THE EFFECT OF BIOREMEDIATION TECHNOLOGIES ON MOBILE PHOSPHORUS CONTENT FROM POLLUTED SOIL WITH CRUDE OIL

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Abstract. *The paper presents the interplay of the studied factors (absorbents, fertilizers, bacterial inoculum) upon the mobile phosphorus content of a soil polluted with 3% crude oil, 45 days from treatments application or 60 days from the pollution moment. The highest mobile phosphorus values were obtained in the variants fertilized with* $N_{200}P_{200}K_{200}$ *mineral fertilizers against the application of 32 kg/ha Zeba absorbent; the values were also high in the variants in which the* $N_{200}P_{200}K_{200}$ *mineral fertilizers were applied together with bacterial inoculum. From the applied new fertilizers AH-SG1 (potassium humates containing fertilizer in an NPK-type matrix with micro elements plus 50 g/l glucose) and AH-SG2 (potassium humates containing fertilizer in an NPK-type matrix with micro elements plus 100 g/l glucose) had the best effect on the soil mobile phosphorus level.*

Key words: *crude oil, bacterial inoculum, fertilizers, absorbents, mobile phosphorus*

INTRODUCTION

As a natural resource at terrestrial scale, soil represents the main capital goods in agriculture and forestry, an essential resource for obtaining basic agricultural food, fibers and fuel that man needs, which confers it the character of irreplaceable mammon of society (FLOREA ET AL., 2008).

The alert consumption pace through fossils fuels burning, followed by the emissions of some organic compounds which lead to carbon dioxide (CO_2) concentration increase and the ozone layer diminishing are global components of resources depletion and Terra pollution (MUNTEAN ET AL., 2005). According to the Agency for Environment, it is estimated that, at the present, the accumulation of crude oil residues amounts to approximately 1.5 million m^3, including those existing in the refineries.

SÎRBU ET AL. (2005) highlighted that, in Gorj County, the polluted surface from crude oil exploitation is 474.80 ha. CREANGĂ (2005) reported that approximately 484 ha agricultural land is polluted with crude oil residues in Argeş County: crude oil, crude oil + brine, brine.

The immediate effect of soil pollution with crude oil and crude oil residues is reflected în soil micro biological activity disturbance. Next, in optimum temperature conditions, a tendency to revert to the normal soil microbiological state shows, as many bacteria are capable to degrade hydrocarbons.

The efficiency of bioremediation is a function of inoculated degrading microorganisms ability to remain active in the natural environment (TA-CHEN LIN ET AL., 2010).

Bioremediation refers to polluted soil inoculation with selected microorganisms with abilities to degrade specific contaminants or groups of contaminants. The most representative bacteria used in crude oil contaminated soils bioremediation are: *Pseudomonas, Escherichia, Micrococcus, Bacillus, Arthobacter* and *Streptomyces*.

The experiments carried out show that the crude oil biodegradation period in soil depends on multiple factors, out of which the crude oil chemical composition, the soil and climatic conditions and also the applied agricultural and soil ameliorative works have the most important role. In case the soil is contaminated both with crude oil and brine, the bioremediation measures are carried out together with other adequate measures for desalinization, appliance for soil leaching on the soil profile, and leached water captation in a drainage system for cleaning before discharge into the emissary (TOTI ET AL., 1999). The following are recommended for crude oil contaminated soils amelioration: liming depending on soil reaction, deep loosening, soil profile homogenization, ameliorative fertilization, input of fine soil, and biological measures for pollution control. DUMITRU (2005) stated that organic fertilization comes not only with a big microbial mass but also with an important nitrogen and other nutritive macro and micro elements source. It is important that, through the applied organic matter decomposition, the soil is supplied with the nitrogen needed by the microorganisms and the agricultural yields for a longer time period.

It is necessary to ensure environment conditions that favor degradative microorganisms growth and reproduction in order to accomplish the bioremediation technologies, as follows: adequate nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, and microelements (copper, zinc, molybdenum, cobalt, iron, manganese) contents, as well as optimum moisture, reaction, and temperature (DALYAN ET AL., 1990; HARDER AND HOPNER, 1991; HARDER ET AL., 1991; WURDEMANN ET AL., 1990).

Nutrients are chemical elements necessary for good growth of microorganisms. Sufficient quantities of nutrients must be available in soils, in specific forms and concentrations for each process, for optimum growth and reproduction of the microorganisms involved in the remediation action.

In a study carried out in Nigeria (ILAH AND ANTAI, 2003) the efficiency of the bioremediation with the help of bacteria from the *Pseudomonas, Bacillius, Vibrio, Micrococus* and *Alcaligenes* genera varied from 26.7 to 43.3% 16 days after application. WODZINSKI AND BERTOLONI (1972) estimated that polycyclic aromatic hydrocarbons (PAHs), for example: naphtalene, anthracene, and phenyl-anthracene are carbon sources for bacteria growth.

Researches carried out by NIKOLOPOULOU ET AL. (2013) through application of treatments with nutrients and microorganisms ascertained that they degraded over 97% C_{12}-C_{13} n-alkanes in a 30 days period, and the polycyclic aromatic hydrocarbons with two or three rings were degraded by 95% in 45 days.

A study accomplished by JEONG et al. (2015) presented a simple technique for crude oil contaminated soils remediation, which implied foam atomization on the soil surface with no additional works. This surfactant agent contained microorganisms and nutrients. The efficiency in eliminating crude oil hydrocarbons was 73.7% 30 days after the treatment.

MATERIAL AND METHOD

In the context enunciated, 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. 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: without absorbent, Peat 16 t/ha , 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.

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

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

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 in Petri dishes. 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.

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.

The bacteria inoculum consisted of strains isolated from soil contaminated with crude oil and tested in the laboratory for its ability to degrade petroleum hydrocarbons as follows: 3 strains belonging to the *Pseudomonas genus*, 2 strains belonging to the *Arthrobacter genus* (*Arthrobacter globiformis* and *Arthrobacter citreus*).

In the experiments performed the potassium humate used to obtain the fertilizer was extracted from coal (lignite) with a solution of potassium carbonate. The mixture humic/fulvic present in the matrix of the fertilizer contained about 70 % organic acids, 50% of which are derived from humic acids and 20% from fulvic acids. At the experimental fertilizer variant AH-SG were introduced two dosages of glucose and thus resulting the fertilizer variants AH-SG1 and AH-SH2. Glucose was obtained by purification and concentration of an aqueous solution of nutritive saccharides obtained from starch.

Zeba is a superabsorbent polymer, on starch based, created to quickly absorb up to 500 times its weight of water to form a hydrogel in which the humidity is maintained, accessible to the plants. It is able to hydrate and dehydrate repeatedly during a season and to balance the retained moisture in function by the plant requirements. It is applied in the soil to a depth of 10-15 cm. The product is non-toxic and completely biodegradable, having beneficial effects on soil microorganisms.

Peat is a natural absorbent peat-based biodegradable, used for the degradation of the hydrocarbons and for absorbing oil. Peat is composed to ± 94 % peat moss and water/various ± 6 %. The absorption capacity is 8-12 times its own weight. Being a product of natural origin, not chemically modified, is non-toxic to living organisms and does not change the characteristics of the environment in which it was applied.

RESULTS AND DISCUSSIONS

The data presented in Figure 1 present the interplay of the studied factors (absorbents, fertilizers, bacterial inoculum) upon the mobile phosphorus evolution in the soil polluted with 3% crude oil, 45 days from the treatment application or 60 days from pollution.

In figure 1A it is noticed that fertilization with AH-SG2 together with bacterial inoculum application against no absorbent had the highest effect in soil mobile phosphorus content increase. The lowest values of mobile phosphorus were obtained following the fertilization with 650 l/ha potassium humates. The application of bacterial inoculum didn't lead to statistically significant alterations of soil mobile phosphorus content as compared to the unfertilized variant. The fact was highlighted that the application of $N_{200}P_{200}K_{200}$ mineral fertilizers led to a significant mobile phosphorus content, but when applied together with the bacterial inoculum the mobile phosphorus content decreased significantly, because of the explosive increase of the total bacteria number. This variant shows the highest biological activity.

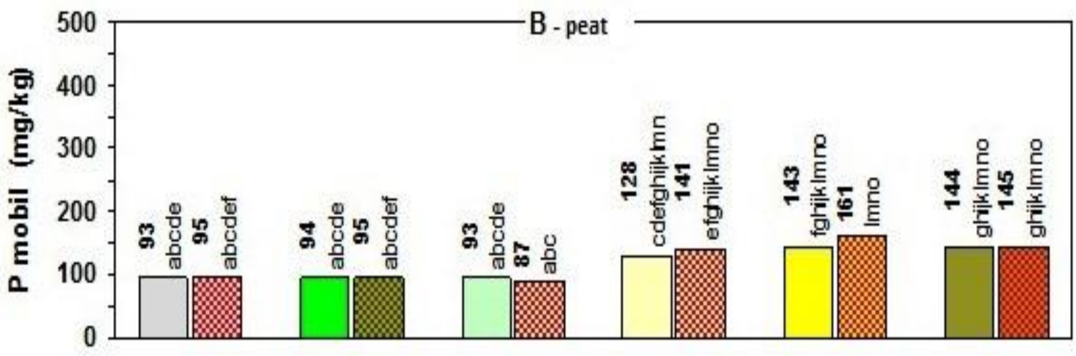
In figure 1B it is noticed that the bacterial inoculum against the application of 16 t/ha peat as absorbent didn't lead to significant alterations of the soil mobile phosphorus as compared to the unfertilized variant.

It is ascertained that, against the application of 16 t/ha peat as absorbent, the highest values of the soil mobile phosphorus were obtained in the variants fertilized with AH-SG1 and in which bacterial inoculum was added; the highest biological activity was also obtained in the same variant; the lowest mobile phosphorus values were identified in the variants treated with potassium humates and bacterial inoculum, against peat; it is appraised that the most efficient fertilizer is AH-SG1.

The application of AH-SG2, which differs from AH-SG1 by the glucose dose, together with the bacterial inoculum led to very significant increases of the phosphorus content in soil.

In figure 1, C data are presented that reflect different fertilizers influence, associated or not with bacterial inoculum, against using the Zeba product as absorbent, in a 16 kg/ha quantity, upon soil mobile phosphorus content.

The administration of the bacterial inoculums' against the application of 16 kg/ha Zeba led to a significant decrease of the soil mobile phosphorus content as compared to the variant without inoculum.


B - peat
P mobil (mg/kg)
500
400
300
200
100
0
93
abcde
95
abcdef
94
abcde
95
abcdef
93
abcde
87
abc
128
cdefghijklmn
141
efghijklmno
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ghijklmno
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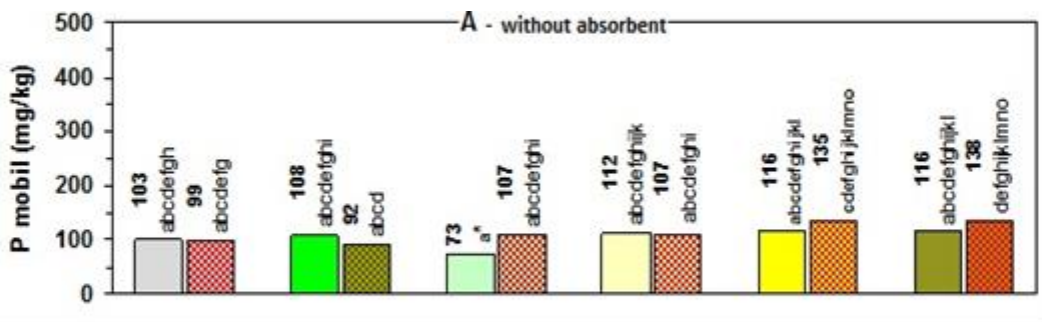
A - without absorbent
P mobil (mg/kg)
500
400
300
200
100
0
103
abcdefgh
99
abcdefg
108
abcdefghi
92
abcd
73
a
107
abcdefghi
112
abcdefghijk
107
abcdefghi
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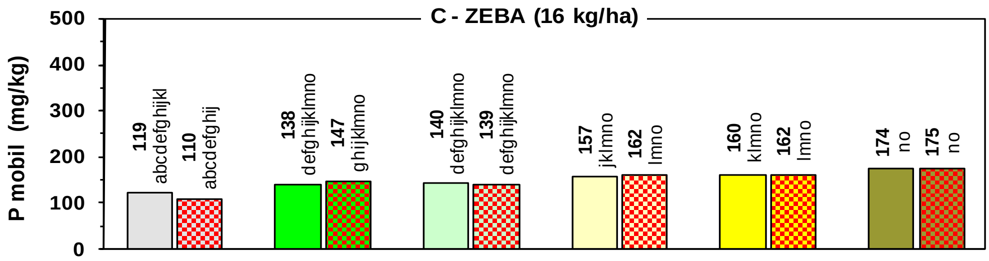
116
abcdefghijkl
138
defghijklmno


C - ZEBA (16 kg/ha)
P mobil (mg/kg)
500
400
300
200
100
0
119
abcdefghijkl
110
abcdefghij
138
defghijklmno
147
ghijklmno
140
defghijklmno
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lmno
174
no
175
no

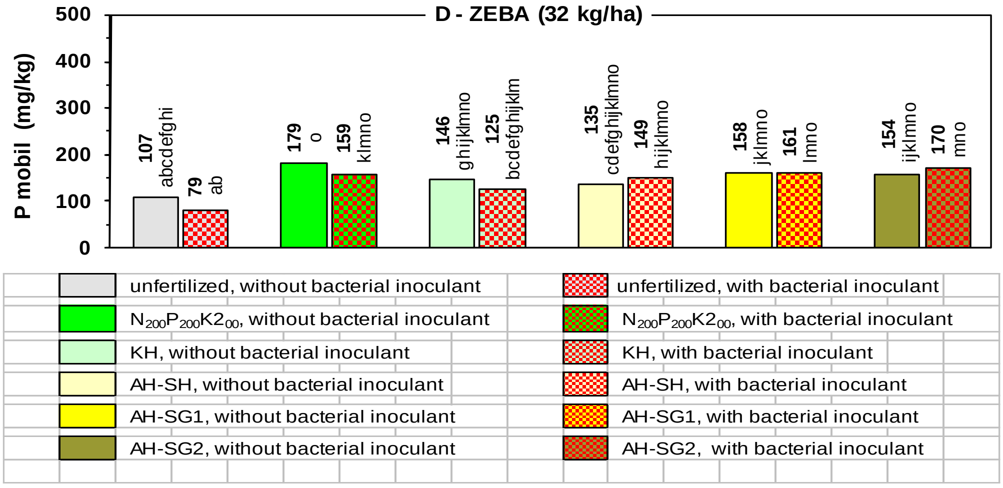

Figure 1. The combined effects of the studied factors on mobile phosphorus content in soil material polluted with 3% crude oil

*For the aggregate formed by the 4 charts (1.A, 1.B, 1.C, and 1.D) the values followed by the same letter (a, b, ...) do not differ significantly from each other (TUKEY multiple correlation method – significance threshold 0.05). DL5%= 48,319.

Given the application of 16 kg/ha Zeba absorbent, the highest soil mobile phosphorus values were identified in the variants fertilized with AH-SG2, associated or not with bacterial inoculum. The lowest mobile phosphorus values were found in the variants in which bacterial inoculum was applied against 16 kg/ha Zeba absorbent.

In Figure 1D data are presented that reflect the influence of different fertilizers, associated or not with bacterial inoculum, against the application of 32 kg/ha Zeba absorbent, upon the soil mobile phosphorus content.

Against the application of 32 kg/ha Zeba absorbent, the highest values of soil mobile phosphorus were obtained in the variants fertilized with $N_{200}P_{200}K_{200}$; the values were also high in the variants in which the $N_{200}P_{200}K_{200}$ mineral fertilizers were applied together with bacterial inoculum; out of the new fertilizers, AH-SG1 and AH-SG2 had the best effect on the soil mobile phosphorus level; their application together with the bacterial inoculum didn't develop the soil mobile phosphorus increase; the lowest soil mobile phosphorus values were found in the variants without fertilizers in which bacterial inoculum was applied against Zeba.

CONCLUSIONS

In the variants without absorbent fertilization with AH-SG2 together with bacterial inoculum application had the highest effect on soil mobile phosphorus content increase; the lowest mobile phosphorus values were obtained following 650 l/ha potassium humates fertilization.

The highest soil mobile phosphorus values against the application of 16 t/ha peat as absorbent were obtained in the variants fertilized with AH-SG1 and in which bacterial inoculum was applied; the highest biological activity was also obtained in this variant; the lowest mobile phosphorus values were identified in the variants treated with potassium humates and bacterial inoculum against peat; it is appraised that the most efficient fertilizer is AH-SG1.

The highest soil mobile phosphorus values under the circumstances of applying 16 kg/ha Zeba absorbent were identified in the variants fertilized with AH-SG2, associated or not with bacterial inoculum; the lowest mobile phosphorus values were found in the variants in which bacterial inoculum was applied against 16 kg/ha Zeba absorbent.

The highest soil mobile phosphorus values under the circumstances of applying 32 kg/ha Zeba absorbent were obtained in the variants fertilized with $N_{200}P_{200}K_{200}$; the values were also high in the variants in which the $N_{200}P_{200}K_{200}$ mineral fertilizers were applied together with bacterial inoculum; out of the new fertilizers, AH-SG1 and AH-SG2 had the best effect on soil mobile phosphorus level; applying these fertilizers together with bacterial inoculum didn't develop the soil mobile phosphorus level increase; the lowest values of mobile phosphorus were found in the variants without fertilizers in which bacterial inoculum was applied against Zeba.

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