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Research in Vegetation House on the Possible Use of Microorganisms in Bioremediation of Soils Contaminated with Crude Oil

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INTRODUCTION

Within the remediation methods experimented all over the world, the bioremediation in situ based on the activity of microorganisms to use the oil hydrocarbons as carbon and energy source, is considered the most effective, especially, because it does not involves the severe and irreversible disruption of structure and, implicitly, the pedogenic characteristics of affected soil and its cost is relatively low.

The microorganisms represent probably the most important component of terrestrial ecosystems, which by their highly diverse physiological activity are the basis of the functions creating soil fertility.

The N100P100K100 fertilization treatment and application of bacterial inoculum proved the most effective so far.

Keywords: pollution, oil, microorganisms, bioremediation.

METHOD AND MATERIALS

The methodology applied in order to bring the polluted soils to the previous fertility state and to investigate the possibility to use the microorganisms for remediation of the soils polluted with hydrocarbons has been fulfilled in vegetation house and laboratory.

A lighter texture soil has been selected to complement the research previously carried out by various authors who generally worked on clay soils.

Experience in the vegetation house has been organized according to the methodology of work. After 7 days following pollution of soil with oil at the rates mentioned in the methodology and after application of treatments (differentiated rates of NPK), the ICPA type bacterial inoculum was applied. After other 7 days, soil sampling was performed for microbiological analyses in order to see how the oil pollution stress affected the population of microorganisms in the soil. The next stage of experimentation will start from this evaluation.

The experiment on soil included treatments with two types of NPK fertilizer and with bacterial inoculum in vegetation pots. Periodical chemical analyses were carried out to know the level of pollutant loading and the evolution of populations of microorganisms in soil, these being quantitatively and qualitatively characterized.

Analyses on the total crude oil content as well as on the crude oil fractions were performed by gravimetrical method.

Each treatment was observed from the phenological viewpoint, and at the end of the experiment period the vegetal mass was evaluated for each treatment.

At the end of the experiment an assessment was carried out on the capability of microorganisms to degrade the oil.

RESULTS AND DISCUSSIONS

In the case of untreated, non-inoculated treatments (V2V8V14), at the first harvest, random values of organic carbon content were observed depending on the degree of infiltration of oil in the soil, varying from 1.21% (control) to 6.35% at the 3% content, the respective humus contents varying from 2.08% to 11.05%. At the second harvest, after some time and application of agri-pedoameliorative measures, an uniformity of contents and a decrease of them is already observed varying from 0.88% (control) to 3.44% as a consequence of the application of measures, on the one hand, and of the oil degradation in time, on the other hand. The untreated inoculated treatments (V5V11V17) show very the influence of inoculum even from the first harvest when the organic carbon content varies between 3.45% and 5.47%, especially with the inoculated treatments where the decrease of organic carbon content is more evident, the values varying from 1.58% to 2.83%. Also a uniformity of contents is observed, particularly with oil concentrations of 3% and 5%, where for the first the decline of organic carbon content is massive (Table 1a). Baldwin (1928) stated that when the crude oil is applied in proportions of 5% the ammonification decreases by 40-45% in first 7 days and 35% in the next 10 days.

Tab.1a

Dynamics of organic C content in vegetation house (V2, V8, V14) şi (V5, V11, V17)

Concentrations	**First harvest 2010**		**Second harvest 2011**		**First harvest 2010**		**Second harvest 2011**	
	C org.%	**Humus %**	**C org.%**	**Humus %**	**C org. %**	**Humus %**	**C org.%**	**Humus**
	NON-TREATED NON-INOCULATED				**NON-TREATED INOCULATED**			
Control	1.21	2.08	0.88	1.52	1.21	2.08	0.88	1.52
1%	4.03	6.94	1.86	3.20	5.54	9.55	2.12	3.65
3%	4.49	7.74	2.22	3.82	2.79	4.80	1.44	2.47
5%	4.76	8.20	2.24	3.86	5.05	8.71	2.72	4.69

In the case of ameliorative mineral fertilization application, situation is changed, if in the case of treatments receiving N100P100K100 but non-inoculated, the organic carbon contents, at the first harvest, are almost uniform, at the second harvest they almost halved. The fact that, at the first harvest, the contents are almost uniform, varying between 4.76% and 4.91%, is explained by the influence of applied nitrogen that favored the development of microbial flora and, at the same time, led to the balance the ratio C/ N (Table 1b).

Tab. 1.b

Dynamics of organic C content in the vegetation house (V3, V9, v15) and V6, V12, V18)

Concentrations	**First harvest 2010**		**Second harvest 2011**		**First harvest 2010**		**Second harvest 2011**	
	C org.%	**Humus %**	**C org.%**	**Humus %**	**C org. %**	**Humus %**	**C org. %**	**Humus**
	$N_{100}P_{100}K_{100}$ NON-INOCULATED				**$N_{100}P_{100}K_{100}$ INOCULATED**			
Control	1.21	2.08	0.88	1.52	1.21	2.08	0.88	1.52
1%	4.91	8.45	2.21	3.81	2.84	4.90	1.40	2.42
3%	4.90	8.44	2.82	4.95	3.49	6.01	1.57	2.71
5%	4.76	8.21	2.45	4.23	4.49	7.74	2.60	4.49

At the second harvest, after the passage of time and under the influence of ameliorative measures, contents vary between 2.21% and 2.82% observing for the first time that the organic carbon content is correlated with the applied rate, presuming that in polluted areas, before the application of agri-pedoameliorative measures, under only natural conditions which may be variable in time, the oil degradation is anarchical.

In the case of treatments fertilized with N100P100K100 and inoculated, the decrease of organic carbon contents is even more striking, the influence of inoculum on increasing being crucial for both the number of bacterial species and the bacterial mass.

At the first harvest, the contents vary from 2.84% to 4.49%, and at the second harvest from 1.40% to 2.60%. The organic carbon content decrease is observed. When the mineral fertilization (N200P200K200)is applied, the increased rate has no a significantly effect in the first two harvests because the bacteria and fungi in soil had not time to increase their bacterial mass, but, however, the effects are visible. So, in the case of the non-inoculated treatments, the same uniformity of organic carbon content was observed at the first harvest (4.03% - 4.76%); in addition, in the case of this mineral fertilizer, the decrease of contents is gradual depending on the applied oil rates, showing higher nitrogen contents that determined the bacteria to work more intensively.

At the second harvest, after the passage of time and the manifestation of the effects of applied measures, it observed that the organic carbon content is less reduced at each oil rate applied (1.86-2.24%) as compared toN100P100K100 fertilization.

At the inoculated treatments (V7V13V19) fertilized with mineral fertilization (N200P200K200) a non-uniformity of the organic carbon content is observed at all the applied rates because the high rate of nitrogen allowed a different and increased mineralization of oil.

After the application of ameliorative measures, in the case of V7V13V19 treatments, the situation returns to normal, observing, however, the maintaining of a small non-uniformity in regarding the contents of 1.44% - 2.72%.

Tab. 1.c

Dynamics of organic C content in the vegetation house (V4, V_{10}, V_{16}) and (V_7, V_{13}, V_{19})

Concentrations	First harvest 2010		Second harvest 2011		First harvest 2010		Second harvest 2011	
	C org.%	Humus %	C org.%	Humus %	C org. %	Humus %	C org. %	Humus
	$N_{200}P_{200}K_{200}$ NON-INOCULATED				$N_{200}P_{200}K_{200}$ INOCULATED			
Control	1.21	2.08	0.88	1.52	1.21	2.08	0.88	1.52
1%	4.03	6.94	1.86	3.20	5.54	9.55	2.12	3.65
3%	4.49	7.74	2.22	3.82	2.79	4.80	1.44	2.47
5%	4.76	8.20	2.24	3.86	5.05	8.71	2.72	4.69

Dynamics of total nitrogen content in non-treated, non-inoculated treatments (V2V8V14) is as follows: at the first harvest, the total nitrogen contents decrease from 0.187% (control) to 0.175% at the level of pollutant loading of 1%; to 0.129%, at the level of pollutant loading of 3%; and to 0.143% at the level of pollutant loading of 5%. The decrease of nitrogen contents is due to the oil degradation action, decreasing as a result of its use by the bacterial flora.

At the second harvest the total nitrogen contents range between 0.134% and 0.147%. It is observed that, in the case of the first rates of 1% and 3% oil, the Nt content is slightly higher than in the control, this situation being a consequence of winter anarchic degradation of vegetation roots grown in pots (table 2a).

In the case of V5V11V17 treatments, inoculation is evident, so that, at the first harvest, a uniformity of values is observed; and, at the second harvest, their decrease is observed. It should be mentioned that, at the pollutant contents of 3% and 5%, a minor increase of the Nt contents is observed as a result of the anarchic degradation of vegetal material.

Tab. 2.a

Dynamics of Nt content and C/N ratio in the vegetation house (V2, V8, V14) and (V5, V11 V17)

Concentrations	**First harvest 2010**		**Second harvest 2011**		**First harvest 2010**		**Second harvest 2011**	
	Nt%	**C/N**	**Nt%**	**C/N**	**Nt%**	**C/N**	**Nt%**	**C/N**
	NOT-TREATATED NOT-INOCULATED				**NOT-TREATATED INOCULATED**			
Control	0.187	7.33	0.139	7.66	0.187	7.33	0.139	7.66
1%	0.175	28.6	0.147	18.66	0.160	25.3	0.138	13.0
3%	0.129	58.6	0.142	22.0	0.146	44.0	0.144	20.66
5%	0.143	37.6	0.134	23.0	0.141	42.0	0.144	21.0

In the case of V3V9V15 treatments mineral fertilized with N100P100K100, but non-inoculated, the Nt contents increased as the result of fertilization and vary between 0.181% and 0.207%.

At the second harvest, they decreased and became uniform (0.151% -0.156%) due to the application of agri-pedoameliorative measures. With the V6V12V18 treatments, the contents of first harvest decrease uniformly depending on the applied oil rates; at the second harvest, the decrease was less ordered. However, it is observed that the application of Nt led to the increase of Nt contents in soil that favors the growth of microorganisms (Table 2b).

Tab. 2.b

Dynamics of Nt content and C/N ratio in the vegetation house (V3, V9, v15) and (V6, V12, V18)

Concentrations	**First harvest 2010**		**Second harvest 2011**		**First harvest 2010**		**Second harvest 2011**	
	Nt%	**C/N**	**Nt%**	**C/N**	**Nt%**	**C/N**	**Nt%**	**C/N**
	$N_{100}P_{100}K_{100}$ NON-INOCULATED				**$N_{100}P_{100}K_{100}$ INOCULATED**			
Control	0.187	7.33	0.139	7.66	0.187	7.33	0.139	7.66
1%	0.207	27.3	0.156	16.33	0.190	17.3	0.156	10.33
3%	0.173	32.6	0.150	21.66	0.169	24.0	0.180	10.66
5%	0.181	30.6	0.151	19.0	0.168	31.3	0.150	20.0

When the N200P200K200 rate increases, a decrease of the Nt content is observed at the first harvest in the case of the non-inoculated treatments (0,189% - 0,166%); and, at the second harvest, a visible decrease of nitrogen content is visible as a result of its use in the biodegradation process (0.164 to 0.146%). The inoculated treatments show a ordered situation of contents due to the pollution with of 1% up to the pollution with 5%, the lowest pollution values being observed at the last level of pollution as a result of intensive use of nitrogen by the microorganisms in the biodegradation process, the microorganisms being more active at higher pollutant concentrations (Table 2c).

Tab. 2.c

Dynamics of Nt content and C/N ratio in the vegetation house (V4, V10, V16) and (V7, V13, V19)

Concentrations	First harvest 2010		Second harvest 2011		First harvest 2010		Second harvest 20112011	
	Nt%	C/N	Nt%	C/N	Nt%	C/N	Nt%	C/N
	$N_{200}P_{200}K_{200}$ NON-INOCULATED				$N_{200}P_{200}K_{200}$ INOCULATED			
Martor	0.187	7,33	0.139	7.66	0.187	7.33	0.139	7.66
1%	0.189	24.6	0.147	14.66	0.203	32.6	0.188	12.66
3%	0.166	31.0	0.164	15.66	0.199	16.3	0.156	10.33
5%	0.173	31.6	0.146	18.0	0.169	35.0	0.140	21.66

Dynamics of C/N is edifying for the biodegradation process. Thus, if in the case of non-treated, non-inoculated treatments (V2V8V14), at the first harvest there are C/N ratios varying between 28.6 and 58.6 as compared to 7.33 (control) and which certify the imbalances installed in the soil as a result of pollution; in case of the second harvest, after applying the agri-pedoameliorative measures and passage of time, the C/N ratio begins to balance itself, however at a much higher level than that of the control (18.66 -23).

In the case of inoculated treatments (V5V11V17), at the first harvest, the C/N ratio already is more reduced than in the case of non-inoculated treatments, it ranging from 25.3 to 42.

This is explained by the action of soil microorganisms and the influence of applied measures.

In the case of the V5V11V17 inoculated treatments, the first harvest shows a C/N ratio decrease at the 1% and 3% levels of pollution and an its increase at the loading degree of 5%. It is normal that the biodegradation, at a higher loading degree, to action more slowly.

At the second harvest, the C/N ratio greatly decreases, the values varying from 13 to 21 due to the inoculum action and applied measures (Table 2a).

Tab. 2.a

Dynamics C/N ratio in the vegetation house (V2, V8, V14) and (V5, V11, V17)

Concentrations	First harvest 2010		Second harvest 2011		First harvest 2010		Second harvest 2011	
	Nt%	C/N	Nt%	C/N	Nt%	C/N	Nt%	C/N
	NON-TREATED NON-INOCULATED				NON-TREATED INOCULATED			
Control	0.187	7.33	0.139	7.66	0.187	7.33	0.139	7.66
1%	0.175	28.6	0.147	18.66	0.160	25.3	0.138	13.0
3%	0.129	58.6	0.142	22.0	0.146	44.0	0.144	20.66
5%	0,143	37.6	0.134	23.0	0.141	42.0	0.144	21.0

Table 2b shows the C/N ratio variation under mineral fertilizer conditions N100P100K100. (V6V12V18).

In the case of the treated, non-inoculated treatments, at the first harvest a uniformity of C/N ratio and its decrease as compared to the non-treated treatments are observed that, and, at the second harvest, the decline it is less obvious.

At the first harvest, in the case of non-inoculated treatments, the ratio decrease in the first two pollution levels the pollution is evident, and the pollution with 5% is roughly the same.

At the second harvest, the C/N ratio at the two first levels of loading is already close to that of the control (10.33 to 10.66), and at a loading of 5% it is lower than in the case of the non-inoculated treatments, being still high (2b).

Tab. 2.b

Dynamics of C/N ratio in the vegetation house (V3, V9, V15) and (V6, V12, V18)

Concentrations	**First harvest 2010**		**Second harvest 2011**		**First harvest 2010**		**Second harvest 2011**	
	Nt%	**C/N**	**Nt%**	**C/N**	**Nt%**	**C/N**	**Nt%**	**C/N**
	$N_{100}P_{100}K_{100}$ NON-INOCULATED				**$N_{100}P_{100}K_{100}$ INOCULATED**			
Control	0.187	7.33	0.139	7.66	0.187	7.33	0.139	7.66
1%	0.207	27.3	0.156	16.33	0.190	17.3	0.156	10.33
3%	0.173	32.6	0.150	21.66	0.169	24.0	0.180	10.66
5%	0.181	30.6	0.151	19.0	0.168	31.3	0.150	20.0

In the case of mineral fertilization with N200P200K200, the variation of C/N ratio is as follows: at the first harvest from, in the case of non-inoculated treatments, the decrease and uniformity are evident (from 24.6 to 31.6) while, for the second harvest, the decline is more pronounced (14.66 to 18.0) (Table 2c).

In the case of inoculated V7V13V19 treatments, the first harvest shows an increase of C/N ratio due to the application of fertilizer rates, and the time passed until the harvest, and at the second harvest the ratio decline is visible showing a biodegradation carried out in the soil mass.the first harvest Top of Form

Tab. 2.c

Dynamics of C/N ratio in the vegetation house (V4, V10, V16) and (V7, V13, V19)

Concentrations	**First harvest 2010**		**Second harvest 2011**		**First harvest 2010**		**Second harvest 2011**	
	Nt%	**C/N**	**Nt%**	**C/N**	**Nt%**	**C/N**	**Nt%**	**C/N**
	$N_{200}P_{200}K_{200}$ NON-INOCULATED				**$N_{200}P_{200}K_{200}$ INOCULATED**			
Control	0.187	7.33	0.139	7.66	0.187	7.33	0.139	7.66
1%	0.189	24.6	0.147	14.66	0.203	32.6	0.188	12.66
3%	0.166	31.0	0.164	15.66	0.199	16.3	0.156	10.33
5%	0.173	31.6	0.146	18.0	0.169	35.0	0.140	21.66

Dynamics of Thp content presents as follows: at the first harvest in 2010 with non-inoculated, non-treated treatments (V2V8V14), the soil sampling was carried out shortly after pollution (trying to simulate a field situation, so that the recorded contents are random). The oil contents have a dynamics corresponding to that of organic carbon content varying between3.03% and 5.14%. At the second harvest, the contents decrease as a result of implemented measures and the passage of time. In the case of non-treated and inoculated variants (V5V11V17), at the first harvest, an obvious decrease of contents in the first two loading levels; and, at an applied oil content of 5% in soil, the oil content in soil is rather similar (3.14% - 3.37%).

At the second harvest, the contents are decreased as compared to both the non-inoculated second harvest and the inoculated harvest. They vary between 0.139% -2.37% (Table 3a).

At the loading levels of 1, 33, 5, 10 or more than 10, the oil fractions susceptible to the microbial attack are degraded (Zoo Bell., 1973).

Tab. 3.a

Dynamics of TPH content in the vegetation house (V2, V8, V14) and (V5, V11, V17)

Concentrations	First harvest 2010	Second harvest 2011	First harvest 2010	Second harvest 2011
	THP %	THP%	THP%	THP%
	NON-TRATATED NON-INOCULATED		NON-TRATATED INOCULATED	
Control	0.00	0.00	0.00	0.00
1%	3.03	1.64	1.84	1.39
3%	5.14	3.51	3.09	2.37
5%	3.14	1.97	3.37	1.83

In the case of the N100P100K100 treatments fertilized with mineral fertilizers and non-inoculated, the first harvest contents are approximately uniform (2.95% -3.37%) showing that, at higher levels of pollutant loading, the content is uniformed (Table 3b).

At the second harvest the contents are lower varying between 1.48% - 2.38% due to the effects of implemented measures and time passage.

In the case of the N100P100K100 treatments fertilized with mineral fertilizers and inoculated (V6V12V18), the first harvest shows a decrease of the contents as compared to from the first harvest (1.42% -2.65%), and, at the second harvest, the contents are reduced much more (0.45% -1.72%). Also, in this latter harvest, the variations of oil content are not observed as compared to the applied rate. This means that inoculated microorganisms have been very effective at these rates (Table 3b).

Tab. 3.b

Dynamics of TPH content in the vegetation house (V3, V9, V15) and (V6, V12, V18)

Concentrations	First harvest 2010	Second harvest 2011	First harvest 2010	Second harvest 2011
	THP%	THP%	THP%	THP%
	$N_{100}P_{100}K_{100}$ NON-INOCULATED		$N_{100}P_{100}K_{100}$ INOCULATED	
Control	0.00	0.00	0.00	0.00
1%	3.37	1.48	1.42	0.45
3%	3.19	2.38	1.84	0.93
5%	2.95	1.63	2.65	1.72

Table 3c shows the results of the mineral fertilized treatments with N200P200K200.

In the case of mineral fertilized treatments (V4V10V16) with N200P200K200 and non-inoculated (V4V10V16), at the first harvest, uniform oil contents are observed, varying between 2.26% -2.95%. At the second harvest, the contents decrease varying between 0.96% - 0.133%. In the case of inoculated treatments, at the first harvest, the contents are lower than in the case of non-inoculated treatments, varying between 2.08% - 2.45%. At the second harvest, a decrease of contents is observed, varying between 1.54% and 2.09%. Within this treatment, a less intensive action of microorganisms was observed as compared to that in the case of mineral fertilization with N100P100K100.

Tab. 3.c

Dynamics of TPH content in the vegetation house (V4, V10, V16) and (V7, V13, V19)

Concentrations	First harvest 2010	Second harvest 2011	First harvest 2010	Second harvest 2011
	THP%	THP %	THP %	THP %
	$N_{200}P_{200}K_{200}$ NON-INOCULATED		$N_{200}P_{200}K_{200}$ INOCULATED	

Control	0.00	0.00	0.00	0.00
1%	2.26	0.96	2.08	1.54
3%	2.70	1.34	2.08	1.54
5%	2.95	1.33	2.45	2.09

CONCLUSIONS

The results of microbiological analyses demonstrate that, in the case of an adequate supply with nutrients, the bacterial populations in soil begin to exceed the initial stress caused by oil pollution and, at the same time, the increase of pollutant contents leads to the lowering the numbers of bacteria.

As the content nutrients is getting higher and a rather balance is established in the soil stronger inoculum effect is manifested.

The time factor is of great importance in evolution regarding both the organic carbon content in oil and the bacterial populations.

Organic carbon content and oil are in a close interrelation decreasing during the experiment as a result of applied biodegradation methodology.The C/N ratio gradually decreases depending on the rates of oil and fertilization, as well as on the applied measures.

The treatment with mineral fertilization (N100P100K100) and inoculum application is most effective so far.In the next research stage we have in view to search how the bacterial populations introduced in the soil act on spilled oil and to establish the best technological links acting into helping the biodegradation.

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