DECONTAMINATION OF A PETROLEUM HYDROCARBONS POLLUTED SOIL BY DIFFERENT BIOREMEDIATION STRATEGIES

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ABSTRACT

Romania, as a country producing oil and with a tradition in its processing, unfortunately, is affected by some accidental, undesired phenomena that lead to the environmental pollution with oil, petroleum products and residues resulted from the oil processing. Over 50 thousand ha of agricultural and forestlands are polluted with petroleum hydrocarbons and brine, within the areas of petroleum extraction, transportation and processing. These pollution phenomena induces important changes at the levels of the soil organisms, especially microorganisms, even the extinction of a number of species, disturbing the bio-geo-chemical elements cycles, organic matter mineralization, with severe reduction effect of soil fertility. By avoiding the pollution effects and rebuilding the initial state of ecosystems, the first stage of the sustainable development concept is applied that, in the soil science field represent the optimization of soil function that generate the fertility. In this view, an experiment for rehabilitation of a petroleum-polluted soil was carried out using the different bioremediation in situ strategies.

INTRODUCTION

As a result of the inventory carried out by the Romanian National Environmental Protection Agency, through subordinate units, between 2007-2008, have been identified a number of 1393 contaminated/potentially contaminated sites representing areas where various large or small-scale industrial activities were conducted, including petroleum activities (HG 683/2015). Romania, as one of the first European countries with crude-oil extraction and processing industry, unfortunately, is affected by some accidental oil or petroleum products spills, that lead to the environment pollution. The development of petroleum industry, both the extraction and processing, including transportation of oil and oil products, often takes together with several secondary, unforeseeable phenomena, with effects that are more or less harmful for the environment. Among these, the most dangerous ones are related to soil pollution by petroleum with or without brine. Over than 50 thousand ha agricultural and forestlands are polluted by petroleum hydrocarbons and brine, within the areas of petroleum extraction, transportation and processing in Romania. Petroleum hydrocarbons radically modifying soil properties (physical, chemical and biological), forming an impermeable film on soil surface, which disturb water circulation in soil and gas exchange between soil and atmosphere, causing the plant roots asphyxiation and favoring the reduction processes. As the conditions become more anaerobic, the number and metabolic activity of bacteria decrease (Hughues and McKenzie, 1975, in Dart &Stretton, 1980). Hugh organic carbon of petroleum (98% hydrocarbons), increase the C: N ratio in soil, exert a negative influence on microbiological activity and plant nitrogen nutrition. The existence of petroleum hydrocarbons on the cultivated soils affect seed germination, plant growth and yield. The volatile components of the crude oil could penetrate the seeds and kill the embryo, or by restrict the water movement towards the seeds and decreasing the necessary oxygen, inhibit its germination (Voiculescu A-R. et al, 2005). Experimental studies carried out by Dumitru et al., (1998) revealed that the maize yield in oil-polluted soil decreasing with increasing of total petroleum hydrocarbons (TPH) concentration, with 36% for 0,5% TPH, and with 57% for 1% TPH. These pollution phenomena induces important changes at the levels of soil organisms, especially microorganisms, leading to the extinction of a large number of species and determining the decrease of soil fertility, the most important property of soil which sustains the vegetal and animal life, human life, respectively.

Human intervention, however, may be required to establish a suitable microbial community at the site or to apply agronomic techniques (such as tillage and fertilization) to enhance petroleum natural degradation or migration processes (Frick et al., 1999, Gomez et al., 2013). For establishing the most appropriate technologies of soil decontamination, a thorough knowledge of physical, chemical and biological characteristics of the polluted soils are necessary (Haghollahi A., et al., 2016, Lacatusu A-R. et al., 2013). The major process contributing to oil polluted soil decontamination is biodegradation that involves the participation of a large variety of microorganisms (Dart &Stretton, 1980). The process consists in the organic contaminants decomposition by the natural microbial inhabitants in soil, which use the pollutants as carbon and energy sources for producing the cell material. Among the microorganisms, some bacteria, yeasts, filamentous fungi and algae possessing the enzymatic equipment able to degrade the petroleum hydrocarbons in soil (Dineen et al., 1989; Prichard, P.H., 1992; Margesin R. & Schinner F., 2001; Viňas, M. et al., 2002). The great diversity and the abundant representation of bacterial and fungal genera with hydrocarbon metabolic capabilities render soil a favorable environment for petroleum biodegradation (Bossert & Bartha, 1986). Soil, as biologic system animated by the immense microbial communities, could influence through their physics and chemical properties the development and activity of these microorganisms. Many technologies developed in the last years for the decontamination of oil-polluted soil rely on the microbial degradation. The amazing physiological versatility of natural communities of microorganisms in various habitats and their ability to metabolize and often mineralize an enormous number of organic molecules has have permitted many decontamination technologies, generically named with the term “bioremediation” (Alexander, 1994). The result of crude oil dispersal on the soil could be, in the first stage, the increasing of bacterial community by proliferation of hydrocarbons degraders’ strains. But, simultaneously, the decreasing of the diversity of populations is occurred. Very soon, after soil pollution with petroleum, the petroleum hydrocarbons degraders, both bacterial and fungal strains, are about 50% bacteria, respectively 60-80% fungi, gradually becoming 100% from the entire microorganisms community, while in unpolluted soils these microorganisms represent only 0.1%. This fact is due to elimination of microorganisms sensitive to oil pollution of soil. More than 200 bacterial, algal, and fungal genera, encompassing over 500 species, have been recognized as capable of hydrocarbon degradation (Kosta et al, 2011). Bioremediation technologies have to include the whole necessary measures to create a favorable environment for development and enhancing activity of those microorganisms that can break down petroleum hydrocarbons, but, if the natural communities are very poor in petroleum degraders, the soil enrichment with selected microorganisms is necessary.

MATERIALS AND METHODS

The in situ bioremediation experiment carried out in the greenhouse was developed on an Albic Luvisols artificially polluted with 3% light crude-oil.

The experimental treatments applied to oil-polluted soil were:

- Organic biostimulation with farmyard manure corresponding to 50 $t.ha^{-1}$;

- Mineral biostimulation with $N_{200}P_{200}K_{100}$ kg.ha^{-1} active substances;
- Augmentation of polluted soil with selected bacteria.

Biostimulation, both organic and mineral, aims, firstly, to raise the total nitrogen content in soil in relation with the total organic carbon (resulted from petroleum hydrocarbons), implicitly, to adjust the C: N ratio to an optimum level.

The Albic Luvisols are native soils poor in nutrients, which, usually, sustain small bacteria communities, being favorable for fungi development. In this case, soil bioaugmentation with selected bacteria was required. Thus, bacteria species isolated from the same soil type, but polluted with very high oil concentration, were purified and multiplied through successive dilution and inoculation in Petri dishes with different nutritive media (Topping, Potato Sugar Peptone Agar). These bacterial strains were tested in laboratory to reveal its capabilities to degrade the petroleum by cultivation on the mineral media without other carbon source than petroleum hydrocarbons. The bacterial strains used for soil augmentation belonging to the genera *Pseudomonas, Arthrobacter, Micrococcus, Flavobacterium*.

By combination of the three treatments above mentioned resulted six different bioremediation strategies namely:

- natural attenuation (NA),
- mineral biostimulation (MB),
- organic biostimulation (OB),
- organo-mineral biostimulation (OMB),
- mineral biostimulation + bioaugmentation (MB+BA) and
- organo-mineral biostimulation + bioaugmentation (OMB+BA).

At the beginning (two weeks after all treatments were been applied) and the final of experiment (after six month), soil samples were analyzed to assess chemicals soil properties: total organic carbon (TOC) - Walkley-Black method (modified by Gogoașă), total nitrogen content (Nt) – Kjeldahl method, and C:N ratio by calculation. Total petroleum hydrocarbons were determined by gravimetric method (ISO 13511:2007). Also, microbiological analyses: quantitative determinations of heterotrophic bacteria (total bacteria number method) using traditional cultivation methods, and taxonomic determinations by usually identification methods, optical microscopy, determination keys and physiological tests, were carried out (Bergey, 1986, Clark, F., 1965, Pitt J.I., 1991)

RESULTS AND DISCUSSIONS

The interpretation of the obtained results aims to reveal the efficiency of each treatment applied on the petroleum hydrocarbons degradation, and the interaction between these, for establish the most appropriate bioremediation strategy for decontamination of an Albic Luvisol in these concrete ecological conditions and pollution level.

The most important indicators of the bioremediation technology efficiency are the total number (TNB) and variety of bacteria, mainly those bacteria with petroleum breakdown metabolic abilities, and the increasing of total petroleum hydrocarbons in polluted soil.

Organo-mineral biostimulation effects.

The native Albic Luvisol used for this experiment being very poor in nutrients and humus, even unpolluted, requires fertilization, both organic and mineral. Farmyard manure, as organic fertilizer, contain all substance necessary to plants growth, increases water hold capacity of soil and buffering capacity to counteract the toxicity of different pollutants, improves the soil structure and permeability, stimulates the microorganisms activity, and by organic matter decomposition release important quantities of nutrients and enzymes into the soil. Farmyard manure, is also, a reservoir of microorganisms active in organic matter decomposition. All these properties recommend its utilization to petroleum-polluted soils bioremediation.

After six month, at the final stage of the experiment, the total organic carbon (TOC) content increases both in organic biostimulation and organo-mineral biostimulation treatments. Obviously, bioaugmentation combined with organic and organo-mineral biostimulation (MBS+BA and OMBS+BA) led to a better and faster mineralization of organic matter both from manure and petroleum hydrocarbons, with correspondent reducing of TOC values (Figure 1). Data suggest that in the absence of bioaugmentation, in this particular polluted soil, the manure acting more than an adsorbent of the petroleum hydrocarbons, fact which could slowing down the pollutant biodegradation.

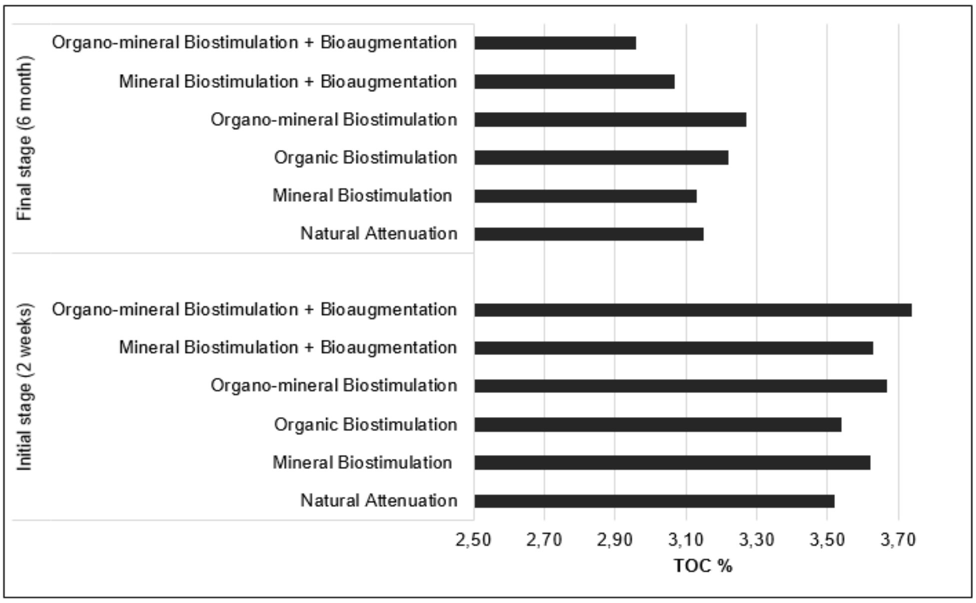

Figure 1. TOC in soil under different bioremediation treatments

The main effect of mineral biostimulation with $N_{200}P_{200}K_{100}$ was to create and maintain, on the entire experimental time period, appropriate levels of nutrients available to the microorganisms, both those natural inhabitants and inoculants, so they be able to multiply and breakdown the petroleum hydrocarbons.

As concerning the total nitrogen content, the highest values was obtained in bioaugmented treatment in both analyzing stages, as a result of nitrogen released from the cellular structures of the dyed microorganisms (Figure 2).

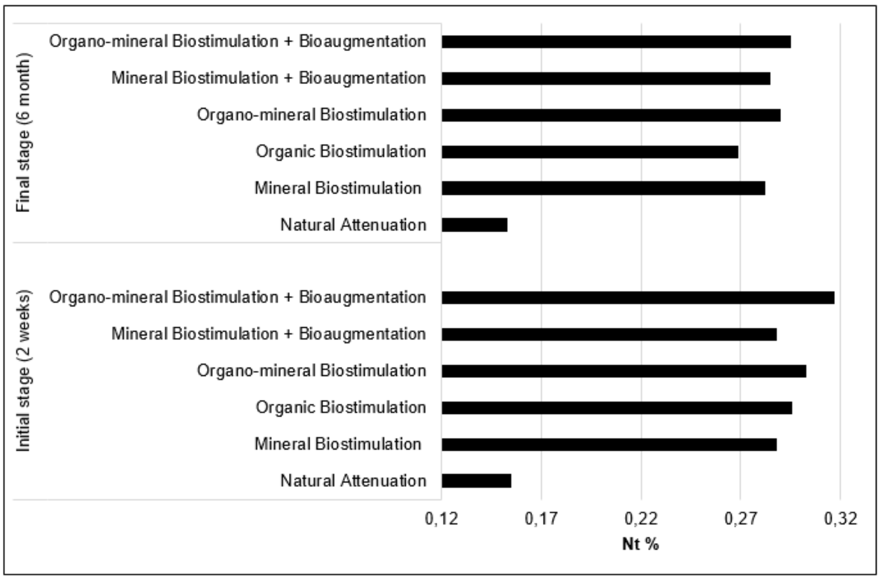

Figure 2. Total nitrogen in soil under different bioremediation treatments

At the fine of experiment the C: N ratio values were, generally, close by those characteristics to an unpolluted soil at the same type, except the natural attenuation treatment (NA), where the C:N ratio still remained very high (24). The best results were obtained in both bioaugmented treatments (MBS+BA and OMBS+BA), where the values of C: N ratio were 12.2, respectively 11.3, reflecting a good balance of these nutrients in soil (Figure 3).

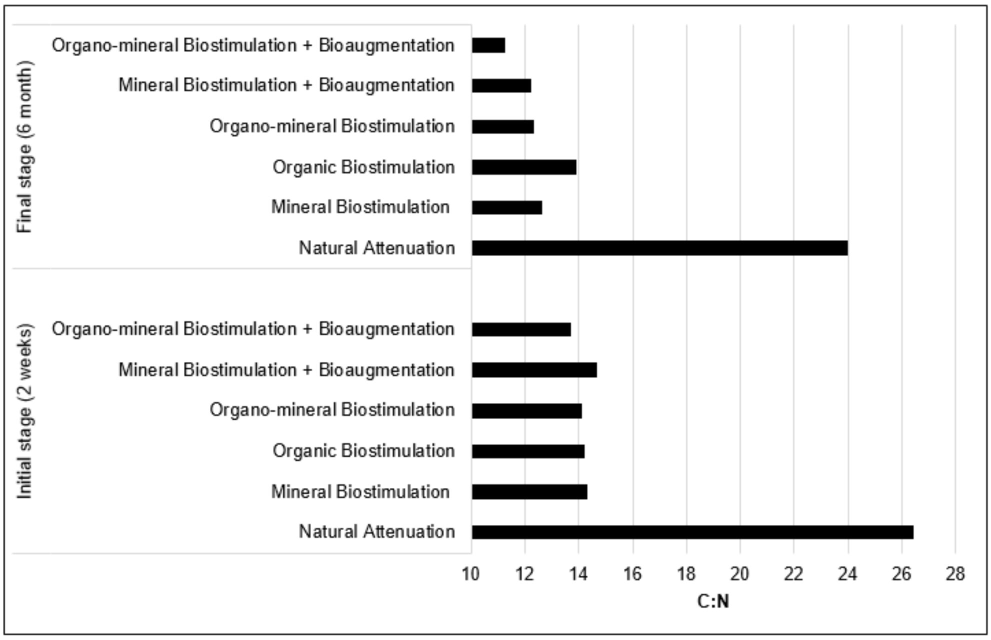

Figure 3. C/N in soil under different bioremediation treatments

Evolution of bacteria communities. In the first experimental stage, two weeks after applying of all treatments, as compared to natural attenuation variant, spectacular values of total bacteria number were recorded, reflecting the influence of every one of bioremediation measures applied to petroleum-polluted soil: mineral and/or organic biostimulation, bioaugmentation and their combinations. Moreover, the data reflect the interrelation between them that intensifying each other. In the treatment with all bioremediation measures applied was obtained the highest value of total number of bacteria, respectively 1,6x10^8/g dry soil (Figure 4). This could be considered a low value for this parameter, but, for a strong oil-polluted Albic Luvisols is quite high.

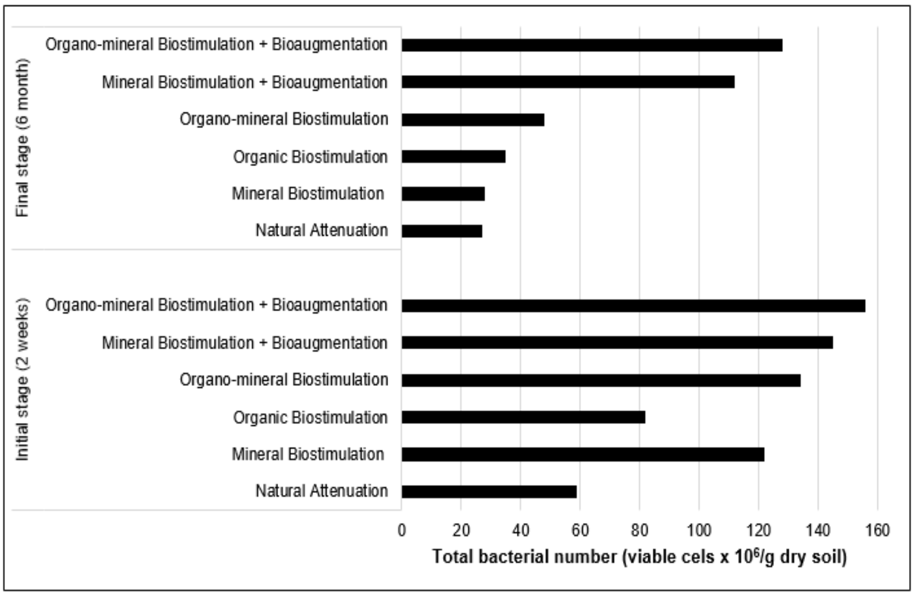

Figure 4. Bacterial communities in soil under different bioremediation treatments

Obviously, this good developing of bacteria is due to proliferation of strains added to the polluted soil by bioaugmentation, and confirms the acclimation of microorganisms to substrate. Moreover, the values reveal the stimulating effect of mineral fertilizers on bacterial communities.

At the final stage of experiment, after six month, there happened a uniformity of values of total number of bacteria at a low level, between 3-5x10^7/g dry soil, in almost all treatments, close to those from natural attenuation treatment (2.7x10^7/g dry soil). Excepted from this rule were bioaugmented treatments, where, the bacterial communities remained at a superior level (1,1-1,3x10^8/g dry soil).

Obviously, the total amount of bacteria in soil is a response to a great number of factors, among which petroleum residues determine new ecological conditions with high impact on the microbial evolution. These conditions influence not only the size, but also the diversity of bacterial communities. The soil becomes inhabited by bacterial genera and species able to use and degrade the petroleum hydrocarbons. Bacterial species frequently isolated from this soil were: *Pseudomonas aeruginosa, Pseudomonas sp., Arthrobacter globiformis, Arthrobacter citreus, Mycobacterium roseum, Micrococcus sp., Flavobacterium sp.* and some *Bacillus* species. These isolated bacteria genera and species are well known as hydrocarbon degraders. Species from all these genera belonging to inoculum utilized for the soil bioaugmentation, and their high frequency indicate a good rate of survive and adaptability to environmental condition.

Also, data confirms good choice of consortium selected bacteria strains for inoculation. In these short experimental period (six month), a selection of adapted and implied in petroleum biodegradation microorganisms, by quantitative and quantitative elimination of that sensitive part of them, was occurred. As consequences, instead of decreasing of the dimension of bacterial populations, this has becomes more and more specialized in petroleum hydrocarbons biodegradation. This is first condition to succeed in decontamination of petroleum-polluted soil.

Evolution of total petroleum hydrocarbons (TPH). The dynamics of the TPH during the experimental time reveals very clearly differences between bioremediation treatments and, also, the best combination between them. In all bioremediation treatments the petroleum breakdown, reflected in the decreasing of total petroleum hydrocarbons, was emphasized by bioaugmentation (Figure 5). That is an evidence for efficiency of selected bacteria using for inoculation in petroleum-polluted Albic Luvisol decontamination.

The total petroleum hydrocarbons contents, constantly decreased during experimental period, but the highest rate of the process was observed in bioaugmented treatment with mineral and organo-mineral biostimulation, where the greatest TPH% reducing occurred. At six months after the experiment has started, TPH concentration in soil of this most performant variant (OMBS+BA) decreased up to 2700 mg·kg-1d.w, that means 9% from initial concentration of 3% crude-oil.

The efficiency of different bioremediation treatments. As we can see in Figure 6, the bioremediation efficiency in the albic Luvisols polluted soil with crude oil was proven by the yield results. During the experiment, the measurements performed after six months, revealed an yield of the decreasing of the TPH concentration in soil up to 91%, as compared to the initial concentration of crude-oil used for artificially pollution of the soil, in the treatment consist in organo-mineral biostimulation and bioaugmentation with selected bacteria (OMBS+BA).

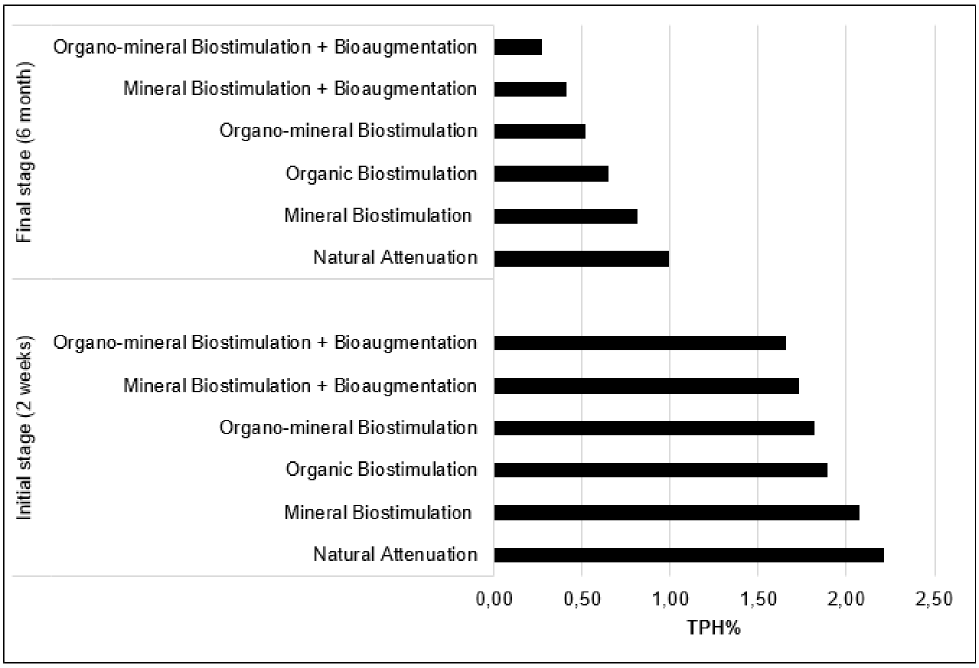

Figure 5. TPH% in soil under different bioremediation treatments

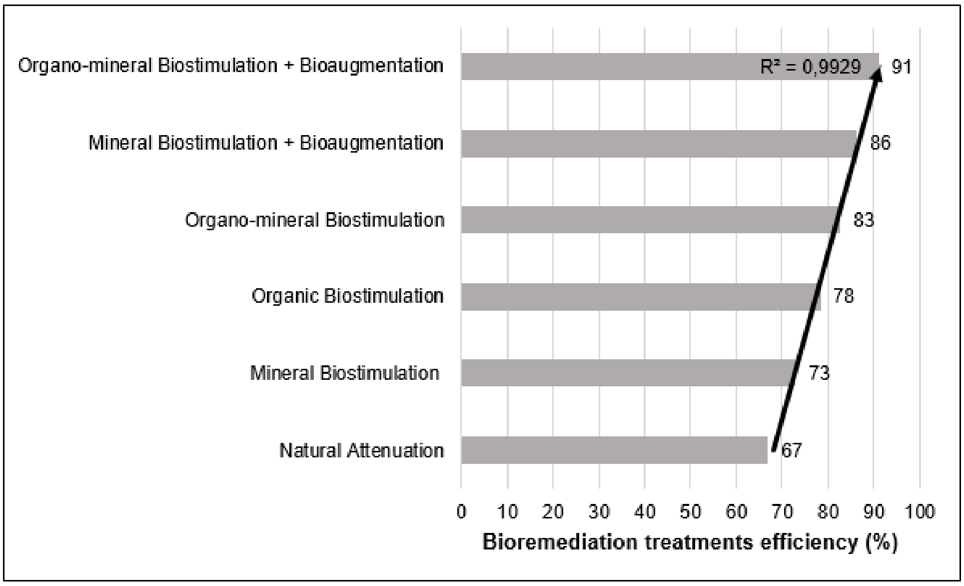

Figure 6. Soil decontamination yield under different bioremediation treatments

Analyzing the dynamics of the TPH decrease in the experimental variant which consisted of natural attenuation (NA) is observed another important aspect, namely that light oil used for artificial pollution of the soil has a component readily degradable and volatile, which disappears in six months only through the own soil self-cleaning mechanisms. In other words, about 33% of the hydrocarbons in the light crude oil used in the experiment represent hardly degradable component, which could be partially removed by the application of bioremediation measures, respectively, biostimulation and augmentation of polluted soil. In the experimental variant with the highest performance in terms of soil decontamination, after 6 months, this highly resistant to degradation crude-oil fraction was reduced up to 9%.

CONCLUSIONS

The experiment conducted in order to decontaminate an Albic Luvisol artificially polluted with 3% light crude oil consisted in six experimental variants resulted from combination of different bioremediation strategies: natural attenuation (control), mineral biostimulation with $N_{200}P_{200}K_{100}$ kg.ha^{-1} active substances, organic biostimulation with farmyard manure corresponding to 50 t.ha^{-1}, and bioaugmentation with selected bacteria. When treated appropriately by optimizing the biodegradation potential of natural-occurring hydrocarbon degraders, the main part of a petroleum hydrocarbons can be eliminated more rapidly.

Adequate biostimulation by mineral, organic or combined fertilization was efficient as compared to untreated soil in natural attenuation variant. Organic and mineral fertilizing contributed to a drastically increasing of C:N ratio, ensuring proper quantities of available nutrients for microorganisms, furnishing an adequate energetic support for microbial activities in soil.

The results achieved in this study proved the efficiency of soil bioaugmentation with selected bacterial consortium to accelerate the biodegradation of petroleum hydrocarbons in crude oil artificial polluted soil. Also, the data reflects that the inoculant microorganisms were able to quickly acclimate, to abundant multiply and effectively breaking down the hydrocarbons from crude oil.

Monitoring of petroleum hydrocarbons concentration in polluted soil shows that in the treatment consist in organo-mineral biostimulation and bioaugmentation with selected bacteria (OMBS+BA), after six months, the TPH concentration decreased up to 0,27% (2700 mg·kg$^{-1}_{d.w.}$), more than three times as compared with those recorded in natural attenuation variant 0,99% (9900 mg·kg$^{-1}_{d.w}$). Also, analysis of the petroleum hydrocarbons degradation yield, in created experimental conditions, shows that in the OMBS+BA treatment, after six months, the TPH concentration decreased up to 91%, as compared to the initial concentration of crude-oil used for artificially pollution of the soil.

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