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INTERACTIONS BETWEEN MICROORGANISMS, SOIL, PLANT IN THE TECHNOGENIC SOIL FROM ROVINARI (GORJ COUNTY)

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Abstract: Soil is doubtless the most complex of all microbial habitats, while the microorganisms are the major participants in this habitat. The technogenic soils represent a special category, where the abiotic components underwent important changes, the same for the microbiotic componence, that is why the study of the relationship soil – microorganisms – plant may represent an important parameter in the evolution of such, within the biological recultivation. The microorganisms are deeply involved in the mineral nutrition of plants, besides the fact that the synthesis of the humic substances, respectively the mineralization of the organic substances depend both on the soil microbiota and on the nature of the cultivated plants. In the present work we looked for the interactions between microorganisms, soil and plants in the experimental variants of a technogenic soil from Rovinari area, a soil resulted after the surface extraction of lignite and subjected then to biological recultivation. There were studied 6 variants cultivated with different plants. The soil samples were analysed both from microbiological and enzymological point of view. It was followed the presence and the dynamic of the levan-forming bacteria involved in the levan synthesis, an important component for the agregation of the soil particles, that is in the formation of a favourable structure from agronomic point of view, close related to the nature of the cultivated plant. It was also determined the number of levan-decomposing bacteria that hydrolise the levan, the equilibrium of the two physiological groups beeing inclined towards the synthesised ones. An other ecological group of studied microorganisms was the one of molecular nitrogen fixing bacteria, aerobic, non-symbiotic (free) that contributes to the enrichment of the soil in nitrogen and that were directly influenced by the nature of the cultivated plant. It was followed the dehydrogenase actual and potential activity that characterises the proliferating microbiota, from enzymological point of view, direct related with the cultivated plant, with the fertilization system and with the applied agrotechnics. The obtained data highlighted the relations between the ecological groups of studied microorganisms, close related to the enzymological (dehydrogenase) activity and with the nature of the cultivated plant.

Introduction

Soil may be considered a biological entity with complex biochemical reactions. Soil is undoubtedly the most complex of all microbial habitats. Primarily because of this complexity, there is insufficient information on how and where most microbial events occur in soil *in situ* and which microorganisms are the major and, physiologically, the most important participants in these events [20]. With the increasing pressure to produce more food, fiber and fuel to meet world demands on a limited land area, there is an unprecedented need to address global concerns about soil degradation. Understanding the underlying biological processes in tandem with identification of early warming indicators of ecosystem stress is need to provide strategies and approaches for land resource managers to promote long-term ecosystem sustainability. Biological and biochemically mediated processes in soils are fundamentaly to terrestrial ecosystem function.

It is generally assumed that soil enzymes are largy of microbial origin, but it is also possible that plants and animals may contribute enzymes in soil [16]. It is difficult to conclusively discriminate between sources of enzymes in soils, and thus, evidence for the primary role of microorganisms as a source of soil enzymes [4]. Vegetation can potentially affect soil enzyme content directly or indirectly. Plant roots can excrete extracellular enzymes [5, 2] showed that, in general, rhizosphere soil increases enzymological activity over nonrhizosphere soil. This studies, however, could not distinguish the enzymes released by roots from enzymes of microbial origin. It seems likely that roots stimulating microbial activity [1] would be a major factor for explaining the rhizosphere effect on soil enzyme activity. Plant residue contains enzymes that may be released into the soil upon residue decomposition or remain active in partially decomposed plant tissue. Furthermore, Dick et al. (1983) showed that fresh corn (*Zea mays)* root tissue exhibiting acid phosphatase activity, when added to soils, was rapidly degraded by soil ptoteases and/or inhibited by soil constituents. Additions of steam sterilized root homogenate (i.e., inactivated acid phosphatase activity) increased acid phosphatase activity, suggesting that plant residue stimulated microbial synthesis of acid phosphatase rather than providing direct additions of corn root phosphatae to the soil. Nannipieri et al. (1983) found similar results when used ryegrass (*Lolium* sp.).

Technogenic soils are soils that form during the technical and biological recultivation of overburdens, tailings, and other spoils and wastes resulted from strip (opencast, surface) and shaft (underground, deep) mining and other industrial activities. The evolution of technogenic soils is, by definition, the process of transforming all these wastes into agricultural and forest soils or into soils used for other purposes (parks, sports fields etc.) [14]. The evolution of technogenic soils, which affects the efficiency of recultivation, is studied using different methods, including many physical, chemical and biological ones. Enzymological methods have also been applied, and it has been found that the level of enzymological activity is a good indicator of the degree of evolution of technogenic soils [15, 3, 18].

After dumping and amelioration mine soils are the begining of soil development. A rapid establishment particularly of an active soil microorganism comunity is a great importance for mine soils fertility and quality. Besides soil microbial biomass N-mineralization and nitrification are some key functions to maintain nutrient requirement of higher plants [8].

Recultivation of quaternary substrates need fertilization according to type and intensity of land-use and in some cases small lime application. However, dominating sands have very low water retention capacities as well as sorption capacities for nutrients [9]. According to Gigon (1984) young mine soils are very susceptible to disturbances from the outside and parameters of stability and resilience are poorly developed.

Schumacher et al. (1993) showed that system of humic substances in young recultivated soils in the Rhinish brown coal mining region was weakly matured but established with time. Microbial biomass and activity and enzyme activities established with the maturity of soil organic matter depending on land-use and tillage practices twenty years after recultivation soils under forest and pasture had higher microbial metabolism potentials than comparable soils under agricultural use. Nevertheless, they did not achieve levels of microbial properties of matured soils from outside recultivated areas. This emphazises the long-term aspect of the development of soil ecosystem and decomposer food webs due to a long-term development and establishment of maturity of organic matter in soils.

The aim of the present work was to study the effects of different cultivated plants on the activity of soil microorganims and on main biochemical processes in mine soils derived from a wide range of deposits in the Rovinari (Gorj county) coal mining area.

Material and Methods

Microbiological and enzymological analyses were carried out on the soil samples collected from 7 variants in spring of year 2005.

The sample sites are located in the Rovinari zone (Gorj county).

Soil samples were collected from the following variants: Variant 1 – Control (grassland), non-technogenic soil; Variant 2 – Plantation of apple trees, technogenic soil; Variant 3 – Plantation of plum trees, technogenic soil; Variant 4 – Plantation of vine, technogenic soil; Variant 5 – Plantation of vine (old 20 years crop), technogenic soil; Variant 6 – Plantation of filbert and birch tree, technogenic soil; Variant 7 – Lot cultivated with alfalfa , technogenic soil.

The soil samples were collected at two depths: 5 -15 cm and 20 – 40 cm. For the microbiological analyses, the samples were collected in aseptic conditions.

As it concerns the microbiological analyses, there were determined the microorganisms involved in the synthesis of levan polysaccharide (the levan-forming ones) and in its decomposing (levan-decomposing ones). There were used elective nutritive media (mineral medium + sucrose for the levan-forming and mineral medium with levan, for those levan-decomposing). The synthesis and decomposition of levan was emphasized by the paper chromatography method [12].

For emphasizing the N_2 fixing bacteria of *Azotobacter* kind it was used an elective media without bound nitrogen. The innoculation was made with soil grains. The colonies set up were analysed also on microscope [7].

As it concerns the enzymological analyses, there was determined the present and potential dehydrogenase activity by the Casida modified method [7]. The levansucrase and the levanase activity were qualitatively determined by paper chromatography [12].

Results and Discussions

The results of microbiological analyses are presented in table 1 and figure 1.

Table 1: Counting of number of levanforming and decomposing microorganisms in technogenic soil

Variant	Name of variant	Depth, (cm)	No.of levanforming microorganisms	No. of levan-decomposing microorganisms
1	Control (grassland), non-technogenic soil	5 – 15	5×10^3	1.9×10^3
2	Plantation of apple trees, technogenic soil	5 – 15	4×10^3	1×10^3
3	Plantation of plum trees, technogenic soil	5 – 15	2×10^3	0.8×10^3
4	Plantation of vine, technogenic soil	5 – 15	1.9×10^3	0.7×10^3
5	Plantation of vine (old 20 years crop), technogenic soil	5 – 15	3.5×10^3	0.8×10^3
6	Plantation of filbert and birch tree, technogenic soil	5 – 15	1.8×10^3	0.6×10^3
7	Lot cultivated with alfalfa , technogenic soil	5 – 15	2.8×10^3	0.8×10^3

Table 1 shows the results obtained in determining the number of levan-forming microorganisms and of those levan-decomposing. From the data analyse it comes out that the number of levan-forming microorganisms is bigger that the one of levan-decomposing microorganisms, for all analysed variants. It is known [12] that levans may have an important role in the aggregation of soil particles, consequently the in the soil pre-existing levansucrase and the proliferant levansucrase-positive microorganisms have an important role in the aggregation, while in the soil pre-existing levanase and the proliferant levan-positive microorganisms have a negative role [12]. Our data confirm that in all variants the microbiological and enzymological capacities encourage levans formation in comparison to their decomposition. As it concerns the effect of the cultivated plant on these two eco-physiological groups, we can remark that the number of levan-forming microorganisms is, generally speaking, relative low – thousands to g^{-1} soil, while the number of levan-decomposing microorganisms is lower, that is tends to hundreds to g^{-1} soil.

Analysing the absolute values of obtained levan-forming microorganisms for the 7 variants, one may remark some small differences, the biggest number being registered for the control variant (5×10^3) and for the technogenic soil variants lower values were registered: 4×10^3 for variant 2, 3.5×10^3 for variant 5, while the lowest for variant 3 (2×10^3) and variant 6 (1.8×10^3).

As it concerns the number of levan-decomposing microorganisms, this is of over 10 times lower, the obtained absolute values are very close, with the highest value for the control variant and the lowest one for variant 6. The lower their number is the poorer the decomposition effect of levan is reduced. Important is the ratio between the number of levan-forming microorganisms and the one of levan-decomposing ones, that remains usually above 2:1.

Certain, to the soil particle aggregation process do participate also other microorganisms, among these, the same importance have those forming the polysaccharide dextrane [13], indirectly do participate also microorganisms producing capsular polymers of proteic or polypeptidic nature, polysaccharid-proteic, respectively.

The figure 1 shows the results of the microbiological analyses making evident the N_2 -fixing bacteria, of *Azotobacter* kind. Our determination settled the percentage of such bacteria, the number of specific colonies for 100 soil particles.

It comes out that for all analysed variants, N_2-fixing bacteria of *Azotobacter* kind were remarked. The obtained percentage is different, depending on the analysed variant, on the depth the soil samples were taken from and certainly, on the nature of the cultivated plant.

For the control variant (grassland soil) was obtained the maximum percentage, both for the depth of 5-15 cm, as well as for 20-40 cm, the same situation being also for the variant 7 technogenic soil, cultivated with alfalfa. A maximum percentage was obtained also for the variant 5, technogenic soil cultivated with vine with 20 years of crops, in the layer of 5-15 cm depth.

For the other variants of technogenic soil, in the layer of 5-15 cm depth were registered percentages of 50-90%, excepting for the variant 6, technogenic soil planted with hazel nut tree and birch tree, where unreasonable, the calculated percentage was of only 9%. In the inferior soil layer (20-40 cm), for all variants of technogenic soil, except for the variant 7, the obtained percentage was quite oscilatory, from 7% for variant 6, to 9% for variant 5 and 2, 10% for variant 3 and 50% for variant 4. As we know that the bacteria in the *Azotobacter* kind are heterotrofic, their growth is intensely influenced by the nature of the cultivated plant. In general, the number of microorganisms is higher in the grassland soils that in the cultivated soils, the total number of microorganisms including also the N_2 – fixing ones. The plot cultivated with alfalfa, with a plant density close to the one of the control variant, presents also an identic percentage. It is interesting the percentage obtained for variant 5, which after a long period of cultivation and fertilization, evolved towards a soil with a good biological potential.

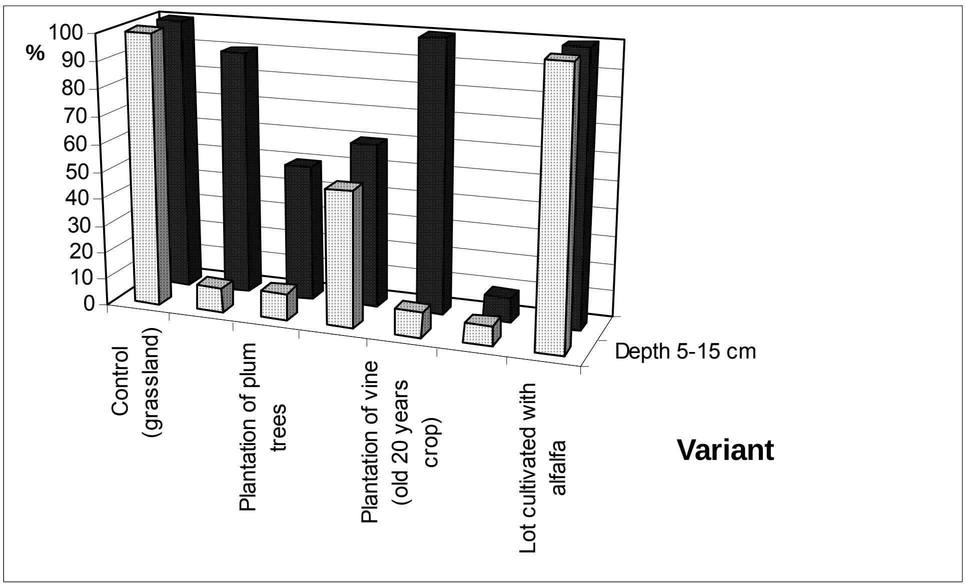

Fig. 1: The presence of *Azotobacter chroccoccum*

The results of enzymological analyses are presented in table 2 and Fig. 2 respectively.

The table 2 presents the values obtained in the determination of the dehydrogenase activity. The first remark is that for all 14 analysed soil samples a dehydrogenase activity was registered, the obtained values being between 0.480 and 0.990 mg formazan / 3 g soil / 48 hours incubation at present dehydrogenase activity, respectively between 1.455 and 2.800 mg formazan / 3 g / 48 hours for the potential dehydrogenase activity.

An other remark was that for all analysed samples the obtained values were lower for the present dehydrogenase activity as for the potential one, where the obtained values were more than twice that high, except for 2 situations – the samples in variant 2 at 5-15 cm depth and the variant 5 at the same depth of 5-15 cm.

Making a comparison between the variants of technogenic soil (2-7) and the control variant (1) soil in the neighbourhood, not affected by the mine working, one may remark that from the view point of the dehydrogenase activity, there are variants more active (e.g. variant 5), variants with an activity close to the control variant (e.g. variant 2, 3 and 7) and variants with lower activity than the control variant (variant 4 and 6). In the case of 20-40 cm depth, the value obtained for the control variant is outrun only by the variant 2.

The results above indirectly show the activity of the proliferant microbiota, which is influenced by the nature of the cultivated plant, by the applied agrotechnics and by the fertilization. To be remarked that the values of the actual dehydrogenase activity for the control variant were outrun by those of variant 5, where the activity was almost double. It indicates that after 20 years of cultivation and fertilization, the technogenic soil evolved towards a fertile soil, especially in the upper layer of 5-15 cm. Other 3 variants presented values close to those of the control variant, which prooves that we had also in these cases an evolution of the soil, that the cultivated plants and fertilizers contributed to the recovery of the soil microbiota and to a microbial activity close to the one of the control variant. The same remarks were made based on the data obtained for the samples where glucose was added, as source of H-donors.

Taking into consideration these data obtained for the dehydrogenase activity, we can assess the status of the technogenic soil in the Rovinari area, as it concerns the microbiological and enzymological potential - which is an indicator of the recovery degree of soil after its destruction through mine working.

Table 2: Dehydrogenase activity of technogenic soils

No. of variant	Name of variant	Depth (cm)	Dehydrogenase activity mg formazan 3 g^{-1}	
			Actual	Potential
1	Control (grassland), non-technogenic soil	5 – 15	0.900	2.100
2	Plantation of apple trees, technogenic soil	5 – 15	0.990	2.050
3	Plantation of plum trees, technogenic soil	5 – 15	0.925	2.400
4	Plantation of vine, technogenic soil	5 – 15	0.490	1.950
5	Plantation of vine (old 20 years crop), technogenic soil	5 – 15	1.950	2.800
6	Plantation of filbert and birch tree, technogenic soil	5 – 15	0.850	1.790
7	Lot cultivated with alfalfa , technogenic soil	5 – 15	0.910	2.080

The figure 2 presents the intensities of the levan-sucrase and levanase activity for the soil samples in the 7 analysed variants in the 5-15 cm deep layer. The determinations were made at two different times (10 days and 30 days respectively).

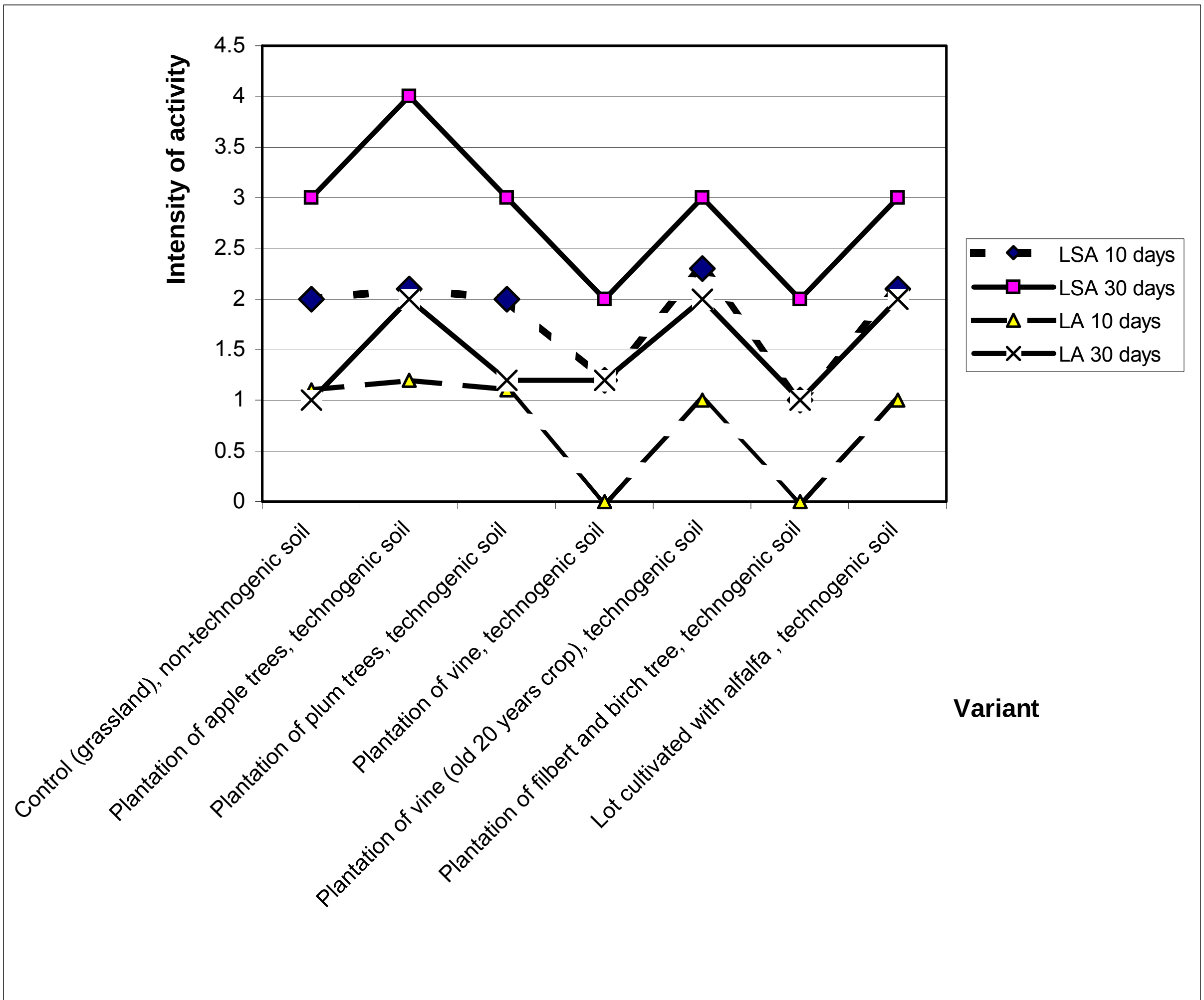

Fig. 2: Evolution of levansucrase activity (LSA) and of levanase activity (LA) in technogenic soils

As for the determination of the number of levan-forming and levan-decomposing microorganisms, the levansucrase activity due to the enzyme accumulated in soil is more intensive than the levanase one. The intensity of the activities increases after a longer incubation period, especially for the levansucrase activity. In general, the intensity of the levansucrase activity was close to the one of the control variant, excepting for two variants, no. 4 and no.6, for both determination periods. The same result is obtained for the levanase activity.

The results we obtained, both the microbiological and the enzymological ones, directed us to the conclusion that the cultivated plant does influence clearly both the microbiota and the enzymological activity and that such may be considered as biological indicators of the evolution of technognic soils.

Conclusions

Microbiology analyses (the determination of the number of levan-forming and levan-decomposing microorganisms) allowed to set a direct relationship between the type of soil (non-technogenic and technogenic) and the nature of the cultivated plant, as well as the N_2-fixing bacteria of *Azotobacter* kind.

Enzymological analyses (the determination of the dehydrogenase and levansucrase activity, respectively levanase one) emphasized the finding on the interactions between the type of soil and the cultivated plant

Both types of analyses (microbiological and enzymological ones) prooved to be valuable biological indicators for the evolution of the technogenic soil in Rovinari towards a fertile soil, with high biological potential.

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INTERACŢIUNI MICROORGANISME-SOL-PLANTE ÎN CADRUL SOLULUI TEHNOGEN DE LA ROVINARI (JUD. GORJ)

(Rezumat)

Solul este, fără îndoială, cel mai complex dintre toate habitatele microbiene, iar la rândul lor microorganismele reprezintă participantul major în cadrul acestui habitat. Solurile tehnogene reprezintă o categorie

specială în care componentele abiotice au suferit modificări importante, la fel şi componenta microbiotică, din acest motiv, studierea relațiilor sol- microorganisme - plante poate constitui un parametru important în evoluția acestora în cadrul recultivării biologice. Microorganismele sunt implicate profund în nutriția minerală a plantelor, pe lângă faptul că sinteza substanțelor humice, respectiv mineralizarea substanțelor organice depinde atât de microbita solului, cât şi de natura plantelor cultivate. In lucrarea de față am urmărit interacțiunile microorganisme-sol- plante în variantele experimentale ale unui sol tehnogen din zona Rovinari, sol rezultat în urma exploatării de suprafață a lignitului şi supus recultivării biologice. Au fost studiate 6 variante cultivate cu diferite plante (graminee, leguminoase, pomi fructiferi, arbuşti), în care probele de sol au fost analizate atât din punct de vedere microbiologic cât şi enzimologic. S-a urmărit prezența şi dinamica bacteriilor levano-formatoare, implicate în sinteza levanului, component important în agregarea particulelor de sol, cu alte cuvinte în formarea unei structuri favorabile din punct de vedere agronomic, în strânsă relație cu natura plantei cultivate. De asemenea, s-a determinat şi numărul bacteriilor levanolitice, ce hidrolizează levanul, echilibrul dintre cele două grupe fiziologice fiind în favoarea celor sintetizante. Un alt grup ecologic de microorganisme studiat a fost cel al bacteriilor fixatoare de azot molecular, aerobe, nesimbiotice (libere) ce contribuie în mod substanțial la îmbogățirea solului în azot, şi care la rândul lor au fost direct influențate de natura plantei cultivate. Din punct de vedere enzimologic s-a urmărit activitatea dehidrogenazică actuală şi potențială, ce caracterizează microbiota proliferantă a solului, în relație directă cu planta cultivată, cu sistemul de fertilizare şi cu agrotehnica aplicată. Datele obținute au scos în evidență relațiile dintre grupele ecologice de microorganisme studiate în strânsă relație cu activitatea enzimatică (dehidrogenazică) şi cu natura plantei cultivate.