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EFFECT OF THE USE OF NEW METHODS FOR THE REMEDIATION OF OIL POLLUTED SOIL

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Abstract

The present study performed the investigation of new environmental friendly methods to remediate polluted soil with crude-oil. The experiment was carried out in greenhouse, in controlled condition using polluted soil and perlite, in different percent. Also, we used five organic fertilizers (Vermiplant, Amalgerol, Poco, Iguana and Formulex). Based on the data obtained under controlled conditions, the best results were obtained in variants where the percentage of perlite used in the soil mix was 25%. The 50% and 75% perlite mix with polluted soil quantities used in the blend did not bring significant differences in plant growth. The research on the influence of remedial measures of the polluted soil on main growing parameters revealed significant differences on physical and chemical properties of the soil, on plant growth, biomass production and plant biochemical composition before and after the treatment. The proposed technology effectively recovered soil properties and plant growth. Microbial populations included 6-15 bacterial strains belonging to Pseudomonas, Bacillaceae and actinomycetes and 5-10 fungal species. Treatments, especially when applied on soil diluted with perlite stimulated species diversity increasing and pathogen inhibition.

Key words: *bean, bioremediation, microbial populations, pollution, perlite.*

INTRODUCTION

The technology for remediation of soil contaminated with petroleum residues or residual salts, which is the subject of this scientific work, is concerned with the use of expanded perlite as a constitutive element of soil quality improvement recipe.

The expanded perlite is a granular, light and porous product based on chemically stable (SiO_2, Al_2O_3, K_2O, Na_2O, Fe_2O_3, CaO, MgO, TiO_2) with density ranging from 40 to 250 kg/m^3. The raw material is the perlitic rock, of volcanic origin, generated by the lava solidified in water. Through this process, the molecular structure of the rock remained chemically bound water. The presence of water molecules causes the explosion of rock granules brought to temperatures of 800-1000^oC. Due to the semiplastic state, where the granules of rock are found at the temperature of the furnace, there is the phenomenon of expansion and breaking of the granules. A granular material is obtained very easily and porous, with various granules, from 0 to 6 mm.

Plants germinate and grow in environments where water, mineral elements and air are available. Oil pollution is a major risk for the biodiversity of the land due to the fact that both crop and bacterial microflora are affected.

Oil reduces soil fertility so nutrients become inaccessible for plants. Oil has a higher density than water and displaces air and water between soil particles, making it difficult for mineral absorption and triggering anaerobic metabolism. Soil properties determine the rate of absorption and plant growth, directly affecting crop production.

Bioremediation and natural rehabilitation occur over a long time, and as such in the rehabilitation of these soils, the scientific community is involved in finding optimal and

rapid solutions (Malschi, 2015; Baek et al., 2004). Aromatic hydrocarbons found in petroleum are large complex high-persistence molecules that typically require powerful reagents to annihilate their action.
Compared with biological methods, physical and chemical methods may produce secondary pollution to reclaim oil contaminated soil (Zhan et al., 2017).

MATERIALS AND METHODS

Experiments were performed in the greenhouse conditions. We used the cultivar Unidor of bean characterized through determined hight, vegetative period of 79 days and resistences at anthracnose, *Colletotrichum lindemuthianum*; BCMV-VMT and PSP-*Pseudomonas syringae* pv. *phaseolicola*.
The polluted soil was collected from Icoana commune, Olt district, situated in the SV part of Romania.
We performed soil mixtures with perlite in varying proportions, and fertilization was performed with Amalgerol, Vermiplant, Poco, Iguana and Formulex in a 2%. The experimental variants are presented in Table 1.

Table 1.The experimental variants

Variant	V1 100% polluted soil	V2 75% polluted soil +25% perlite	V3 50% polluted soil +50% perlite	V4 25% polluted soil +75% perlite
Control	V1/1	V2/1	V3/1	V4/1
Amalgerol	V1/2	V2/2	V3/2	V4/2
Vermiplant	V1/3	V2/3	V3/3	V4/3
Poco	V1/4	V2/4	V3/4	V4/4
Iguana	V1/5	V2/5	V3/5	V4/5
Formulex	V1/6	V2/6	V3/6	V4/6

We determined: the amount of water required to soak up the soil; the amount of water consumed by plants (throughout the experiment); growth dynamics of plants; the total vegetative mass resulting; distribution of the root system (at the end of growth).
The caracteristic of fertilizers used in experiment. **Amalgerol** is a product with that contain natural oils with benefic effects on the plant regarding increases the root mass of plants and the absorption of nutrients.
VermiPLANT is a totally natural product obtained from earthworms that contains microelements such as barium, zinc, iron, manganese and amino acids. **POCO** is a totally natural product with pH: 8.5-9.5 that contained: Calcium 0.04-0.05%; Iodine: 6.30-12.70 mg/l; Magnesium 0,50-0,80 mg/l; Nitrogen (N): 0.025-0.038 mg/l; Potassium: 0.50-0.64%; Sodium (Na): 0.088-0.120%; Sulfur 0.028-0.050%. **Iguana** is a organic product with 4% nitrogen, 3% phosphorus, 6% potassium. **Formulex** is also an organic product that contains: N 2.40%; P_2O_5 0.85%; K_2O 3.36%; CaO 1.85%; B 0.0108% and microelements.

RESULTS AND DISCUSSIONS

Data obtained from bean plants grown under controlled conditions showed that the best results were obtained in variants where the percentage of perlite used in the soil mix was 25%. The 50% or 75% perlite quantities used in the blend did not bring significant differences in plant growth (Figure 1).

Figure 1. Experimental variants

In the case of polluted soil (V1), the biomass of bean plants was higher in the variant that we administered the Vermiplant product (176.25 g V1/3). This was with 16.75 g higher than the control variant (159.5 g V1/1)). The variant we improved the soil with 25% perlite and fertilized with Vermiplant produced an average plant biomass of 201.67 g, with 32.67 g more than the control variant (Figure 2).

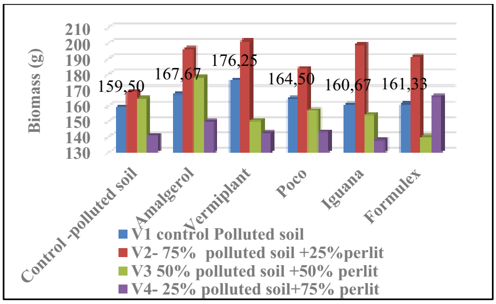

Figure 2. Influence of substrate on plant biomass

Statistically analyzing the obtained data, we found that, in the case of 25% perlite soil improvement and organic fertilizers, we obtained increases in the mass of plants, in all experimental variants, the differences being very positive at V2 (Table 2).

Table 2. Total biomass of bean plants

Variants	V1	V2	V3	V4
Control	159.50	169.00***	164.67**	141.00 ooo
Differences	100.00	105.96	103.24	88.40
	DL5%= 2.690		DL5% in % = 1.6865	
	DL1% = 4.080		DL1% in % = 2.5580	
	DL0.1%= 6.50		DL0.1% in %= 4.0752	
Amalgerol	167.67	196.33***	178.00***	150.00 ooo
Differences	100.00	117.09	106.16	89.46
	DL5% = 2.050		DL5% in % = 1.2226;	
	DL1% = 3.100		DL1% in % = 1.8489;	
	DL0.1% =4.950		DL0.1% in %= 2.9522	
Vermiplant	176.25	201.67***	150.67 ooo	142.50 ooo
Differences	100.00	114.42	85.49	80.85
	DL5% = 0.110		DL5% in % = 0.0624;	
	DL1%= 0.170;		DL1% in % = 0.0965;	
	DL0.1% = 0.280		DL0.1% in %= 0.1589	
Poco	164.50	184.00***	157.33 oo	143.25 ooo
Differences	100.00	111.85	95.64	87.08
	DL5% = 4.230		DL5% in % = 2.5714	
	DL1% = 6.400		DL1% in % = 3.8906	
	DL0.1% = 10.190		DL0.1% in %= 6.1945	
Iguana	147.56	199.00	154.20	138.17
Differences	100.00	134.86 **	104.50	93.64
	DL5% = 21.350		DL5% in % = 14.4690;	
	DL1% = 32.300		DL1% in % = 21.8899	
Formulex	161.33	191.25	140.00	166.00
Differences	100.00	118.55***	86.78 ooo	102.89 **
	DL5% =1.970		DL5% in % = 1.2211;	
	DL1% = 2.980		DL1% in % = 1.8471;	
	DL0.1% = 4.760		DL0.1% in %= 2.9505	

V1 - contaminated soil
V2 - contaminated soil 75%+25% perlite
V3 - contaminated soil 50%+50% perlite
V4 - contaminated soil 25%+75% perlite

In the case of applying a higher amount of perlite to the soil by applying the fertilizer dose, we obtained statistically significant negative results in most of the variants except for the variant to which the Formulex product was applied.

The correlations between the applied product and the perlite-polluted soil mixture, in varying percentages (Figures 3-8), led to the conclusion that there was a significant correlation with the Vermiplant product (R^2=0.5386) (Figure 5). For Formulex, the relationship was insignificant (R^2 = 0.0522) (Figure 8).

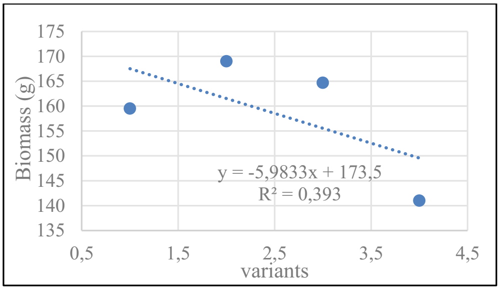

Figure 3 Influence of soil improvement with perlite at the control variant on plant biomass

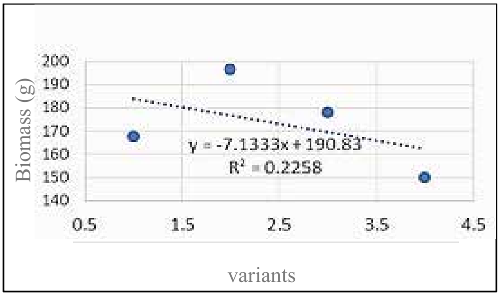

Figure 4. Influence of soil improvement with perlite at variant fertilized with Amalgerol on plant biomass

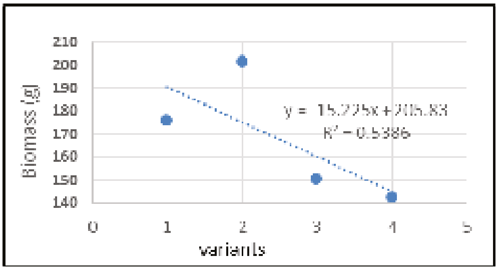

Figure 5. Influence of soil improvement with perlite at variant fertilized with Vermiplant on plant biomass

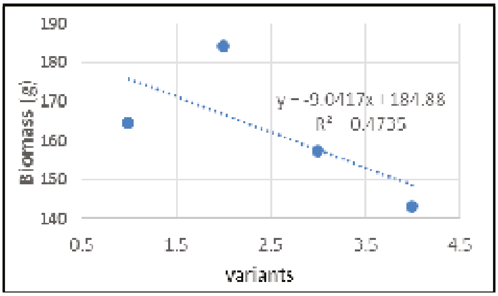

Figure 6. Influence of soil improvement with perlite at variant fertilized with Poco on plant biomass

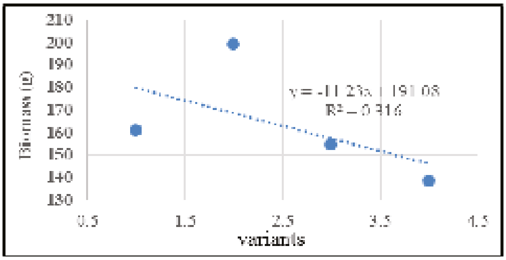

Figure 7. Influence of soil improvement with perlite at variant fertilized with Iguana on plant biomass

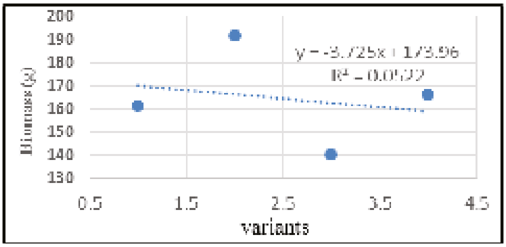

Figure 8. Influence of soil improvement with perlite at variant fertilized with Formulex on plant biomass

Analyzing the influence of the above mentioned fertilizers on the perlite-soil combinations (Figures 9-12), we estimate that for the control variant (polluted soil), the influence of fertilization application showed insignificant relationship (R^2=0.0405) (Figure 9).

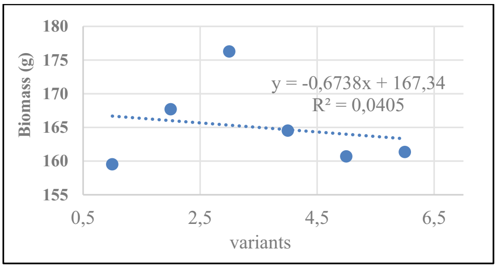

Figure 9. Influence of fertilizers on polluted soil

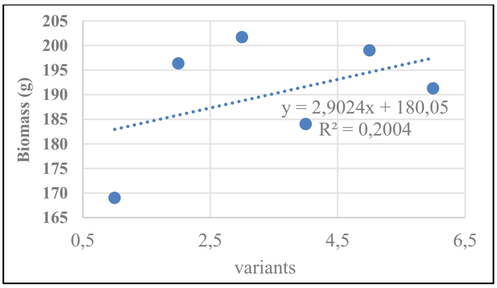

Figure 10. Influence of fertilizers on polluted soil ameliorated with perlite in percent of 25%

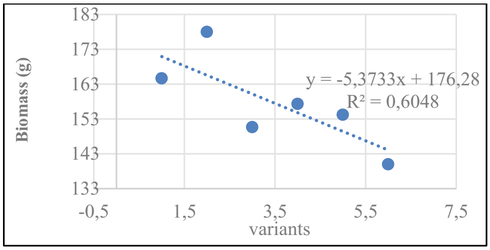

Figure 11. Influence of fertilizers on polluted soil ameliorated with perlite in percent of 50%

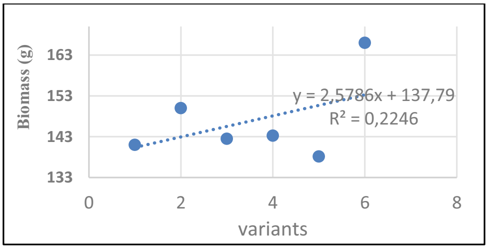

Figure 12. Influence of fertilizers on polluted soil ameliorated with perlite in percent of 75%

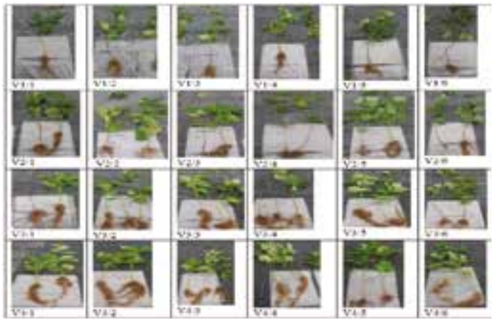

Figure 13 Aspect of bean plants in experiment

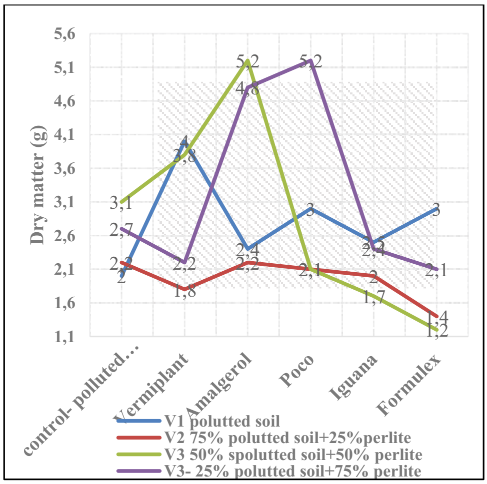

Figure 14. Dry matter content of the roots

Analyzing the aspect of plants (Figure13) and dry matter content of the roots, we found that the highest values were recorded in soil variants improved by 50% perlite, and fertilized with Vermiplant (5.2 g) and the variant improved with 75% perlite and treated with Poco (Figure 14).
The highest dry matter content of the leaves was recorded in the 25% perlite-enhanced soil variant, and fertilized with Iguana (Figure15).

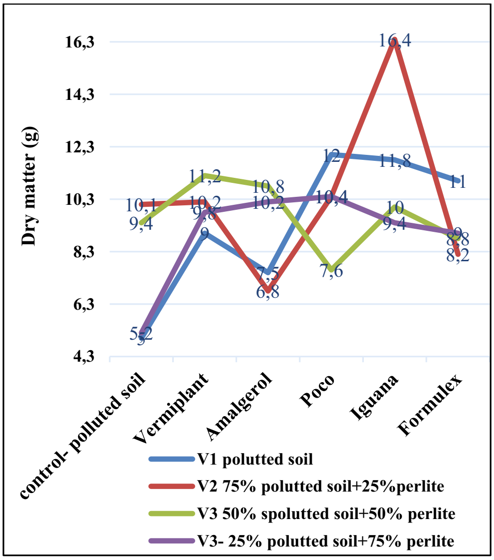

Figure 15. Dry matter content of the leaves

Microbial populations in experimental variants were represented by 6 to15 bacterial species including actinomycetes belonging to 1-4 Series. The stimulation of species diversity was noticed and ecologic ameliorative treatments were added to soil, especially in variants mixed with various proportions of perlite.
In many variants, *Pseudomonas fluorescens* was dominant, followed by different species of *Bacillaceae* (Figure 16). Fungal microflora included communities of 5 to maximum 10 species with variation as a function of experimental factors (substrate type and the nature of stimulating treatment applied). An inhibition of *Fusarium* pathogenic species as compared with control was evidenced in polluted soil under the influence of various stimulate treatments and the dominance of cellulolytic and hydrocarbon degrading species *Cunninghamella elegans*. Species of genus *Aspergillus*, accompanied by antagonists *Trichoderma, Paecilomyces* and other species with high ability for enzymatic degradation of various organic substrates were identified with high frequency in soil with perlite and stimulating treatments (Figure 17).

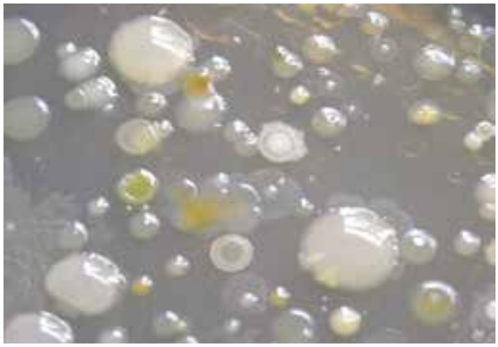

Figure16. Aspects of the bacteria developed in 75% polluted soil + 25% perlite and Iguana (V2/5)

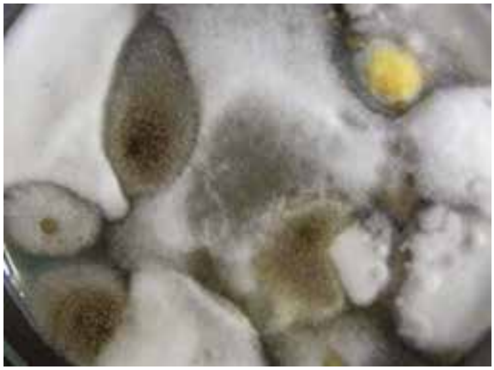

Figure17. Aspects of the fungi developed in 25% polluted soil + 75% perlite (V4/2) and Amalgerol

Results of the present study confirm our previous research that showed the involvement of pseudomonads (Matei et al., 2007; Scarlat et al., 2015) and *Aspergillus* species in hydrocarbon degradative processes.
Other authors reported beneficial effect of bioremediation technologies including bacterial and fungal biodegraders with stimulating treatments for soil conditions improvement and plant growth and enhancing (Potin et al., 2004; Verma et al., 2006).

CONCLUSIONS

Application of organic fertilizers has led to the increase of plant mass in all variants. When

Vermiplant was applied (V2/3), the highest plant mass was 201.67 g compared with the rest of the variants.

There were direct relationships between the polluted soil mixture (75% polluted soil and 25% perlite) and the Vermiplant product (V2/3).

Regarding the dry matter content of the roots, it was result that the variants cultivated on a mixture of 50% polluted soil mixed with 50% perlite with application of Amalgerol (V3/2) and also in the 25% polluted soil mixed with 75% perlite with application of Poco products (V4/4), the dry matter content was the highest (5.2 g for both variants).

The highest dry matter content at the leaves was recorded in the fertilized variant with the Iguana product, plants grown on the substrate with polluted soil (75%) and perlite (25%) (V2/5).

Microbial populations included 6-15 bacterial species dominated by *Pseudomonas, Bacillaceae* and actinomycetes and 5-10 fungal species.

Treatments, especially when applied on soil diluted with perlite stimulated species diversity increasing and pathogen inhibition.

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