PRELIMINARY STUDY OF MICROBIAL COMMUNITIES IN SOIL CONTAMINATED WITH OIL HYDROCARBONS FROM ICOANA

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Abstract

Soil contamination with crude oil or petroleum residues is worldwide the major problem occurred consequently to exploitation, transport and processing. The presence of hydrocarbons leads to increased quantities of carbon and imbalances in C:N ratio with consequences on microbial life from soil. A wide variety of edaphic microorganisms including bacterial and fungal species are known as hydrocarbon bio-degraders, contributing to soil decontamination. Research has been carried out to characterize the microbial communities in soil contaminated with oil hydrocarbons by an accidental spill from broken pipes in Icoana, Olt county. The results revealed important decrease in both bacterial and fungal counts under the impact of contaminant hydrocarbons as compared to non-contaminated soil, accompanied by biodiversity loss (SR_2 values 1.49 for bacteria and 2.32 for fungi comparatively with 7.02 and respectively 3.27 in non-contaminated soil). Low levels of global physiological activity of microorganisms registered in contaminated soil and 2.34 times higher levels in non-contaminated soil. Changes in species composition of microbial populations indicated moderate similarity between bacterial species lists (SI=67) and important dissimilarity between fungal species lists (SI=16). Hydrocarbon utilizers from microflora included pseudomonads, bacilli and cosmopolitan fungal species from genera *Cladosporium, Fusarium, Sporothrix* in contaminated soil and *Trichoderma, Cladosporium, Aspergillus, Penicillium* in non-contaminated soil.

Key words: microbial communities, biodegradation, oil hydrocarbons, soil respiration, decontamination

Crude oil exploitation, industrial processing and transport can cause soil pollution, affecting environment and human health. Many hydrocarbons with low biodegradability, especially polycyclic aromatics are considered as being involved in human cancer and by the long persistence into the soil causing major injuries to edaphic microorganisms and plants, too (Demnerova K.*et al*., 2005). Important yield loss caused by contamination with hydrocarbons occurs when agricultural soils are affected. In Romania, from 50100 hectares polluted with petroleum, 49 570 hectares are agricultural lands (Toti M. *et al.*, 2001). The main process in remediation of polluted soils is biodegradation of hydrocarbons mediated by the microorganisms. Chorom M. *et al.* (2010) cites data revealing that hydrocarbon biodegradation in soil can be influenced by many factors (microbial species, nutrients availability, pH, temperature, moisture, oxygen, soil properties and contaminant concentration).

Among microbial species able to act as efficient petroleum hydrocarbon degraders, literature cites certain representatives of bacterial genera *Pseudomonas, Yarroia, Acinetobacter, Corynebacterium, Sphingomonas, Flavobacterium, Bacillus* (Matei S. *et al.,* 2004, 2007; Kishore D., Ashisk M., 2007; Xu J. *et al.*, 2007; Lahel A. *et al.*, 2016) and fungal genera *Aspergillus* (Scarlat V. *et al.*, 2015), *Trichoderma* (Potin A. *et al.*, 2004). Performant selected strains can be successfully involved in bioremediation technologies broadly classified as in situ and ex situ (Boopathy R., 2000).

A preliminary stage in starting a bioremediation technology is the study of microbial populations of the contaminated soil that, corroborated with data on physical and chemical properties, can contribute to choose the most appropriate methods of approaching the problem.

Research has been carried out to characterize the microbial communities in soil contaminated with oil hydrocarbons by a previous accidental spill from broken pipes in Icoana, Olt county comparatively with non-contaminated soil and to evidence the presence of microorganisms able to use the hydrocarbons as carbon source in own metabolism.

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MATERIAL AND METHOD

Soil samples collected from Icoana, Olt county, accidentally contaminated with total petroleum concentration of 72.87g x kg^{-1} dry soil by spillage from deteriorated pipes were analysed comparatively with non-contaminated soil to assess the modifications induced by the presence of hydrocarbons in edaphic microbial communities.

Microbiological parameters utilised for characterization of microbial communities were: total counts of aerobic heterotrophic bacteria (on Topping medium), total counts of fungi (on PDA medium) repported to 1g dry soil and estimated by soil dillution method.

The capability of microorganisms to utilize hydrocarbons in own metabolism was assessed by cultivation on selective media (HC) with oil as sole source of carbon (Patowary K*. et al.,* 2017).

Taxonomic identification of microorganisms developed on specific culture media after incubation in the dark at 25^{o}C was carried out on the basis of cultural, morphological and physiological characteristics, according to specific determinative manuals of Bergey D.(1994), Watanabe T.(2002), Domsch K. and Gams W. (1970).

The global physiological activities of edaphic microflora were determined by substrate induced respiration method (SIR) and expressed as mg CO_2x100g^{-1} soil (Matei S., 2011).

Similarity Index (SI) of Sorenssen calculated for bacterial or fungal lists of species from non-contaminated and contaminated soil was utilized as a measure of changes in microbial communities composition as response to the presence of hydrocarbons (Tiwari S. *et al.,* 1994).

RESULTS AND DISCUSSION

The results of microbial counts revealed important decrease in fungal and especially in bacterial community effectives in soil contaminated with hydrocarbons comparatively to the counts in non-contaminated soil. Thus, fungal effectives were less than half from non-affected community and bacteria counts decreased with one order of magnitude, being seriously affected by contamination with hydrocarbons.

As a consequence of population loss, soil respiration level, as a measure of global physiological activity of edaphic microflora in contaminated soil was low (24.653mg CO_2x100 g^{-1} soil) and 2.34 times higher levels registered in non-contaminated soil (*Table 1*).

From the taxonomic point of view, in contaminated soil were present associations of genera *Pseudomonas* (non-fluorescent and fluorescent) with bacillaceae, *Arthrobacter globiformis* and Actinomycetes Series Fuscus (*Fig.1*), confirming a concomitant loss of diversity (*Table 2*). SR_2 value was 1.49 comparatively with 7.02 in bacterial community from non-contaminated soil.

A number of 14 bacterial species dominated by *Bacillus megaterium* accompanied by species of *Pseudomonas,* three species from genus *Arthrobacter* and actinomycetes from Series, Albus, Fuscus, Griseus and Ruber were identified in non-contaminated soil.

Similarity Index (SI) between the two bacterial communities revealed that 67% were common species.

In Petri plates with PDA culture medium plated with soil dilutions from contaminated soil were identified 6 species of fungi with SR_2=2.32 and 19 species with SR_2=3.27 in non-contaminated soil.

Changes in species composition of fungal populations indicated important dissimilarity between fungal species lists (SI=16%).

The species shared between the two soils were representatives of potential pathogenic genus *Fusarium* and *Cladosporium herbarum.*

Other cosmopolite species belonging to antagonistic genera *Penicillium, Trichoderma, Paecilomyces* or to strong cellulolytic *Myrothecium, Aspergillus* (*Fig.3*), as well as *Humicola* with abundant growth on Petri plates from non-contaminated soil samples were not identified when hydrocarbons contaminated the soil.

Other species with simple morphology and metabolic behaviour (*Geotrichum candidum, Rhizopus stolonifer*) or *Sporothrix schenckii* (*Fig. 2*), following specific pathways in utilization of carbon sources were able to colonize the soil contaminated with hydrocarbons.

Table 1

Microbial counts and potential soil respiration in soil samples from Icoana

Soil	Fungal counts *x 10^3cfu x g^{-1} dry soil*	Bacterial counts *x 10^6 viable cells x g^{-1} dry soil*	Soil respiration *mg CO_2x100 g^{-1} soil*
Contaminated soil	25.864	4.695	24.653
Non-Contaminated soil	58.017	19.923	57.878

Table 2

Taxonomic composition of bacterial and fungal microflora in soil samples from Icoana

Soil	**Bacterial species** (Topping)	**Fungal species** (PDA)
Contaminated soil	*Pseudomonas* sp., *Bacillus circulans,* *Bacillus megaterium,* *Pseudomonas fluorescens,* *Bacillus cereus,* *Arthrobacter globiformis,* Actinomycetes Series Fuscus	*Fusarium sp.,* *Cladosporium herbarum,* *Fusarium culmorum,* *Geotrichum candidum* *Rhizopus stolonifer,* *Sporothrix schenckii*
Species richness	S=7 SR_2=1.49	S=6 SR_2=2.32
Non-contaminated soil	*Bacillus megaterium,* *Pseudomonas fluorescens,* *Pseudomonas* sp., *Bacillus cereus,* *Bacillus circulans,* *Bacillus cereus* var. *mycoides,* *Arthrobacter citreus,* *Bacillus polymixa,* *Arthrobacter globiformis,* *Arthrobacter simplex* Actinomycetes Series Albus, Fuscus, Griseus and Ruber	*Penicillium sp.,* *Penicillium janthinellum,* *Trichoderma viride,* *Aspergillus niger,* *Paecilomyces marquandii,* *Trichoderma harzianum,* *Humicola grisea,* *Penicillium glabrum,* *Aspergillus terreus,* *Cladosporium herbarum,* *Aspergillus fumigatus,* *Myrothecium verrucaria,* *Fusarium oxysporum,* *Acremonium strictum,* *Alternaria alternata,* *Fusarium verticillioides,* *Actinomucor elegans,* *Verticillium tenerum,* *Fusarium culmorum*
Species richness	S=14 SR_2=7.02	S=19 SR_2=3.27
SI	**67**	**16**

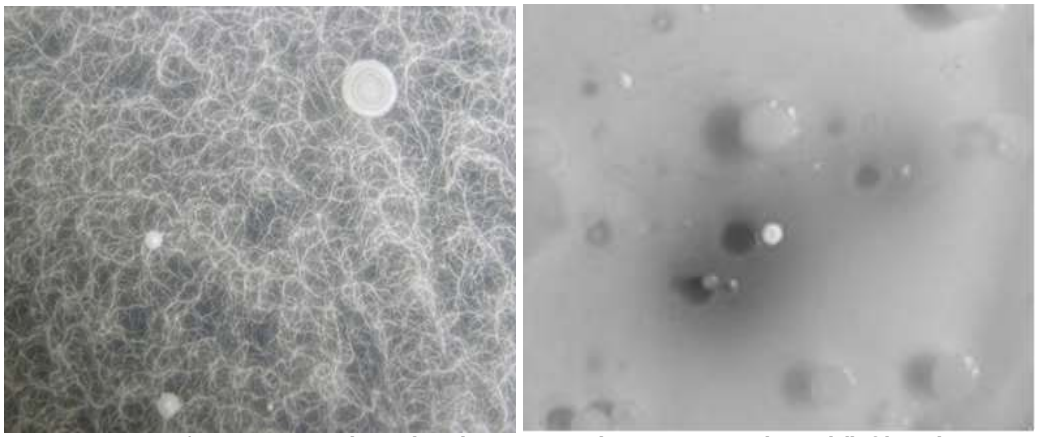

Figure 1. **Bacteria and actinomycetes in non-contaminated (left) and hydrocarbon-contaminated soil (right)– Icoana**

Hydrocarbon utilizers from microflora included pseudomonads, bacilli and cosmopolitan fungal species from genera *Cladosporium*, *Fusarium*, *Sporothrix* in contaminated soil and *Trichoderma*, *Cladosporium*, *Aspergillus*, *Penicillium* in non-contaminated soil.

These species of bacteria and fungi were able to form colonies on media with hydrocarbons from crude oil as single source of carbon confirming that they act as biodegraders with role in soil self-cleaning processes.

On the other hand, the presence of soil-borne plant pathogens in soil from Icoana–Olt as dominant species in communities from contaminated soil is a factor of risk for plants, in conditions of disequilibria caused by the absence of efficient antagonists such as *Trichoderma* and *Paecilomyces*.

In non-contaminated soil, the development of potential patogenic species from genera *Fusarium* and *Alternaria* was kept under the biocontrol by well-represented populations of antagonists *Trichoderma viride, Trichoderma harzianum, Paecilomyces marquandii, Verticillium tenerum.*

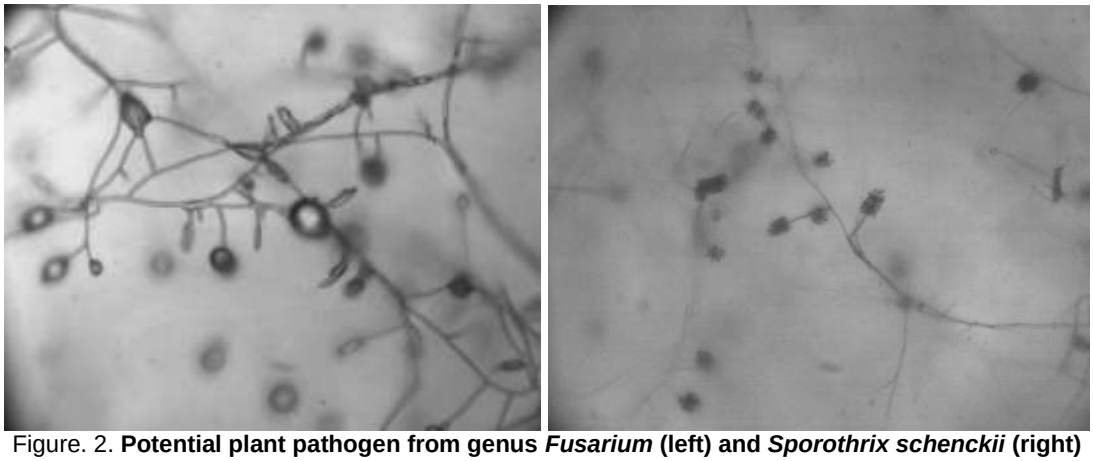

Figure. 2. **Potential plant pathogen from genus *Fusarium* (left) and *Sporothrix schenckii* (right) in hydrocarbon-contaminated soil – Icoana (300x)**

Biochemical antagonism and hyper-parasitism was evidenced between *Trichoderma viride* and the pathogens *Fusarium* and *Alternaria* by optic microscopy examination.

Trichoderma released yellow metabolites towards colonies belonging to pathogen and developed coiled hyphae around it emitting haustoria to feed.

In contaminated soil, the pathogen developed important effectives on the basis of hydrocarbon decomposition and the other species, excepting *Cladosporium herbarum* had a status of accidental or accessory species.

This structure with few species with many individuals and many species with few individuals is characteristic for unbalanced communities unlike non-contaminated soil where the number of colony forming units was more uniform distributed on species.

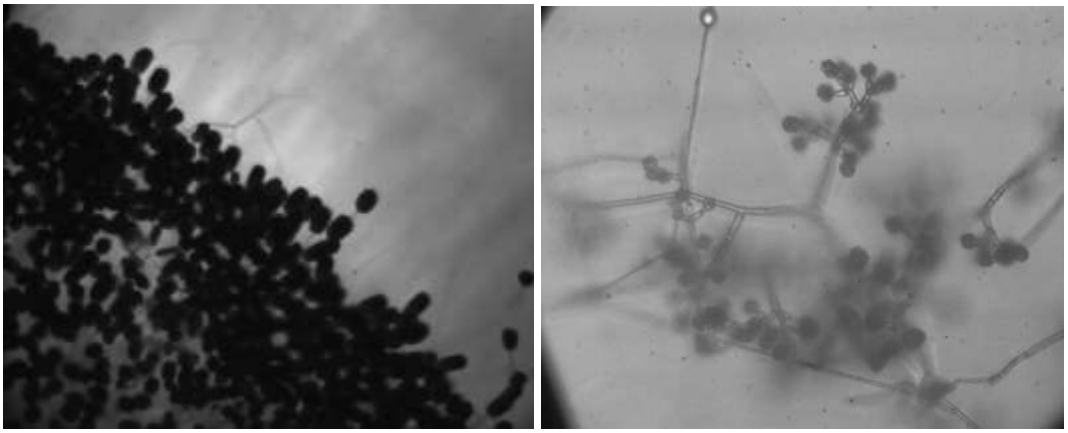

Fig. 3. *Aspergillus terreus* (left) (60x) and *Trichoderma viride* (right) (600x) from non-contaminated soil – Icoana

Results from the present research are in concordance with other data from literature reporting quantitative and qualitative changes in microbial communities in soils contaminated with hydrocarbons from crude oil exploitation, processing and transport.

As in our taxonomic determinations, various fluorescent and non-fluorescent pseudomonads, bacilli, actinomycetes and fungi from genera *Fusarium*, *Cladosporium*, *Aspergillus* and *Penicillium* were reported as hydrocarbon bio-degraders (Toti M. *et al.*, 2001; Kishore D., Ashisk M., 2007; Scarlat V. *et al*., 2015; Xu J. *et al.*, 2007).

Their bio-degradative ability was explained by the capacity of the microorganisms to produce biosurfactants involved in soil decontamination processes (Techaoei S. *et al.*, 2007; Sumiardy A. *et al.*, 2012; Patowary K. *et al.*, 2017).

Research results in non-polluted ecosystems showed that antagonists belonging to the actinomycetes, pseudomonads or to fungal strains of *Trichoderma* conferred efficient biocontrol of pathogens, but in microbial communities from polluted soils their absence was necessary to be replaced by inoculation with performant species to improve their number and metabolic activity or by adequate management practices (Bonilla N. *et al.,* 2012, Lahel A. *et al.,* 2016).

In conditions of contaminated soil from Icoana, the capacity of self-cleaning is low, microbial populations are insufficient and we recommend inoculation with performant consortia biosurfactant-producing and management of soil physical-chemical conditions to improve hydrocarbon biodegradation.

CONCLUSIONS

Important decrease in both bacterial and fungal counts were determined under the impact of contaminant hydrocarbons as compared to non-contaminated soil,

The presence of hydrocarbons induced biodiversity loss in microbial communities, with SR_2 values of 1.49 for bacteria and 2.32 for fungi comparatively with 7.02 and respectively 3.27 in non-contaminated soil.

Low levels of global physiological activity of microorganisms registered in contaminated soil and 2.34 times higher levels in non-contaminated soil.

Changes in species composition of microbial populations indicated moderate similarity between bacterial species lists (SI=67) and important dissimilarity between fungal species lists (SI=16).

Hydrocarbon utilizers from microflora included pseudomonads, bacilli and cosmopolitan fungal species from genera *Cladosporium, Fusarium, Sporothrix* in contaminated soil and *Trichoderma, Cladosporium, Aspergillus, Penicillium* in non-contaminated soil.

Inoculation with supplementary performant microbial consortia biosurfactant-producing and management of soil physical-chemical conditions to improve hydrocarbon biodegradation is recommended for contaminated soil from Icoana.

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REFERENCES

Bergey D.H., Holt J.G., 1994. Bergey's *Manual of Systematic Bacteriology 9*. vol. 2, Wiliams and Wiliams Eds., Baltimore, USA, 787p.

Bonilla N., Cazorla F.M., Martinez-Alonso M., Hermoso J.M., Gonzales-Fernandez J., Gaju N., Landa B.B., de Vicente A., 2012. *Organic amendments and land management affect bacterial community composition, diversity and biomass in avocado crop soils*. Plant and Soil, 357, p.215-226.

Boopathy R., 2000. *Factors limiting bioremediation technologes*. Bioresource Technology, 74, 1, p. 63-67.

Chorom M., Shariff H.S., Montamedi H., 2010. *Bioremediation of a crudeoil-polluted soil by application of fertilizers***.** Iranian Journal of Environment Health Science and Engineering, 7, 4, p.319-326.

Demnerova K., Mackova M., Spevakova V., Beranova K., Kokankova L., Loveka P., Rislava E., Macek T., 2005. *Tow approachs to biological decontamination of groundwater and soil polluted by aromatics-characterization of microbial population.* International Microbiology, 8, 2, p. 205-211.

Domsch K.H., Gams W., 1970. *Fungi in agricultural soils*. T&A Constable Ltd., Edinburg, London, 290p.

Kishore D., Ashisk M., 2007. *Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India*. Bioresource Technology, 98 (7), p. 1339-1345.

Lahel A., Fanta A.B., Sergienko Natalia, Lopez Estefania, Behera S., Rene E., Park H.S., 2016. *Effect of process parameters on the bioremediation of diesel contaminated soil by mixed microbial consortia*. International biodeterioration & Biodegradation, 113, p. 375-385.

Matei S., **2011.** *Determinarea respiratiei solului si a biomasei microbiene In: Dumitru, M., Manea, A.(coord.), 2011 - Metode de analiză chimică și microbiologică (utilizate în sistemul de monitorizare a solurilor)*. Ed. SITECH, Craiova, p. 283-288.

Matei S., **Matei Gabi-Mirela, Mocanu Adina.**, **2004.** *Research on hydrocarbons biodegradation by four bacterial strains inoculated in soil experimentally polluted with Diesel oil.* Publicațiile SNRSS, 34A, 2, p. 140-150.

Matei S., **Matei Gabi-Mirela, Mocanu Adina.**, **2007.** *The dynamic modifications of Diesel oil fractions in a Chromic Luvisol under remedial measures.Soil Science, 41, 1, p. 88-100.*

Potin O., Rafin C., Veignie E., 2004. *Bioremediation of an aged poly ciclic aromatic hydrocarbons (PAHs)-contaminated soil by filamentous fungi isolated from the soil.* International biodeterioration & Biodegradation, 54, p. 45-52.

Patowary K., Patowary R., Kalita M.C., Deka S., 2017. *Characterization of biosurfactant produced during degradation of hydrocarbons using crude oil as sole source of carbon*. Front in Microbiol; 8, p. 279.

Scarlat V., Pele Maria, Drăghici Elena Maria, 2015. *Evaluation the ability of the fungus Aspergillus to*

remove oil from contaminated soils. Scientific Papers. Series B, Horticulture,59, p. 463- 466.

Sumiardi A., Mangunwardoyo W., Hudiyono S., Susilaningsih D., 2012. *Biosurfactant characterization of bacterial consortium from soil contaminated hydrocarbon in Cepu area, Central Java, Indonesia*. Int J Sci and Res Pub, 2(7), p. 1-7.

Techaoei S., Leelapornpisid P., Santiarwarn D., Lumyong S., **2007.** *Preliminary screening of biosurfactant producing microorganisms isolated from hot spring and garages in Northern Thailand.* KMITL Sci and Technol J , 7, p. 38-43.

Watanabe T., **2002.** *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species.* 2nd ed., CRC PRESS, Boca Raton London, New York, Washington D.C., 486p.

Xu J., Tinglin H., Zhixin T., et al., 2007. *Isolation of petoleum degradation bacteria and its application to bioremediation of petroleum-contaminated soils.* Actz Scientiae Circumstantiae, 27, 4, p. 622-628 (in Chinese).

Tiwari S.C., Tiwari B.C., Mishra R.R., 1994. *Succession of fungi associated with the decomposing litters of pineapple (Ananas comosus)*. Pedobiologia, 38, p. 185-192.

Toti M., Dumitru M., Motelică D.M., Căpitanu V., Constantin Carolina, Mihalache Gabriela, Bălăceanu Claudia, Matei S., Matei Mirela, 2001. *Spill Sorb product (Canada) and its use for amelioration of soils polluted with petroleum.* Procedings Symposium Restoration Ecology,Uviversity of Agricultural Sciences, p.218-226.