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# EFFECT OF PERLITE AND NATURAL BIOSTIMULATORS AND FERTILIZERS ON MICROBIAL ACTIVITY IN OIL-POLLUTED SOIL

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***Abstract***

*The presence of hydrocarbons and salts in oil-polluted soils is responsible for inappropriate water, air and nutrients regimes, with negative consequences for plants growth. The aim of this paper is to present the results of research carried out to improve soil conditions for microbial communities, using a mix of oil-polluted soil from Icoana farm, Olt county with Perlite and natural stimulators and fertilizers (AMALGEROL, VERMIPLANT, POCO, IGUANA and FORMULEX) in greenhouse experiments with bean (Phaseolus vulgaris L. cultivar UNIDOR). The paper presents the total counts of bacteria and fungi (estimated by dilution plate) and soil respiration (by substrate-induced respiration method). All the natural products and perlite significantly increased bacterial populations and reduced the fungal counts, especially pathogenic species. Biodiversity was stimulated in bacterial communities, generally dominated by Pseudomonas fluorescens, Bacillaceae and Actinomycetes. The dominance of antagonistic fungi Trichoderma viride and Fusarium oxysporum was recorded in myco-coenoses from variants with perlite and in variants treated with VERMIPLANT, IGUANA or POCO. Soil respiration was stimulated by the better substrate aeration with perlite and the natural stimulators and fertilizers FORMULEX and IGUANA.*

***Key words**: natural fertilizers, biostimulators, perlite, microbial activity, oil-polluted soil.*

## INTRODUCTION

Oil hydrocarbons are considered as persistent organic pollutants with negative effect on environment and human health (Varjani, 2017). The presence of hydrocarbons and salts in oil-polluted soils is responsible for inappropriate water, air and nutrients regimes, with negative consequences for plants growth. Microbial life from soil is also affected by the increased quantities of carbon that cause imbalances in C:N ratio. Edaphic microorganisms (bacterial and fungal species) contribute to soil decontamination by the biodegradation of hydrocarbons depending on nutrient availability, moisture content, temperature and soil pH (Abed et al., 2015; Lahel et al., 2016; Zhao et al., 2018; Galitskaya et al., 2021).

The effect of fertilizers and soil aeration on oil degradation was evidenced (Odu 1984; Margesin and Schinner, 2001; Amadi & Ukpaka, 2016; Tangahu et al., 2017; Fagnano et al., 2020). Many results reported their beneficial impact on biodegradative activity of edaphic microorganisms such as fluorescent bacteria from genus *Pseudomonas*, Bacillaceae or fungi from genera *Aspergillus* (Scarlat et al., 2015), *Penicillium* (Techaoei et al., 2007), frequently used as bioinoculants in bioaugmentation technologies for decontamination of oil-polluted soils (Bonilla et al., 2012; Patowary et al., 2017). Literature reported the antagonistic capacity of various bio-fertilizers against seed-borne myco-pathogens of tomato plants (Mogle & Mane, 2010; Jaiswal et al., 2017; Bonanomi et al., 2020) or stimulation of plant growth and resistance to pathogens for *Eruca sativa* L. plants cultivated on polluted soil under bioinoculation with commercial products based on *Trichoderma harzianum* Rifai (Al-Rajhi, 2013). Research demonstrated that biosurfactants with various chemical composition produced by the consortium of bacterial strains from soil contaminated with hydrocarbon in Cepu area, Central Java, Indonesia, were involved in decomposition of oil residues (Sumiardi et al., 2012). Xu et al.

(2005) reported bioremediation of oil-contaminated sediments on an inter-tidal shoreline using a slow-release fertilizer and chitosan.

Research has been carried out to assess the quantitative and qualitative changes in microbial communities from soil contaminated with hydrocarbons under the influence of perlite added to dilute the soil for improving aeration and with various natural biostimulators and plant fertilizers.

## MATERIALS AND METHODS

Soil for greenhouse experiment was collected from a private farm located to Icoana, Olt county, accidentally contaminated with total petroleum concentration of 72.87g x kg$^{-1}$ dry soil by spillage from deteriorated pipes. Microbial communities in polluted soil have been characterized comparatively with those from non-polluted soil (Matei & Matei 2017).

In order to improve soil conditions for microbial communities, a mix in proportion of 50% each (v/v) of surface (A horizon) oil-polluted soil from Icoana with perlite (provided by PROCEMA SRL) was used in the greenhouse experiment with bean (*Phaseolus vulgaris* L. cultivar UNIDOR) as test plant. Expanded perlite obtained by thermic treatment has twenty times increased volume comparatively with initial material. It is a white, hard and very porous material utilisable as additive for soil. Perlite for horticultural utilization 5 (0-5 mm in diameter) has the density 0.1-0.9 gxcm$^{-3}$, humidity 0.5%, pH 6.5-8, refraction index 1.5, thermic conductivity at 24ºC 0.04-0.06 W/m*K, solubility in water <1%, weak acids, contains 3% bound water, silicon 33.8%, aluminium 7.2%, potassium 3.6%, sodium 3.4%, iron 0.6%, calcium 0.6%, magnesium 0.2%, microelements 0.2% (Drăghici et al., 2016a). Available water in expanded perlite was 36.5-43.2% from its volume. This water can be released in time helping plants to survive during drought conditions. Five natural stimulators and fertilizers (AMALGEROL, VERMIPLANT, POCO, IGUANA and FORMULEX) were added to.

AMALGEROL (Hechenbichler, Austria) is a natural product with vegetal oils and hormones that stimulates plant growth, mycorrhizal symbiosis, $N_2$-fixing, microbial activity, vegetal debris decomposition, improves soil structure and fertility (Retrieved from https://www.amalgerol.com/).

VERMIPLANT (Doctor Plant Morile Mătieș, Romania) is a biofertilizer enriched in natural nutrients from earthworms, containing microelements (barium, iron, zinc, manganese) and amino acids that stimulate microbial activity and plant growth (Retrieved from https://doctorplant.ro/ingrasaminte/201-304-vermiplant-ingrasamant-foliar.html).

POCO (Wise Use International BV, Netherlands) is a natural product of herbs and plant extracts (utilized for pollution control), stimulating and accelerating the growth and metabolic activity of microorganisms by micro-nutrients and trace elements (Retrieved from https://www.wiseuse.nl./wiseuseeng/pocoeng.html).

IGUANA (Advanced Nutrients, Canada) is a natural organic product of algae with macro and microelements plus other co-factors necessary for improving soil conditions, stimulating plant growth and yields (Retrieved fromhttps://www.advancednutrients.com/secret-menu/iguana-juice-organic-oim/).

FORMULEX (Growth Technology, England) is a natural complete, balanced and stabilized nutrient solution of all macro and microelements for optimum plant growth and rooting in horticulture (Retrieved from https://www.growthtechnology.com/product/formulex/).

Microbiological analyses were performed by soil dilution method on specific culture media with agar-agar (Topping for aerobic heterotrophic bacteria and PDA for fungi).

After 7 days incubation at dark, colonies were counted and microbial density was reported to gram of dry soil.

Taxonomic identification was done using morphologic criteria, according to Bergey's manual (Bergey & Holt 1994) for heterotrophic bacteria and to Domsch & Gams (1970) and Watanabe (2002) determinative manuals for fungi.

The total number of species in community (S) was recorded for each experimental variant. The ratio between microbial effectives and the number of species in communities expressed species richness ($SR_2$ index).

The global physiological activities of microflora were determined by substrate induced respiration method (SIR) and results were expressed as mg $CO_2$ x 100 $g^{-1}$ soil (Matei, 2011).

All assays were carried out in triplicate. Results were interpreted by one-way analysis of variance (ANOVA). The value $p<0.05$ was considered statistic significant (Student test).

## RESULTS AND DISCUSSIONS

The results showed that all the natural products added and perlite significantly increased bacterial populations (Figure 1) and reduced the fungal counts (Figure 2), especially pathogenic species that dominated in untreated control (polluted soil).

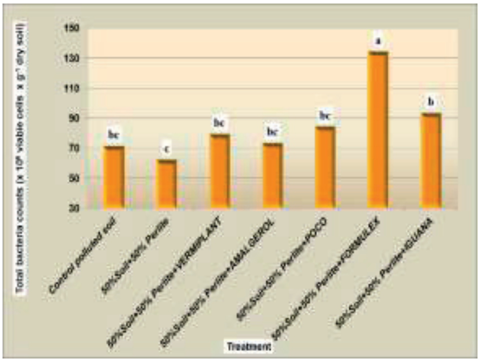

Figure 1. Influence of perlite and natural stimulators and fertilizers on bacteria

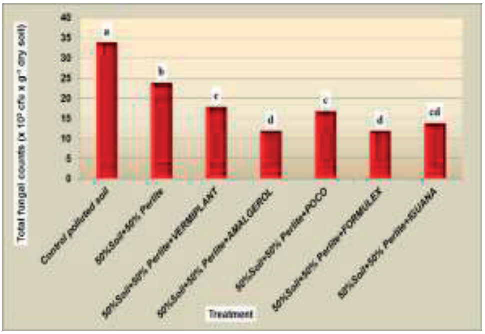

Figure 2. Influence of perlite and natural stimulators and fertilizers on fungi

Biodiversity was stimulated in bacterial communities, generally dominated by *Pseudomonas fluorescens*, Bacillaceae and Actinomycetes, with a maximum of 15 species for the variant with 50% polluted soil + 50% perlite (Fig. 3).

It is well-known the complex role of siderophore-producing fluorescent bacteria (*Pseudomonas*) and Actinomycetes in plant growth promotion, biocontrol of pathogens and bioremediation (Verma et al., 2011; Sah & Singh, 2020).

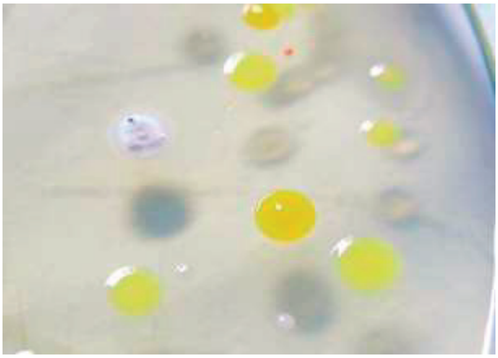

Figure 3. Bacteria from the variant with 50% polluted soil + 50%Perlite

The dominance of antagonistic fungi *Trichoderma viride* (Figure 4) and *Fusarium oxysporum* was recorded in myco-coenoses from variants with 50% perlite and in variants treated with VERMIPLANT, IGUANA or POCO.

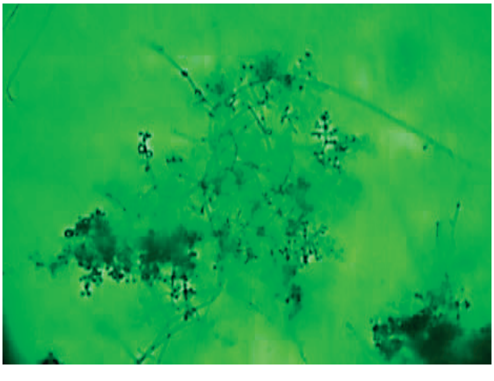

Figure 4. *Trichoderma viride* from the variant with 50% polluted soil + 50% Perlite + VERMIPLANT (150x)

The presence of natural biostimulators and fertilizers induced increased biodiversity in microbial communities, with maximum $SR_2$ values of 0.814 for bacteria at variant with FORMULEX.

Similar values of this index (0.500) were recorded for fungi at variants with AMALGEROL, POCO and IGUANA comparatively with 0.083 and respectively 0.294 in non-treated control (Table 1).

Physiological activities of microbiota were stimulated by the better substrate aeration with perlite and the natural stimulators and fertilizers FORMULEX and IGUANA (Figure 5).

Table 1. Taxonomic composition and biodiversity indices of bacterial and fungal microflora in greenhouse experiment

| **Experimental variant** | **Bacterial species** | **Fungal species** |
|---|---|---|
| Control<br>Polluted soil | *Pseudomonas fluorescens,*<br>*Bacillus polymixa,*<br>*Bacillus circulans,*<br>*Bacillus cereus*<br>Actinomycetes Series Albus, Fuscus | *Fusarium verticillioides,*<br>*Aspergillus ochraceus,*<br>*Fusarium avenaceus,*<br>*Aspergillus flavus,*<br>*Aspergillus terreus,*<br>*Fusarium* sp.,<br>Non-identified,<br>*Rhizopus stolnifer,*<br>*Eurotium herbariorum,*<br>*Fusarium sporotrichioides* |
| | S=6 $SR_2$=0.083 | S=10 $SR_2$=0.294 |
| 50% polluted soil+ 50% Perlite | *Bacillus megaterium,*<br>*Pseudomonas fluorescens,*<br>*Arthrobacter globiformis,*<br>*Pseudomonas aeruginosa,*<br>*Bacillus circulans,*<br>*Pseudomonas striata,*<br>*Arthrobacter citreus,*<br>*Arthrobacter simplex,*<br>*Bacillus subtilis,*<br>*Bacillus polymixa,*<br>*Micrococcus* sp.<br>Actinomycetes Series Albus, Griseus, Fuscus, Coeruleo-griseus | *Fusarium oxysporum,*<br>*Aspergillus niger,*<br>*Trichocladium* sp.,<br>*Penicillium* sp.,<br>*Eurotium herbariorum,*<br>*Mortierella minutissima,*<br>*Acremonium chartarum* |
| | S=15 $SR_2$=0.237 | S=7 $SR_2$=0.291 |
| 50% polluted soil+ 50% Perlite<br>+ VERMIPLANT | *Pseudomonas fluorescens,*<br>*Bacillus cereus var. mycoides,*<br>*Bacillus megaterium,*<br>*Bacillus circulans,*<br>*Pseudomonas pseudogleyi,*<br>*Bacillus* sp.,<br>*Pseudomonas* sp.,<br>*Arthrobacter oxydans* | *Trichoderma viride,*<br>*Aspergillus flavus,*<br>*Aspergillus fumigatus,*<br>*Fusarium* sp.,<br>*Penicillium vermiculatum,*<br>*Fusarium equisetii* |
| | S=8 $SR_2$=0.100 | S=6 $SR_2$=0.333 |
| 50% polluted soil+ 50% Perlite<br>+ AMALGEROL | *Pseudomonas fluorescens,*<br>*Bacillus megaterium,*<br>*Bacillus cereus* var. *mycoides,*<br>*Pseudomonas aeruginosa,*<br>*Arthrobacter globiformis,*<br>*Bacillus cereus,*<br>*Arthrobacter citreus,*<br>*Pseudomonas* sp.,<br>*Micrococcus* sp.<br>Actinomycetes Series Albus | *Cunninghamella elegans,*<br>*Trichocladium* sp.,<br>*Fusarium oxysporum,*<br>*Acremonium* sp.,<br>*Fusarium* sp.,<br>*Monocillium indicum* |
| | S=10 $SR_2$=0.135 | S=6 $SR_2$=0.500 |
| 50% polluted soil+50% Perlite<br>+ POCO | *Bacillus megaterium,*<br>*Pseudomonas fluorescens,*<br>*Bacillus cereus* var. *mycoides,*<br>*Pseudomonas* sp.,<br>*Bacillus cereus,*<br>*Pseudomonas striata,*<br>*Bacillus circulans,*<br>*Arthrobacter globiformis*<br>Actinomycetes Series Fuscus, Albus, Ruber | *Fusarium* sp.,<br>*Trichocladium* sp.,<br>*Penicillium vinaceus,*<br>*Aspergillus niger,*<br>*Trichoderma* sp.,<br>*Fusarium culmorum,*<br>*Myrothecium catenulatum* |
| | S=11 $SR_2$=0.129 | S=7 $SR_2$=0.500 |

| **Experimental variant** | **Bacterial species** | **Fungal species** |
|---|---|---|
| 50% polluted soil+50% Perlite<br>+ FORMULEX | *Pseudomonas fluorescens,*<br>*Arthrobacter globiformis,*<br>*Bacillus cereus* var. *mycoides,*<br>*Arthrobacter citreus,*<br>*Bacillus circulans,*<br>*Arthrobacter simplex,*<br>*Micrococcus* sp.<br>Actinomycetes Series Albus, Griseus, Fuscus, Luteus | *Trichoderma viride,*<br>*Fusarium oxysporum,*<br>*Trichocladium* sp.,<br>*Paecilomyces marquandii,*<br>*Neosartoria fischeri* (sin. *Aspergillus fischeri*) |
|  | S=11 $SR_2$=0.814 | S=5 $SR_2$=0.416 |
| 50% polluted soil+ 50% Perlite<br>+ IGUANA | *Pseudomonas fluorescens,*<br>*Bacillus megaterium,*<br>*Bacillus subtilis,*<br>*Pseudomonas pseudogleyi,*<br>*Arthrobacter citreus,*<br>*Bacillus circulans,*<br>*Bacillus cereus*<br>Actinomycetes Series Fuscus | *Fusarium oxysporum,*<br>*Myrothecium roridum,*<br>*Aspergillus candidus,*<br>*Trichocladium* sp.,<br>*Cladosporium cladosporioides,*<br>*Verticillium leccani,*<br>*Neosartoria fischeri* (sin. *Aspergillus fischeri*) |
|  | S=8 $SR_2$=0.084 | S=7 $SR_2$=0.500 |

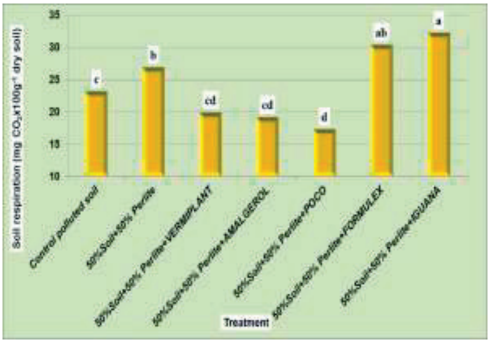

Figure 5. Influence of perlite and natural stimulators and fertilizers on soil respiration

Total plant biomass increased when used 50% perlite and in the variant with AMALGEROL as compared to non-treated control (Figure 6).

Figure 6. Bean plants grown in polluted soil (control), in the variant with perlite and with perlite and AMALGEROL

Results from the present research are in concordance with data from literature reporting bioremediation of a crude oil-polluted soil by application of fertilizers (Chorom et al., 2010). Margesin and Schinner (2001) found increased microbial counts and soil respiration, as well as enzyme activity in fertilized soil that induced a 70% hydrocarbon loss comparatively with 50% in unfertilized alpine soil polluted with Diesel oil hydrocarbons. Other research revealed that combined use of organic soil amendments (poultry dung) and phytoremediation with five plant species significantly improved the activity of microbial community, promoting the restoration of ecosystem (Nwaichi et al., 2015). Rahman et al. (2003) reported enhanced bioremediation of n-alkane in petroleum sludge using a bacterial consortium amended with rhamnolipid and micronutrients.

Previous research evidenced the beneficial effect of fertilizers, aeration condition and inoculation with hydrocarbon degrading microbial strains on the decontamination of oil-polluted soil and restoration of microbial diversity and physiological activity of edaphic microbiota (Dumitru et al., 2004). This data evidences the importance of the technology for stimulating microbial biodiversity and activity of natural hydrocarbon degraders and accelerating decontamination process (Xu & Lu, 2010; Chandran et al., 2020;).

In conditions of contaminated soil from experiment, we recommend management of physical and chemical conditions to improve the microbial activity and hydrocarbon biodegradation by using mixtures with perlite and natural biostimulators and plant fertilizers.

Perlite was chosen to be used in mixture with polluted soil because it is a porous material, with both excellent water retention and drainage capabilities, provides proper aeration, also acting as an efficient insulator or protector against temperature changes.

Perlite is an inert and sterile medium, can be used without fear of tracking in pests or plant pathogenic microorganisms, it is guaranteed to last for years, inexpensive and environment friendly.

Previous research on application of perlite in oil-contaminated sandy soil evidenced its beneficial effect on improving humidity conditions, water uptake and stimulating potato growth during decontamination process by including it in phytoremediation technology (Drăghici et al., 2016b).

In the present study, the process of biostimulation involved the supplying of polluted soil-perlite mix with nutrients as various natural organic or inorganic fertilizers that stimulated microbial proliferation and activation of hydrocarbon degraders from indigenous microflora. The effect on bean plants utilised for phytoremediation was presented elsewhere (Matei et al., 2018).

It is assumed that the hydrocarbons can be more rapidly degraded by the higher microbial counts induced by the nutrients added to soil as compared with natural attenuation process (Wu et al., 2019).

Similar results were obtained by Ruperto et al. (2003) using biostimulation and bioaugmentation to increase the bioremediation of a hydrocarbon contaminated Antarctic soil comparatively with effectiveness of natural microflora or phytoremediation and bioaugmentation with oil-degrading strains for remediating saline soil contaminated by heavy crude oil (Cai et al., 2016).

As in the present experiment, recent results on remedial efficiency of bioaugmentation with microbial consortia and biostimulation for improving diesel-contaminated soils evidenced specific response of bacterial diversity, metabolic activity and biodegradation pathway as a function of fertilizers or amendment variants and suggested that holistic approach including both consortia bioaugmentation and biostimulation was the most adequate option (Wu et al., 2016a; Chaudari et al., 2021).

## CONCLUSIONS

All the natural products and perlite significantly increased bacterial populations and reduced the fungal counts.

The presence of natural biostimulators and fertilizers induced increased biodiversity in microbial communities, with maximum $SR_2$ values of 0.814 for bacteria at variant with FORMULEX and 0.500 for fungi at variants with AMALGEROL, POCO and IGUANA comparatively with 0.083 and respectively 0.294 in non-treated control.

Microbial communities were dominated by *Pseudomonas fluorescens*, bacillaceae and actinomycetes, and antagonistic fungi *Trichoderma viride*, *Fusarium oxysporum*, accompanied by *Aspergillus* or *Penicillium*.

Physiological activities of microbiota were stimulated by the perlite and the natural stimulators and fertilizers FORMULEX and IGUANA.

Total plant biomass increased when used the mixture with perlite and in the variant with AMALGEROL.

Management of soil physical and chemical conditions with perlite and natural biostimulators and plant fertilizers to improve hydrocarbon biodegradation by microbial communities is recommended for reclaiming the contaminated soil from Icoana.

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