RESEARCH REGARDING BIOREMEDIATION OF THE SOIL POLLUTED WITH PETROLEUM HYDROCARBONS, IN THE GREEN HOUSE

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Abstract

On the background where no absorbent was used, the highest rate of degradation of the petroleum hydrocarbons (47%) was attained in the variants treated with the AH-SH fertilizer, the highest rate of degradation of the hydrocarbons (45%) on a background without absorbent was attained in the variants treated with potassium humate and microelements along with the bacterial inoculant. Upon utilization of 16 t/ha peat as absorbent without using any microbial inoculant, the highest rate of degradation (43%) of the petroleum hydrocarbons was attained following the fertilization with AH-SG1, upon application of the bacterial inoculant, the highest rate of degradation of the petroleum hydrocarbons (47%) was provided by fertilization with AH-SG2. Upon application of a 16 kg/ha dose of Zeba absorbent without any bacterial inoculant, the highest rate of degradation (47%) of the petroleum hydrocarbons, 45 days after the application of the treatments, was provided by the fertilizer AH-SG2. Upon treatment with 16 kg/ha Zeba absorbent and application of the bacterial inoculant, the highest rate of degradation (52%) was provided by the fertilizer AH-SG1. The second highest rate of degradation, 51%, was provided by the fertilizer AH-SG1. Upon application of 32 kg/ha Zeba absorbent without any bacterial inoculant, the best results in the bioremediation process were obtained following the treatment with the fertilizer AH-SG2 in a dose of 650 l/ha plus 64 kg/ha glucose. In these variants, the level of the petroleum hydrocarbons dropped by 59% after just 45 days of treatment or 60 days after the pollution. Upon application of 32 kg/ha Zeba absorbent along with the bacterial inoculant, the highest rate of degradation of the petroleum hydrocarbons (54%) was provided by the fertilizer AH-SG1. The second highest rate – 53% – was provided by the fertilizer AH-SG2.

Key words: petroleum hydrocarbons, bacterial inoculant, liquid fertilizers, absorbents, bioremediation

In Romania, the damages caused to agriculture as a result of pollution with oil products are up to 50,000 t/year, not to take into consideration the loss caused indirectly to the environmental factors (Toti et al., 1999). The physical and chemical remediation methods for the soils polluted with petroleum proved to be inefficient after 1990 (Abu et al., 1996) on the reason that they could cause a secondary pollution of the soil after their utilization, but also on reason of their extremely high costs.

Biological decontamination consists of stimulating the natural phenomena of microorganism development in order to accelerate the process of metabolization of the pollutants (Toti et al., 2001). Henis (1997) deems that any chemical compound whose accumulation in nature becomes hazardous to the environment can be regarded as a pollutant and its removal by means of microorganisms can be regarded as bioremediation. The efficiency of bioremediation depends on the inoculated degrading microorganisms' ability to remain active in the natural environment (Ta-Chen Lin et al., 2010). The soil contaminated with hydrocarbons, subjected to the bioremediation technology, must be supplemented with macroelements. The nitrogen and phosphorus quantities necessary to the microorganisms' multiplication is estimated based on the bacterial biomass requirement and on the concentration of hydrocarbons (Fan et al., 1998). Dumitru (2005) pointed out that organic fertilization comes not only with a large microbial mass, but also with a significant source of nitrogen and other nutrient macro- and microelements. The application of an increased concentration of nutrients resulted in higher rates of hydrocarbon biodegradation on the farming lands in Nigeria (Ayotamuno et al., 2006). Over 200 species of microorganisms were identified until now

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(bacteria, yeasts, microscopic fungi) able to transform crude oil residues into degradation products (Sirvius şi Tranier, 1985). According to Atlas and Bartha (1972), the most active bacteria in biodegradation are: *Pseudomonas, Arthobacter, Acinetobacter, Flavobacterium, Corynebacterium.* Franche (1974) researched the process of biological assimilation for the residual petroleum products at Oak Ridge – Tennessee, calculating the crude oil degradation rate at 1.425% during the first month and 61.4 % after three months. In the soils of the oil fields in Kuwait *Rhodococcus rhodococcous* was also isolated and it proved to be one of the most efficient bacteria in terms of crude oil degradation (Sorkhoh et al., 1990). As far as degradation of crude oil at the soil surface is concerned, Larter and Alpin (2003) and Larter et al. (2003) demonstrated that the crude oil biodegradation rates range between 10^{-6} and 10^{-7}/kg/m^2/year (for anaerobic biodegradation at the temperature of 60°C) and between 10^{-2} and 10^{-1}kg petroleum/m^2/year. In all the cases, biodegradation is efficient only within the limits of certain pollutant concentrations. If a certain pollutant concentration limit is exceeded in the environment, the microorganisms might remain at the outer edge of the contaminated area or may be destroyed as a result of the pollutant's high toxicity (Vintilescu, 1994).

The efficienty of bioremediation by means of the bacteria of the *Pseudomonas, Bacillius, Vibrio, Micrococus* and *Alcaligenes* species varied from 26.7% to 43.3% within 16 days from application, in a research conducted in Nigeria (Ilah and Antai, 2003). Wodzinski and Bertoloni (1972) estimated that the polycyclic aromatic hydrocarbons (PAHs) such as: naphthalene, anthracene and phenylanthracene serve as a carbon source to the growth of bacteria.

MATERIAL AND METHOD

A trifactorial experiment was organized in the vegetation house of ICPA Bucharest, using the upper horizon (Am) of an aluviosol taken from Comana – Giurgiu and polluted with 3% crude oil, which experiment consisted of 48 variants and 3 repetitions. For performance of the bioremediation experiments on the soil polluted with oil products, four fertilizers were used, which contain humic substances, namely: KH – fertilizer containing potassium humates and microelements; AH-SG1 – fertilizer containing potassium humates in an NPK-type matrix with microelements and 4% monosaccharides, in which nitrogen is in amidic

form; AH-SG2 – fertilizer containing potassium humates in an NPK-type matrix with microelements and 8% monosaccharides, in which nitrogen is in amidic form; AH-SH – fertilizer containing potassium humates in an NPK-type matrix and magnesium, in which nitrogen is in amidic form.

Factor 1: absorbents with 4 graduations peat 0 and 10 g, Zeba 16 kg/ha, Zeba 32 kg/ha.

Factor 2: fertilization with 6 graduations:

- $N_0P_0K_0$
- $N_{200}P_{200}K_{200}$
- Potassium humate (KH)
- AH-SH
- AH-SG1
- AH-SG2

Factor 3: inoculation with 2 graduations, i.e. without inoculation and with inoculation

NPK dose applied: $N_{200}P_{200}K_{200}$ kg/ha;

KH (potassium humate) dose applied: 650 l/ha;

AH-SH (potassium humate in NPK-type matrix) dose applied: 650 l/ha;

AH-SG1 (potassium humate in NPK-type matrix with 50 g glucose/l) dose applied: 650 l/ha with 32 kg glucose;

AH-SG2 (potassium humate in NPK-type matrix with 100 g glucose/l) dose applied: 650 l/ha with 64 kg glucose;

Peat = 16t/ha

Zeba = 16 kg/ha, Zeba = 32 kg/ha

The quantitative determinations of the heterothrophic bacteria were made by means of the technique of decimal serial soil dilution dispersion on Topping agarized nutrient medium (Clarck, 1965, Pochon, 1954, Franser, 1976, Papacostea, 1976) in Petri dishes. The inseminated dishes were incubated at the temperature of 27°C, for 5-7 days. The colonies developed on the surface of the culture media were counted and included in a formula to establish the value of the total number of bacteria (TNB):

$$TNB = \frac{n \times dilution \times 10 \times 100}{100 - H}$$

where: x = average of colonies on the medium (Topping or Czapeck); 10 = coefficient for balancing the 0.1 ml inoculant to which the soil dilution degree is prorated; U% = soil humidity.

The qualitative determinations of the heterothrophic bacteria were made by usual identification techniques: macroscopic techniques (appearance of the colonies: shine, colour, consistency, transparency form, relief, colony edge, pigments diffused in the medium etc.) and microscopic (form, organization, cell dimension etc.), cultures on selective media, physiological diagnostic tests (Bergey, 1986, Papacostea, 1976).

For the determination of the total nitrogen (N%), we used the Kjeldahl method,

disaggregation with H_2SO_4 at 350°C, potassium sulphate and copper sulphate as catalyst – SR ISO 11261:2000, accessible phosphorus (mobile P): according to the Egner-Riehm-Domingo method and colorimetrically dosed with molybdenum blue according to the Murphy-Riley method (reduction with ascorbic acid). Accessible (mobile) potassium: extraction according to the Egner-Riehm-Domingo method and dosing by flame photometry, base saturation degree V% by calculation, petroleum residues determined by the gravimetric method.

RESULTS AND DISCUSSIONS

It was found that without application of absorbents and bacterial inoculant *(Figure 1)*, the highest degradation tate of the petroleum hydrocarbons (47%) was attained in the variants treated with the AH-SH fertilizer (potassium humates in an NPK-type matrix and magnesium) in which the nitrogen is in amidic form).

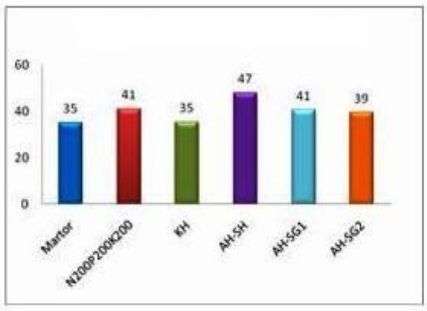

Figure 1. **Petroleum hydrocarbon degradation rate upon application of the treatments without absorbent and without inoculation**

The data presented in *Figure 2* show that the highest petroleum hydrocarbon degradation rate (45%), without absorbent, was attained in the variants treated with potassium humate (KH) and microelements along with the bacterial inoculant.

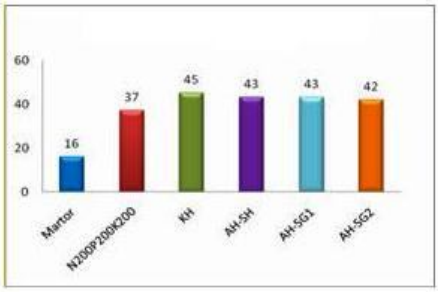

Figure 2. **Petroleum hydrocarbon degradation rate upon application of the treatments without absorbent, with inoculation**

Upon using 16 t/ha peat as absorbent, without using any microbial inoculant, the highest degradation rate (43%) of the petroleum hydrocarbons was attained following the fertilization with AH-SG1 (liquid fertilizer containing potassium humates in an NPK-type matrix with microelements and 50 g/l glucose, applied in a dose of 650 l/ha plus 32 kg/ha glucose). Close results (42%) were attained also in the variants treated with the AH-SG2 fertilizer, which has a structure similar to AH-SG1, but which contains o double quantity of glucose *(Figure 3.)*.

It was found that upon using 16 t/ha peat as absorbent and application of the bacterial inoculant, the highest petroleum hydrocarbon degradation rate (47%) was provided by the fertilization with AH-SG2 (fertilizer containing potassium humates in an NPK-type matrix with microelements and 100 g/l glucose, applied in a dose of 650 l/ha plus 64 kg/ha glucose) *(Figure 4)*.

A 46% degradation rate was attained by the fertilizer containing potassium humate and microelements (KH), and a 45% degradation rate, ranking the third, was attained by the mineral fertilizers applied in an $N_{200}P_{200}K_{200}$ dose.

The data presented in Figure 1.4 show that upon application of a 16 kg/ha dose of Zeba absorbent, in the variants without a bacterial inoculant, the highest petroleum hydrocarbon degradation rate (47%) 45 days after the application of the treatments was attained with the AH-SG2 fertilizer.

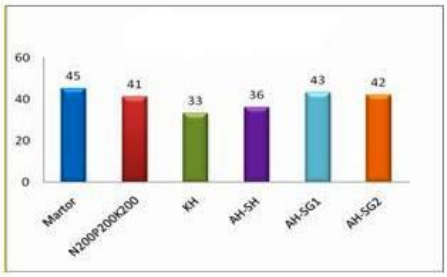

Figure 3. **Petroleum hydrocarbon degradation rate upon application of the treatments when using peat, without inoculation**

The variants treated with mineral fertilizers in an $N_{200}P_{200}K_{200}$ dose ranked the second with a 46% degradation rate.

Upon treatment with 16 kg/ha Zeba absorbent and application of the bacterial inoculant, the highest degradation rate (52%) was attained with the AH-SG1 fertilizer.

The AH-SG1 fertilizer ranked the second with a 51% degradation rate *(Figure 5.)*.

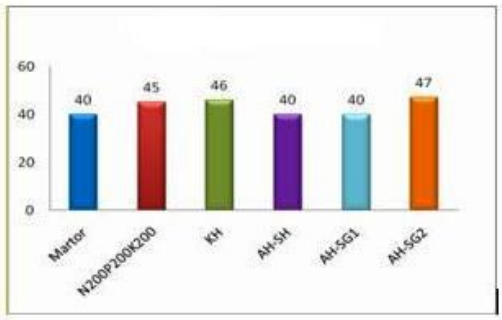

Figure 4. **Petroleum hydrocarbon degradation rate upon application of the treatments when using peat, with inoculation**

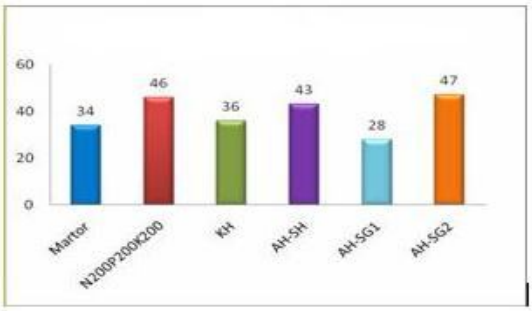

Figure 1.5. **Petroleum hydrocarbon degradation rate upon application of the treatments when using Zeba 16 kg/ha**

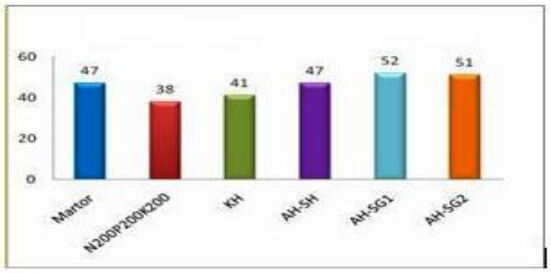

Figure 1.6**. Petroleum hydrocarbon degradation rate upon application of the treatments when using Zeba 16 kg/ha with inoculation**

The data presented in *Figure 6*. point out the fact that upon treatment of the soil poluted with 3% crude oil, using 32 kg/ha Zeba absorbent, wothout bacterial inoculant, the best results in the bioremediation process were attained by treatment with the AH-SG2 fertilizer in a dose of 650 l/ha plus 64 kg/ha glucose.

In these variants, the petroleum hydrocarbon level dropped by 59% just 45 days after the treatment or 60 days after the pollution.The fertilizer AH-SG1 ranked the second with a 52% efficiency.Upon application of 32 kg/ha Zeba absorbent and bacterial inoculation, the highest petroleum hydrocarbon degradation rate (54%) was attained with the AH-SG1 fertilizer. The AH-SG2 fertilizer ranked the second with a 53% degradation rate *(Figure 7.)*. It can be estimated that 60 days after the pollution and 45 days after the application of the treatments the highest petroleum hydrocarbon degradation rate (over 50%) is attained by the use of Zeba absorbent in a dose of 32 kg/ha, plus the application of the bacterial inoculant and the fertilization with AH-SG1 or AH-SG2.

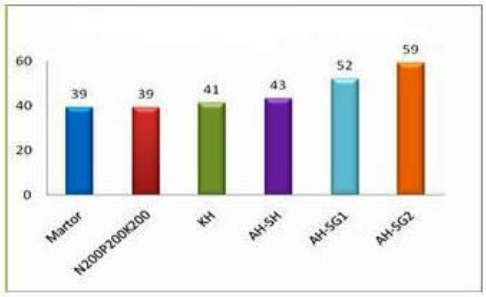

Figure 7. **Petroleum hydrocarbon degradation rate upon application of the treatments when using Zeba 32 kg/ha without inoculation**

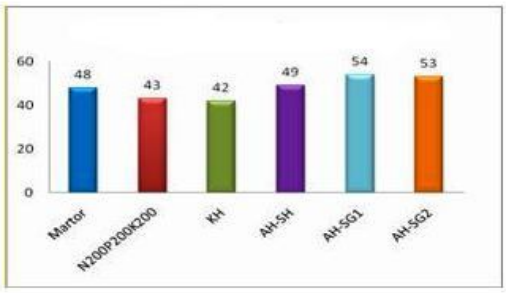

Figura 1.8. **Petroleum hydrocarbon degradation rate upon application of the treatments when using Zeba 32 kg/ha with inoculation**

CONCLUSIONS

- It was found that when no absorbent and no bacterial inoculant are used, the application of the treatments with the liquid fertilizer AH-SH results in the degradation of the petroleum hydrocarbons in the soil (47%) in just 45 days after the treatment or 60 days after the pollution;

- Without any absorbent and using the bacterial inoculant, the application of the treatments with potassium humate resulted in a 45% degradation rate in just 45 days after the treatment or 60 days after the pollution;

- When peat was used as absorbent without bacterial inoculation, the highest petroleum hydrocarbon degradation rate was attained by application of AH-SG1 (43%) in just 45 days after the treatment or 60 days after the pollution;

- When peat was used as absorbent along with bacterial inoculation, the highest petroleum hydrocarbon degradation rate was attained by

application of AH-SG2 (47%) in just 45 days after the treatment or 60 days after the pollution;

- Upon application of 16 kg/ha of Zeba absorbent without bacterial inoculation, the AH-SG2 liquid fertilizer degraded the petroleum hydrocarbons in the soil at a 47% rate in just 45 days after the treatment or 60 days after the pollution;

- Upon application of 16 kg/ha Zeba absorbent along with bacterial inoculation, the AH-SG2 liquid fertilizer degraded the petroleum hydrocarbons in the soil at a 52% rate in just 45 days after the treatment or 60 days after the pollution;

- Upon application of 32 kg/ha of Zeba absorbent without bacterial inoculation, the best results in the bioremediation of the aluviosol polluted with 3% crude oil were attained by treatment with the AH-SG2 fertilizer (59%) in just 45 days after the treatment or 60 days after the pollution;

- Upon application of 32 kg/ha Zeba absorbent along with bacterial inoculation, the treatments with AH-SG2 fertilizer resulted in the degradation of the petroleum hydrocarbons in the soil at a 54% rate in just 45 days after the treatment or 60 days after the pollution.

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