> , <i>B. cereus</i> , <i>B. macerans</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter citreus</i> , <i>Bacillus circulans</i> , Enterobacter , <i>Streptomyces</i> sp.
V ₈ – soil polluted with 10% crude oil + 0.5% Ecosol	<i>Arthrobacter globiformis</i> , <i>Pseudomonas</i> sp., <i>Streptomyces</i> sp.	<i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>B. cereus</i> , <i>Mycobacterium</i> sp.	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> , <i>Streptomyces</i> sp.	<i>Bacillus circulans</i> , <i>Mycobacterium</i> sp., <i>Bacillus megaterium</i> .	<i>Bacillus cereus</i> var. <i>mycoides</i> , <i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Mycobacterium phley</i> , <i>Bacillus megaterium</i> .	<i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> , <i>Mycobacterium roseum</i> , <i>Bacillus megaterium</i> .	<i>Arthrobacter globiformis</i> , <i>Pseudomonas</i> sp., <i>Bacillus polymyxa</i> , <i>Bacillus circulans</i> .
V ₉ – soil polluted with 10% crude oil + 0.5% Ecosol + bacterial inoculum	<i>Pseudomonas</i> sp., <i>Mycobacterium roseum</i> .	<i>Pseudomonas</i> sp., <i>Mycobacterium</i> sp., <i>Bacillus circulans</i> .	<i>Mycobacterium phley</i> , <i>Pseudomonas</i> sp., <i>Bacillus circulans</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter simplex</i> , <i>Bacillus circulans</i> , <i>B. polymyxa</i> .	<i>Pseudomonas</i> sp., <i>Bacillus circulans</i> , Micrococcus , <i>Bacillus polymyxa</i> , <i>Arthrobacter globiformis</i> , <i>Mycobacterium phley</i> , <i>Bacillus megaterium</i> , <i>Arthrobacter citreus</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter simplex</i> , <i>Bacillus polymyxa</i> , <i>B. circulans</i> , <i>Arthrobacter citreus</i> , <i>Mycobacterium phley</i> , <i>Bacillus megaterium</i> , <i>B. sphaericus</i> , <i>B. cereus</i> .	<i>Pseudomonas</i> sp., <i>Mycobacterium phley</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>Arthrobacter citreus</i> , <i>Bacillus megaterium</i> .
V ₁₀ – soil polluted with 10% crude oil + 1% Ecosol	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Mycobacterium</i> sp.	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> , <i>B. polymyxa</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus sphaericus</i> , <i>B. circulans</i> , <i>Mycobacterium</i> sp., <i>Bacillus megaterium</i> , <i>Arthrobacter simplex</i> , <i>Bacillus polymyxa</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter citreus</i> , <i>Bacillus circulans</i> , <i>B. polymyxa</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> , <i>Mycobacterium roseum</i> , <i>Bacillus sphaericus</i> , <i>Arthrobacter simplex</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Mycobacterium roseum</i> , <i>Bacillus circulans</i> .
V ₁₁ – soil polluted with 10% crude oil + 1% Ecosol + bacterial inoculum	<i>Pseudomonas</i> sp., <i>Mycobacterium phley</i> .	<i>Pseudomonas</i> sp., <i>Mycobacterium</i> sp., <i>Arthrobacter globiformis</i> .	<i>Pseudomonas</i> sp., <i>Mycobacterium roseum</i> , <i>Arthrobacter globiformis</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Mycobacterium</i> sp., <i>Bacillus megaterium</i> , <i>B. circulans</i> .	<i>Pseudomonas</i> sp., <i>Mycobacterium phley</i> , <i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus megaterium</i> , <i>B. polymyxa</i> , <i>Arthrobacter citreus</i> , <i>Bacillus macerans</i> .	<i>Pseudomonas</i> sp., <i>Mycobacterium phley</i> , <i>Bacillus circulans</i> , <i>Arthrobacter globiformis</i> , <i>Bacillus polymyxa</i> , Flavobacterium , <i>Bacillus sphaericus</i> , <i>B. megaterium</i> .	<i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Bacillus circulans</i> .

Fig. 3. Bacterial microcosm from control variant

Fig. 4. *Pseudomonas* sp. – growth on CZPA medium

Survival of microorganisms in crude oil in uninoculated variants and after bacterial inoculation in the inoculated variants is a key deciding factor in the rate of biodegradation of hydrocarbons in soils.

CONCLUSION

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 crude oil, and bioaugmentation based on enriching the soil with specific biodegrading hydrocarbons microorganisms. For soil polluted with 5% crude oil, the bacterial top, including those placed in the soil by inoculation was 30 days after impact, respectively 7 days after inoculum application, while in soil polluted with 10% crude oil, multiplication top of bacteria was observed in the determination made at 45 days after impact

and 21 days after inoculum application, showing once again how necessary is for microorganisms habituation and adaptation to environment being a function of pollutant concentration. In the most determination periods were determined *Pseudomonas*, *Arthrobacter*, *Mycobacterium* as frequent bacterial isolated from experimental variants. 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*.

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