ROLE OF MINERAL FERTILIZATION IN ACCELERATING THE BIOREMEDIATION PROCESS OF A SOIL POLLUTED WITH CRUDE OIL

ROLUL FERTILIZĂRII MINERALE ÎN ACCELERAREA PROCESULUI DE BIOREMEDIERE A UNUI SOL POLUAT CU ŢIŢEI

Anca-Rovena LACATUSU [1], Radu LACATUSU[1, 2], Mihail DUMITRU[1], Mihaela LUNGU[1]

[1] National Research & Development Institute for Soil Science, Agrochemistry and Environment Protection Bucharest; [2] "Al. I. Cuza" University, Iassy
anca@icpa.ro, bd. Mărăşti, 61, 011464, Sector 1, Bucureşti

Abstract: *A field experiment for bioremediation of a crude oil polluted soil was limited on a Stagnic Vertic Luvosols to the Albota - Arges County. The bioremediation in situ experiment included agronomical and microbiological techniques. For this has been set a controlled pollution of soil from the experimental site, up to 5% volume of crude oil in the volume of soil, on the 20 cm depth. Experimental factors were: treating the crude oil-polluted soil with limestone amendments to reduce the acidity level (6 t/ha $CaCO_3$); deep scarification of soil up to a 40 cm depth in order to improve the regime of ear and water circulation in soil; organic fertilization with 150, and 300 tons/ha of stable manure fermented, chemical fertilization with NPK and, soil inoculation with selected micro organisms. The results presented further relates the importance of mineral fertilization with different doses of NPK ratio on the decontamination of crude oil polluted soil, exclusively referring to variants in which the only measure applied to soil was mineral fertilization with doses up to 200 kg N/ha, 200 kg P/ha and 100 kg K/ha. The experimental data show that for this type of soil, heavily polluted with crude-oil, application of mineral fertilizers with NPK is a prerequisite for initiating the processes of degradation and biodegradation of petroleum hydrocarbons. Optimal doses of mineral fertilizers established in the experiment, this supported by the disappearance of pollutants in soil and production results obtained, are 200 kg / ha nitrogen, 100 kg / ha phosphorus and 100 kg / ha potassium.*

Rezumat: *Pe un luvosol stagnic vertic poluat cu ţiţei de la Albota, Arges, a fost organizat un experiment de bioremediere in situ care a inclus atat tehnici agronomice, cât si microbiologice. A fost aplicată o poluare controlată a solului cu concentratia de 5% ţiţei din volumul de so pe adâncimea de 20cm. Factorii experimentali au fost: amendarea solului cu 6 t/ha $CaCO_3$ pentru reducerea nivelului acidităţii, scarificarea pâna la adâncimea de 40 cm în vederea îmbunătăţirii regimului circulaţiei apei şi aerului în sol, fertilizarea organică cu 150 si 300 t/ha gunoi de grajd, fertilizarea minerală cu NPK si inocularea cu microorganisme selecţionate. Rezultatele prezintă importanţa fertilizării cu diferite doze de NPK in decontaminarea solului poluat cu ţiţei, lucrarea referindu-se exclusiv la variantele experimentale in care singura masură aplicată solului a fost fertilizarea cu N si P în doze de pâna la 200 kg/ha, si cu 100 kg/ha K. Datele experimentale au arătat ca în cazul unui sol puternic poluat cu ţiţei, fertilizarea minerală cu NPK este o condiţie absolut obligatorie pentru iniţierea proceselor de degradare şi biodegradare a hidrocarburilor petroliere. Dozele optime de de fertilizanţi minerali stabilite prin acest experiment, susţinute de rata dispariţiei poluantului în sol si rezultatele de producţie obţinute sunt: 200 kg/ha azot, 100 kg/ha fosfor si 100 kg/ha potasiu.*

Keywords: *crude oil, bioremediation, mineral fertilizers*
Cuvinte cheie*: ţiţei, bioremediere, fertilizare minerală*

INTRODUCTION

Pollution by crude-oil radically changes soil properties, both the physical, chemical and biological ones. It forms an impermeable film on the surface of the soil, preventing water movement in soil and the exchange of gases between soil and atmosphere, producing asphyxia of the roots, and increasing the reducing processes. As the soil becomes more anaerobic, the number and metabolic activity of bacteria decreasing, with all the consequences arising out of conduct of the circuit elements biogeochemical mediated by them (DART and STRETTON, 1980; STUCKI G. & ALEXANDER M., 1987; SIMS et al., 1990). Crude oil is being very rich in organic carbon (98% oil), is increasing the C/N in the soil, that adversely affecting the microbiological activity and plant nitrogen nutrition (HALE et al., 2001; KAUFMANN, K., et al., 2002).

Eliminating the effects of pollution and restoring the original ecosystem to implement the concept of sustainable development, which in soil science is optimizing the generating functions of soil fertility.

Technologies for soil decontamination, consisting primarily in the chemical and physical treatments carried out in order to promote and accelerate the biodegradative processes of petroleum waste discharged. They should include all the works necessary to ensure conditions favourable to intensive development of those populations of micro organisms from soil, with abilities in the metabolic degradation of petroleum hydrocarbons. Deep aeration of soil is needed for micro organisms (predominantly aerobic) be adequately supplied with oxygen and appropriate fertilization with organic and mineral fertilizers, especially nitrogen, because the microbial metabolism can be consumed and disposed excessive reserves of soil carbon derived from hydrocarbons of crude oil.

Moreover, the issue of restoration of land with soils polluted by organic compounds from crude oil can be successfully solved only by detailed study of mechanisms of loss of fertility, the quantitative characteristics of one or other of the changes that happen in the conduct of soil processes.

Microorganisms able to degrade xenobiotic substances are present in general in these polluted environments, but natural biodegradation rate is very low. Therefore, it have been developed various bioremediation technologies that are involving: a good knowledge of ways to optimize the biodegradation, behaviour and effects of chemicals into the soil on the ecosystem and selection of microorganisms with superior degradation abilities (BLUESTONE, 1986; ZITRIDIS, 1990; VERSTRAETE W. & TOP E.M., 1999; MEGHARAJ M. et al., 2000; VOICULESCU A-R. et al., 2001).

The most important way to optimize the biodegradation consist in ensuring the environmental conditions favoured the growth and multiplication of microorganisms involved in biodegradation, namely: adequate levels of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and microelements: copper, zinc, molybdenum, cobalt, iron, manganese, and moisture, and more an optimum reaction and temperature (DALYAN et al., 1990; HARDER and HOPNER, 1991; FOGHT et al., 1990; WALWORTH et al., 1995; WURDEMANN et al., 1990; JANA, T.K. et al., 2000). All these parameters exert a direct influence on the growth rate, on the number and physiological activity of microorganisms' populations, and their optimization is the main mechanism for stimulating xenobiotic polluting substances biodegradation.

Thus, there are two basic technologies approached in bioremediation: first, called **ecological**, it is based on activation of degradative microflora existing in soil (most often through the addition of nutrients) and the second, the **inoculation** with microorganisms selected in the laboratory because their increased degradation capacity, the option for each of them being made according to the results of the analysis performed.

If degradative microflora is present in adequate quantities, ecological method is preferred. On the other hand, if degradative microflora is absent or cannot be stimulated, the inoculation it is recommended (VANDEPITTE et al., 1995; VAN VEEN et al., 1997).

Option for any of the two bioremediation strategies must be preceded by microbiological, chemical and physical analysis, according to the results which polluted soil should be amended to achieve optimum conditions for microflora involved in degradation.

All the efforts of science in recent years have shown that bioremediation is a method of soil decontamination more efficient and cheaper as compared to known physical and chemical methods.

The following presents the effect of mineral fertilization with NPK on the bioremediation of a Stagnig Vertic Luvosols polluted with crude-oil.

MATHERIAL AND METHODS

For testing the *in situ* bioremediation of an oil-polluted stagnic vertic luvosols, a field experience which included agrotechnics measures and microbiological inoculation with selected microorganisms from autochthonous microflora, was carried out at the Albota territory, Argeş County. For this purpose, the soil of experimental site was polluted with crude-oil, up to 5% concentration by volume of oil in the soil, to a depth of 20 cm.

Considering that the soil layer between 0-20 cm is sufficient for the development of plant roots and at the same time represents the layer with the highest fertility in the soil specific testing. Meanwhile, the thickness of soil unaffected by pollution up to the horizon Btyw (specific for the type of soil that has been experimenting) is sufficient to observe the migration and percolation of the crude-oil in deep soil. The experience was developed on three years time period, between 2000 and 2002.

The results presented in this paper refer to the importance of mineral fertilization with different doses of NPK on the decontamination rate of crude oil polluted soil. Analysis refers only to experimental treatments in which mineral fertilization with doses of up to 200 kg N / ha, 200 kg P / ha and 100 kg K / ha, as follows: $V1=N_0P_0K_0$, $V2=N_{100}P_{100}K_{100}$, $V3=N_{200}P_{100}K_{100}$, $V4 = N_{200}P_{200}K_{100}$, was the only technologic measure applied to polluted soil.

RESULTS AND DISCUSSIONS

Organic carbon content distribution

The organic carbon content distribution in different variants of mineral fertilization applied to crude oil-polluted stagnic vertic luvosols, at the beginning and the end of bioremediation experience, illustrated in Figures 1 and 2 reveal a close relationship between the total concentration of petroleum residues and the organic carbon content.

Data presented in Figure 1 represents the values determined at 6 months from the pollution of soil, in the first experimental year autumn. After six months since the pollution occurred, the degradation processes of crude oil are ongoing, but the rate is obviously different depending on the dose of mineral fertilizers applied.

The lowest rate of oil degradation is evident in the unfertilized treatment, where the total petroleum hydrocarbons concentration is 2.32%. These represent the hard degradable oil fraction that is still present after volatilization and decomposition of volatile hydrocarbons and those with few carbon atoms and the low degree of ramification chains. In treatments in which NPK mineral fertilizers have been applied levels of total petroleum hydrocarbons decreased by 22%, 60% and 30% as compared with unfertilized treatment. The most pronounced degradation of crude oil occurred in the $N_{200}P_{100}K_{100}$ treatment.

In the third experimental year, although the organic carbon content varies between variants can clearly see the curve of crude oil degradation, with the highest rates in the mineral fertilized variants $N_{200}P_{100}K_{100}$ and $N_{200}P_{200}K_{100}$. One aspect worth to be noted is that the higher dose of phosphorus does not increase to take into account the rate of degradation of crude oil, the difference being only 0.03% (figure 2).

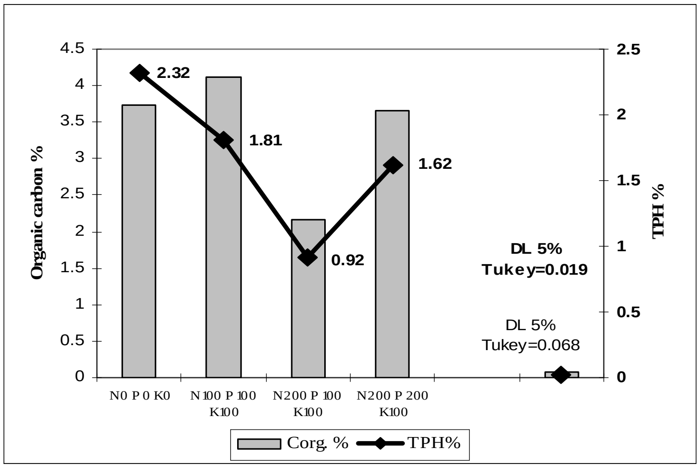

Figure 1. Distribution of organic carbon content relative to the total petroleum hydrocarbons concentration-Irst experimental year

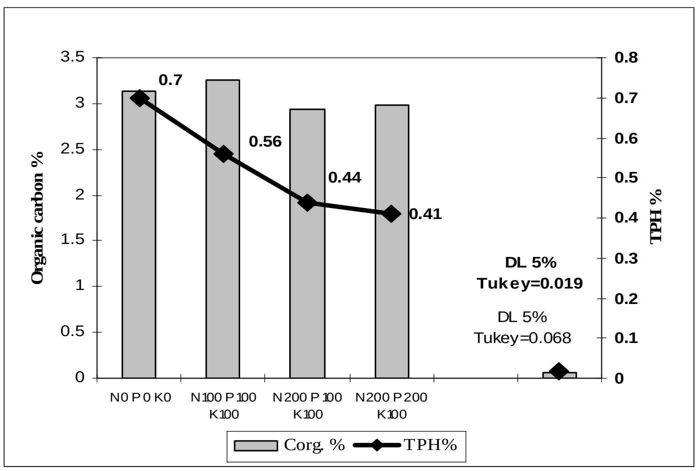

Figure 2. Distribution of organic carbon content relative to the total petroleum hydrocarbons concentration -III[rd] experimental year

Total nitrogen content distribution

Six months after the soil pollution, the distribution of total nitrogen content is very similar to that of total carbon (Figure 3). Thus, in the first experimental year autumn, among the mineral fertilized variants, the highest nitrogen content, 0.317%, value placed on high-class content, was determined in variant with minimum doses of nitrogen and phosphorus ($N_{100}P_{100}K_{100}$).

Increasing the nitrogen dose at 200 kg/ha resulted, paradoxically at first sight, the decrease in total nitrogen content in soil. In reality, this is a high recovery of nitrogen applied by microorganisms involved in biodegradation of petroleum hydrocarbons and the corn plants, means of production values obtained in these variants.

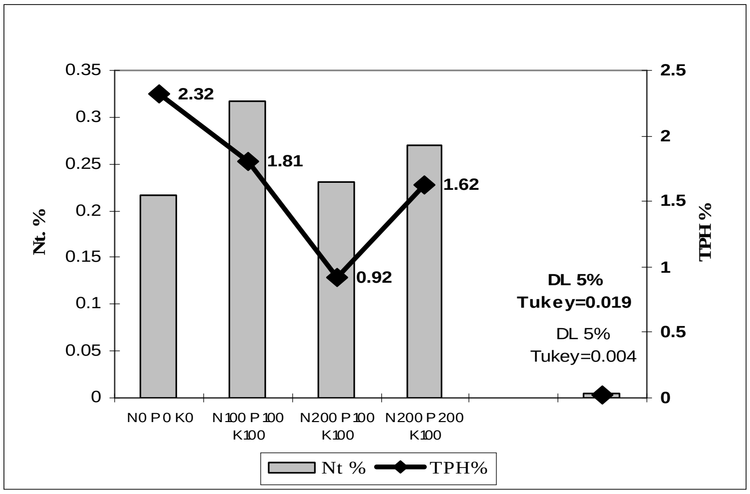

Figure 3. The content of total nitrogen and total petroleum hydrocarbons in different mineral fertilization treatments - I[rst] experimental year

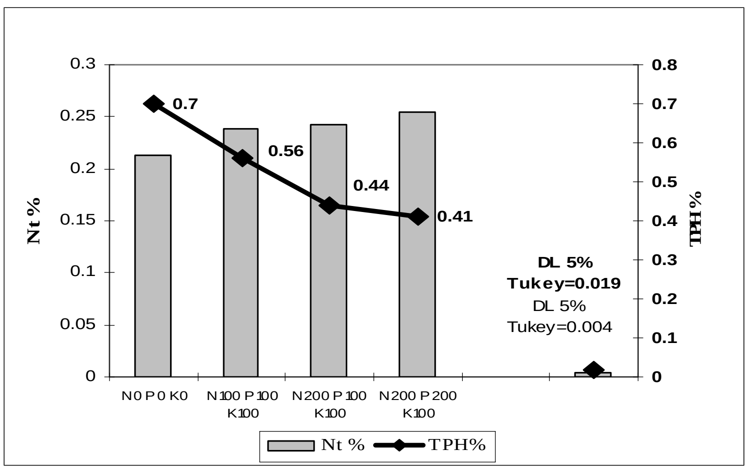

Figure 4. The content of total nitrogen and total petroleum hydrocarbons in different mineral fertilization treatments III[rd] experimental year

At the end of experience, in the third year autumn, the total nitrogen content of soil were uniform in mineral fertilized variants and the variation range was 0238-0255% (Figure 4). In all fertilized variants total nitrogen content values were higher than in unfertilized ones (0213%). All values obtained are placed in middle-class content

C/N ratio

At 6 months of soil pollution, in unfertilized, control variant, the ratio C / N ratio still remains at a high level, 20 respectively (Figure 5). Other values indicate that mineral balance is restored quickly in the variant fertilized with $N_{100}P_{100}K_{100}$. At the end of the experience the C / N ratio is reduced in proportion to the decrease in petroleum hydrocarbon concentrations, to values close to normal (Figure 6).

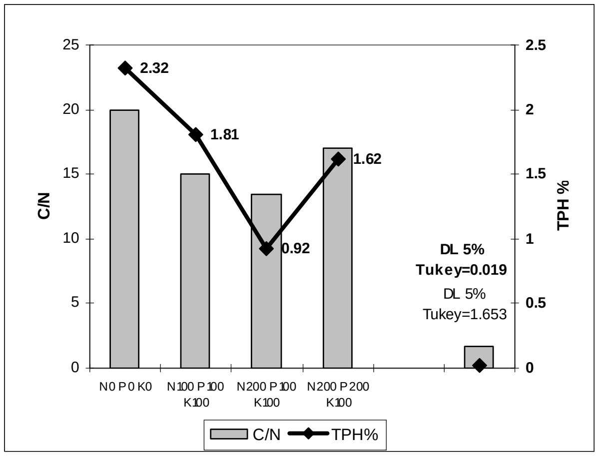

Figure 5. C / N ratio in the soil polluted with crude oil in different mineral fertilization treatments-
I^rst^ experimental year

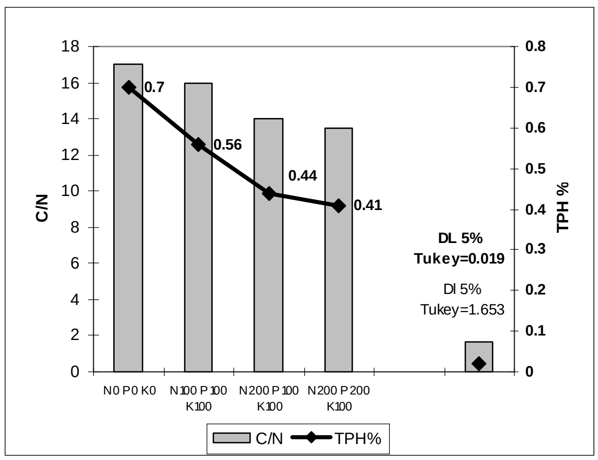

Figure 6. C / N ratio in the soil polluted with crude oil in different mineral fertilization treatments
III^rd^ experimental year

Mobile phosphorus content distribution

In the first experimental year, the mobile phosphorus content (Figures 7) show the same trend values as those obtained in the case of nitrogen, the highest (P_{AL} = 54mg/kg) was obtained in a $N_{100}P_{100}K_{100}$ treatment, about 2, 45 times higher than in unfertilized ones (22 mg/kg). In control treatment the mobile phosphorus is in the lower limit of middle class content and the maximum value of the $N_{100}P_{100}K_{100}$ treatment, already mobile phosphorus in high class content. In variants with $N_{200}P_{100}K_{100}$ and $N_{200}P_{200}K_{100}$ soluble phosphorus values are somewhat low and close in size (41 and 43 mg/kg) also placed in high-class content.

As for nitrogen, low phosphorus content in the mobile versions in which the dose of fertilizer applied was higher, to reflect better recovery by microorganisms and plants, supported by oil degradation efficiency and production data obtained. At the end of the experience most accumulation of soluble phosphorus in soil was in the variant with

$N_{200}P_{100}K_{100}$ (42 mg/kg), followed by the variant $N_{200}P_{200}K_{100}$ (35 mg/kg) both placed in high - class content (figure 8).

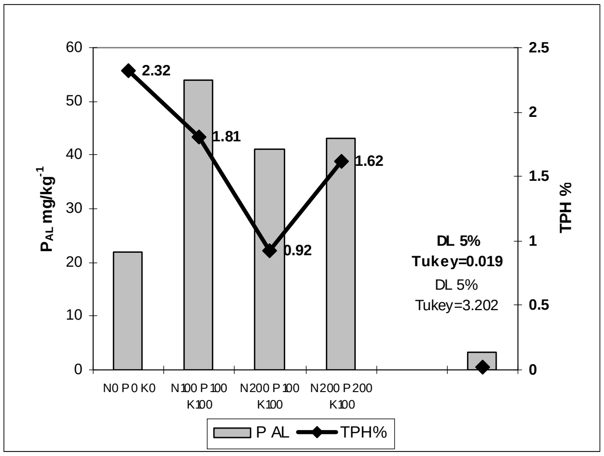

Figure 7. The content of mobile phosphorous and total petroleum hydrocarbons in different mineral fertilization treatments I[rst] experimental year

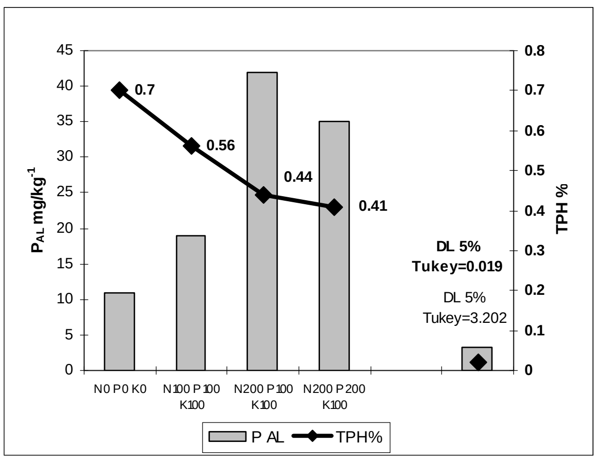

Figure 8. The content of mobile phosphorous and total petroleum hydrocarbons in different mineral fertilization treatments III[rd] experimental year

Mobile potassium content distribution

The content of mobile potassium (Figures 9 and 10) is precisely that of mobile phosphorus, both in the first experimental year and the end experience. As to phosphorus, the highest value of the first experimental year was obtained in a $N_{100}P_{100}K_{100}$ treatment (128 mg/kg) and the lowest, even lower than in unfertilized soil, in $N_{200}P_{100}K_{100}$ (64 mg/kg).

In the third experimental year soil accumulates the highest amount of mobile potassium in $N_{200}P_{100}K_{100}$ treatment (168 mg/kg) followed closely by $N_{200}P_{200}K_{100}$ variant (146 mg/kg).

In the unfertilized variant, mobile potassium content is low at both the beginning and the end experience. At the end experience, in variants with $N_{200}P_{100}K_{100}$ and with $N_{200}P_{200}K_{100}$

the mobile potassium content is classified as middle-class content.

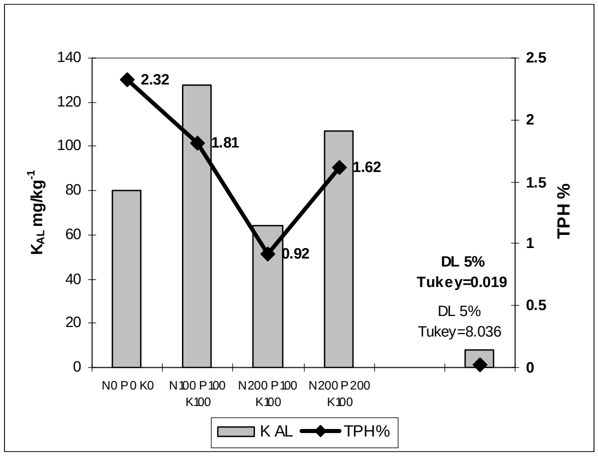

Figure 9. The content of mobile potassium and total petroleum hydrocarbons in different mineral fertilization treatments-I[rst] experimental year

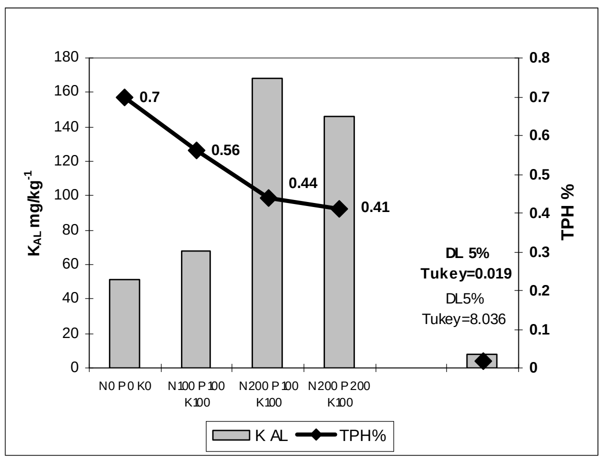

Figure 10. The content of mobile potassium and total petroleum hydrocarbons in different mineral fertilization treatments-III[rd] experimental year

Maize production results

Results of production (kg / ha grain) presented in Figures 11 and 12 shows obtaining maize production significantly greater in the variant mineral fertilized with $N_{200}P_{100}K_{100}$, since from the first experimental year.

This aspect is closely correlated with the largest decrease in the total petroleum hydrocarbons. The significance is that this variant of fertilization offers the most favourable conditions to the development of bacteria with abilities in petroleum hydrocarbon degradation. These naturally colonizing any soil, and application of mineral fertilizers with nitrogen in the 200 kg / ha dose, restores mineral balance of the soil, which stimulates growth and activity of microorganisms, reducing the period of acclimatization and adaptation to their new environment affected by pollution.

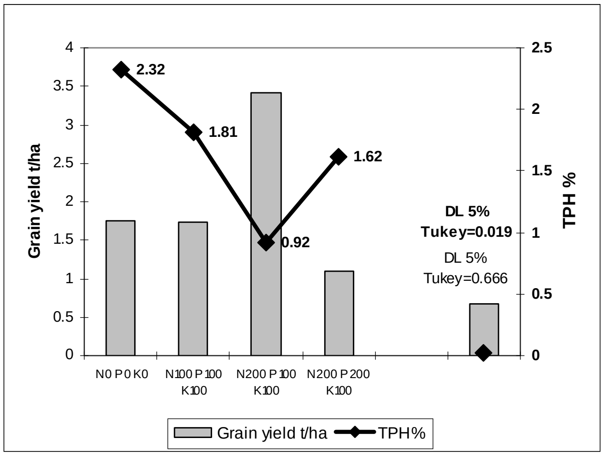

Figure 11. Maize yield (t/ha grain) in the soil polluted with crude oil in different mineral fertilization treatments - I[rst] experimental year

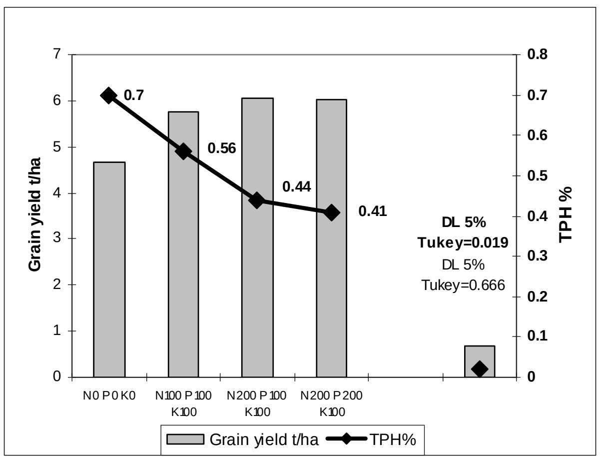

Figure 12. Maize yield (t/ha grain) in the soil polluted with crude oil in different mineral fertilization treatments III[rd] experimental year

Grain yield results obtained at the end of the experience shows values very close between the variants fertilized with NPK, and significantly higher as compared with untreated ones. Also, we could see a clear correlation between concentrations of petroleum hydrocarbons in soil and crops (fig. 13).

Another outstanding at the end of the experiment is that increasing the dose of phosphorus applied at 200 kg / ha, has not generated the growth of grain production. It also has not brought about any increase efficiency of soil decontamination, which would be reflected in a further decrease of the total petroleum hydrocarbons content.

At the global level of production can be found a very significant increase, from 2.003 t / ha maize grain harvested in the first year to 5.628 t / ha in the third experimental year.

All this shows that for decontamination of a strongly oil-polluted stagnic vertic luvosols, application of NPK mineral fertilizers is an absolutely necessary condition for initiating the processes of degradation and biodegradation of petroleum hydrocarbons.

Optimal doses of mineral fertilizers established in this experiment, supported by the disappearance of the pollutant from the soil and the production obtained are: 200 kg / ha nitrogen, 100 kg / ha phosphorus and 100 kg / ha potassium.

It is important to noted high degree of statistical assurance of results (table 1).

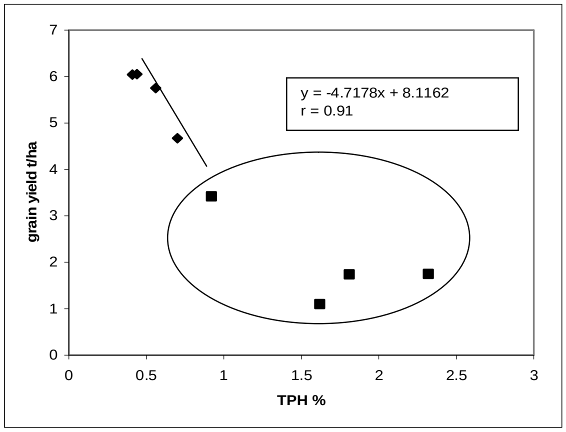

Figure 13. Relations between the production of maize and the total petroleum hydrocarbons in the first year of experimentation (■) and second (♦)

Table 1.

The semnification of the results obtained in a bioremediation experiment of a stagnic vertic luvosols polluted with crude oil according with Fisher, Student and Tukey tests (ANOVA model)

Variable	Fisher test	Student test DL 5%	Tukey test DL 5%
Corg. %	**	0.05	0.068
Nt %	**	0.003	0.004
C/N	**	1.224	1.653
P_{AL} (mg/kg)	**	2.370	3.202
K_{AL} (mg/kg)	**	5.949	8.036
TPH %	**	0.014	0.019
Grain yield (t/ha)	**	0.493	0.666

CONCLUSIONS

Application of NPK mineral fertilizers in doses $N_{100}P_{100}K_{100}$; $N_{200}P_{100}K_{100}$ and $N_{200}P_{200}K_{100}$ on a stagnic vertic luvosols strongly polluted with crude oil contributed to decrease of the total of petroleum hydrocarbons content by 22%, 60% and 30%.

Maximum dose of nitrogen applied (200 kg/ha) on a moderate soil assurance found with phosphorus and potassium, provides a favourable environment for development of bacteria with abilities in the crude oil biodegradation.

At the end of the experiment, between the contents of main macro-elements of soil (N, P, K) and concentrations of petroleum hydrocarbons an inverse proportionality relationship has been recorded.

Maximum dose of nitrogen associated with moderate doses of phosphorus and potassium, has contributed to significant reducing of the petroleum hydrocarbons content, and has increased, also significant, the production of maize.

Mineral NPK fertilizers have contributed to initiate processes of degradation and biodegradation of petroleum hydrocarbons in investigated stagnic vertic luvosols heavily polluted with crude-oil.

BIBLIOGRAPHY

1. BLUESTONE M.,1986 - Microbes to the rescue. Chemichal Week, 139: 567-572.
2. DALYAN U., HARDER H. , HOPNER TH., 1990 - Hydrocarbon biodegradation in sediments and soils. A systematic examination of physical and chemichal conditions - Part II. pH values. Erdol & Kohle, Erdgas, Petrochemie, 43: 435-342.
3. DART R.K. and R.J. STRETTON, 1980 - Microbiological aspects of pollution control. Elsevier Sci. Publ. Comp., Amsterdam - Oxford - New York, 202-216.
4. FOGHT J., FEDORAK P.M., and WESTLAKE D.W.S., 1990 - Testing the efficacy of a bacterial consortium in oil-polluted soil decontamination. Can. J. Microbiol., 36: 169-175.
5. HALE BOOTHE, D.D., SMITH, M.C., GATTIE, D.K., DAS, K.C., 2001 - Characterization of microbial populations in landfill leachate and bulk samples during aerobic bioreduction. 5: 285-294.
6. HARDER H., KURZEL-SEIDEL B., HOPNER TH., 1991 - Hydrocarbon biodegradation in sediments and soils. A systematic examination of physical and chemichal conditions - Part IV. Special aspects of nutrient demand. Erdol & Kohle, Erdgas, Petrochemie, 44: pp. 59-62.
7. JANA, T.K.; A.K. SRIVASTAVA, K. CSERY, D.K. ARORA, 2000 - Influence of growth and environmental conditions on cell surface hydrophobicity of Pseudomonas fluorescens in non-specific adhesion, Journal of Microbiology, Revue canadienne de microbiologie, 46 (1): 28-37.
8. KAUFMANN, K., DAKHEL, N., HARMS, H. HÖHENER, P., 2002 - Assessment of microbial communities in the unsaturated zone during natural attenuation of petroleum hydrocarbons. In: D. Halm & P. Grathwohl (Eds.), Proceedings of the 1st International Workshop on Groundwater risk assessment at contaminated sites, Tübinger Geowissenschaftliche Arbeiten, Heft, 61: 249-253.
9. MEGHARAJ M., I. SINGLETON, N.C. MCCLURE, R. NAIDU, 2000 - Influence of Petroleum Hydrocarbon Contamination on Microalgae and Microbial Activity in a Long-Term Contaminated Soil, Archive of Environmental and Toxicology. Springer-Verlag New York, Inc. 38: 439-445.
10. SIMS, J.L., SIMS, R.C., MATEWS, J.E., 1990 - Approach to bioremediation of contaminated soil. Hazard. Waste Hazard. Mater. 7: 117-145.
11. STUCKI G., AND ALEXANDER M., 1987 - Bioremediation of Oil-Polluted Soils, Appl. Environ. Microbiol., 53: 292-297.
12. VAN VEEN, J.A., VAN OVERBEEK, L.S., VAN ELSAS, J.D., 1997 - Fate and activity of microorganisms introduced into soil. Microbiology and Molecular Biology Reviews 61: 121-135.
13. VANDEPITTE, V., QUATAERT, P., DE RORE, H., VERSTRAETE, W., 1995 - Evaluation of the Gomperty function to model survival of bacteria introduced into soil. Soil Biology and Biochemistry 27: 365-372.
14. VERSTRAETE W., E.M. TOP, 1999 - Soil clean-up: lessons to remember, International Biodeterioration & Biodegradation, 43: 147-153.
15. VOICULESCU ANCA ROVENA, M. DUMITRU, M. TOTI, GABRIELA MIHALACHE, CAROLINA CONSTANTIN, 2001 - Quantitative and Qualitative Distribution of Microflora in Petroleum and Salty Water Polluted Soils, Știința Solului, vol. XXXV, nr. 1-2, p.68-75.
16. WALWORTH, J.L., REYNOLDS, C.M., 1995 - Bioremediation of a petroleum-contaminated eryie soil: effects of phosphorus, nitrogen and temperature. J. Soil Contam., 4: 299-310.
17. WURDEMANN S, HARDER H.,HOPNER TH. 1990 - Hydrocarbon biodegradation in sediments and soils. A systematic examination of physical and chemichal conditions - Part I. Grain size, surface area and soil type. Erdol & Kohle, Erdgas, Petrochemie, 44: 217-224.
18. ZITRIDES T.G., 1990 - Bioremediation comes of age. Pollution Engineering, 12: 59-60