“BABEŞ – BOLYAI” UNIVERSITY OF CLUJ – NAPOCA
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DOCTORAL DISSERTATION

THE MICROBIOLOGICAL AND ENZYMOLOGICAL ASSESSMENT OF THE BIOLOGICAL POTENTIAL OF THE TECHNOGENIC SOIL IN ROVINARI, GORJ COUNTY

(abstract)

DOCTORAL SUPERVISOR:
PROF. MIHAIL DRĂGAN – BULARDA, PhD

DOCTORAL STUDENT:

GORNOAVĂ MONICA
(married COPÂNDEAN)

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INTRODUCTION

Technogenic soils are soils that form as a result of the biological, technical and mining recultivation of outcropping materials, sterile dumps and other waste materials resulting from opencast and surface mining operations as well as other industrial activities. Concurrently, all these waste materials represent a dangerous source of environmental pollution (Kiss and collab., 1993).

The recultivation of lands degraded by opencast mining operations is the act of restoration of the usable water holding capacity and production capacity of soils using technical and biological treatments.

The refertilization and recultivation of degraded lands is a first-order preoccupation for specialists and requires a harmonious collaboration of a large number of scientific and technical disciplines, from chemistry, biology and corporate work organization to geology, hydrogeology, botany and meteorology to field and plant cultivation, mining engineering and pedology (Huidu and Jescu, 1987).

The term of biological recultivation refers to the actual cultivation of crop fields.

At this stage, cultivation is carried out on agriculture and forestry surfaces.

Agricultural recultivation is based on a complex refertilization, harvest, drainage and seed selection plan and, in particular, a competent hayfield and grassland management plan to prevent soil degradation by livestock.

Agricultural recultivation is carried out in two stages.

The first stage targets the restoration of soil fertility – after that of the land – through the cultivation of plants with low soil demands, high vegetal productivity and high resistance to disease and weeds.

The plants in the Trifolieae tribe are the most recommended (alfalfa, clover).

The second stage involves the cultivation of crops that permit the attainment of normal harvests after the soil fertility has been restored (Huidu and Jescu, 1987).

Prior to the sowing, the land is harrowed using different harrows (the disc harrow). At first, the land is sowed with grass-seed or alfalfa. Generally, a second harrowing is carried out in the fourth or fifth year, depending on the pedological conditions, and then another sowing. Alternatively, a crop rotation system can be adjusted for arable lands (Huidu and Jescu, 1987).

It is necessary that double quantities of fertilizer than usual be used during the restoration period, based on the field experiments. In some cases, it is necessary that an abundance of fertilizers be used for several years after the restoration period as well.

Water treatment, active aeration and the application of fertilizers play a very important role in agricultural recultivation.

Forest recultivation can be initiated upon the leveling and harrowing of the land.

Forestation on harrowed land is usually done directly with seedlings of trees of different essences, according to the local silvo-climatic conditions and the nature of the dumps.

The seedlings are generally planted 4 meters apart.

In the case of elm and locust trees, the forest is practically restored within approximately 4 years.

If the land still requires humus activation, decay or enrichment, then cultivation starts with varieties of fast-growing and undemanding pre-plant trees (poplars, hybrids, alders, locust trees), which are then replaced with varieties of superior economic value.

Mature tree transplanting is also common.

In Rovinari Basin (Cicani pit), fruit trees (apple trees, cheery trees) have been planted both on ameliorated and nonameliorated lands, with very positive results being obtained in both cases (Huidu and Jescu, 1987).

SCOPE AND OBJECTIVES

In the present paper, we have tracked the interactions between the micro-organisms, soil and plants in the experimental versions of a technogenic soil in Rovinari area resulted from surface brown coal mining activities and subjected to biological recultivation.

In this context, the main objectives of the doctoral dissertation have been the following:

To study the abundance, diversity, dynamics and ecological significance of groups of bacteria in the technogenic soils (aerobic heterotrophic bacteria, ammonifying bacteria, denitrifying bacteria, desulfating bacteria, iron-reducing bacteria, levan synthesizing bacteria, levan lytic bacteria) with the purpose of obtaining data to establish the soil quality bacteriologic index. This is the first time this index has been calculated for this type of soil in Romania;

1) To assess the quantitative enzymatic activities (phosphatase, catalase, non-enzymatic cathalysis, actual and potential dehydrogenase, urease) and the qualitative enzymatic activities (amylase, dextranase, levanase, cellulose, inulinase);
2) To determine the soil quality bacteriologic index (BI) and enzymatic index (EI), which allow the comparison and hierarchization of the samples;
3) To analyze the relationships between the numerical density variation of the groups of bacteria in the technogenic soil in Rovinari, Gorj county and the season, depth and sampling site.
4) To analyze the relationships between the values of the quantitative enzymatic activities in the technogenic soil in Rovinari, Gorj county and the season, depth and sampling site.
5) To estimate the bioremediation of the technogenic soil in Rovinari, Gorj using biological (microbiological and enzymological) tests with the purpose of returning significant land surfaces to the agricultural circuit using biological recultivation.
6) To establish the biological potential of the technogenic soil. This is strongly related to its fertility rate. In the present case, the biological potential of the technogenic soil in Rovinari, Gorj was estimated by calculating the soil quality bacteriologic and enzymatic indices.

The doctoral dissertation is organized in 9 chapters. Part I contains a presentation of the theoretical notions and descriptions of the methods of analysis whereas Part II contains the results, the discussions on the scientific research conducted and the afferent conclusions.

The doctoral dissertation brings contributions of real scientific value on the biological potential of the technogenic soil in Rovinari, Gorj county, which is strongly related to the recultivation technology and the type of plants cultivated and is influenced by the season changes. The soil quality bacteriologic and enzymatic indices are analyzed in an attempt to emphasize the evolution of the biological potential, of the remediation and improvement of the biological characteristics of this technogenic soil resulted from surface coal mining activities.

CHAPTER 6. THE CHARACTERIZATION OF THE SOIL AND LITHOLOGICAL MATERIALS

6.1. The morphological description of the lithological openings and open pits

The soil in **Cicani pit** is a brown alluvial, poorly humified, sandy clay, non-carbonate soil with a thickness ranging between 1 and 1,5 meters. A coarse sand, gravel and rock deposit spreads down to 6 meters below the topsoil and a clay deposit 40 cm thick spreads below it. The coal is present at a depth of 640 meters (Munteanu, 1998).

6.2. The physical and hydro-physical characterization of the soil and lithological materials

In the A horizons (0-40 cm), natural soils present a sandy clay texture. In the inferior part of the profile, the texture is generally sandy or sandy clay, the content of clay ranging between 4 and 19% and the bulk density ranging between 0/82 and 1,34 g/cm^3 in relation to the granulometric content (Munteanu, 1998).

The physical and hydro-physical characteristics of natural soils and each lithological layer are positive in general, being affected only by the interference with grit and gravel layers, which impair the normal execution of agricultural work (Munteanu, 1998).

6.3. The chemical characterization of the soil and lithological materials

The reaction of the soils in the meadows and inferior terrace of the Jiu River is varied. Thus, the reaction of the soils on the inferior terrace is low acid (pH 6,3-6,8 in water) whereas the reaction of those in the meadows is neutral or low alkaline (pH 7,2) at the surface and low acid below.

Evolved natural soils generally have an average content of nutrients, a good hydro-physical regime and are suitable for crops (Munteanu, 1998).

CHAPTER 7. MATERIALS AND METHODS OF ANALYSIS

7.1. Characteristics of the work area

7.1.1. Geographical position

Over 80% of the country's brown coal reserve is concentrated in its southern part, in Rovinari, Motru, Jilţ, Albeni, Seciuri (Gorj county), Berbeşti (Vâlcea county) and Mehedinţi basins.

The most important are Rovinari and Motru-Jilţ.

Rovinari Basin is situated in the sub-Carpathian area of the Getic Depression and mainly includes the areas in the proximity of the middle stream of the Jiu River. The geological research has identified 17 layers in this basin, with only layers V-XII having any economic relevance (Munteanu, 1998).

There are two well individualized coal mining areas in Rovinari mining basin. They are:

a) ***the meadow area,*** where brown coal is extracted in Gârla, Roşia de Jiu, Peşteana Sud and Peşteana Nord pits;

b) ***the hill area,*** where brown coal is extracted in East Rovinari - Poiana Tismana I and II, Pinoasa and Urdari pits (Munteanu, 1998).

Cicani pit was localized on the left bank of the Jiu River, within the radius of Bâlteni Township. The proceedings for opening Cicani pit were initiated in 1963 and the coal excavations started in 1965. Initially, the sterile excavations were carried out using bucket excavators and auto transportation (Huidu, 2000).

The continuous excavation technology was introduced in 1967. This technology used rotor excavators, belt conveyors for the transportation of the sterile materials and coal and dumpers for their deposition.

The coal was stored in a 1,600-ton concrete silo and loaded in CFR (Romanian Railways) wagons. The machinery and means of transportation at Cicani pit were made by a German manufacturer. In April 1973, the coal mining technological flux was inverted and the coal production was routed to Rogojelu through a coal main. The pit was closed because of reserve exhaustion in 1974. The closing documentation was approved by the leaders of the Ministry of Mines, Petroleum and Geology on January 1, 1974.

The balance reserves had been exhausted completely by the time of the closing.

In the 1970's, the dump of Cicani pit was used for experimental grain cultivation. Today, it has been recultivated with trees, vines and grain, the production being comparable to that of regular lands (Huidu, 2000).

7.1.2. Main climate characteristics of Oltenia's mining area

The average air temperature ranges between 9 and 10 degrees Celsius, the winters generally being warm (-2° C in January) and the summers characterized by a limited thermal regime (21° C in July), although the absolute maximum values can reach 38-40° C. Consequently, there are 70-90 summer days per year and 20-30 tropical days. In autumn, the average temperature exceeds 11° C because of the southern circulation which is predominant, particularly in the first half of October. The same dominant atmospheric circulation lies at the basis of the positive temperatures registered at the beginning of winter in December. The maximum precipitation in a 24-hour period is among the highest in the Sub-Carpathians, as is the exceptional precipitation intensity, which reaches 5.6 mm/min/m^2 in Târgu-Jiu (Huidu, 2000).

7.1.3. Vegetation

The brown coal deposits in Oltenia are located in the forest vegetation region of the mixed sessile oak and beech floor and that of oak forests. The vegetation regions overlap, with oak forests penetrating the beech and sessile oak ones, particularly along the river valleys (Munteanu, 1998).

The lands unaffected by mining activities in the surface coal mining area in the Jiu's meadows are occupied by annual crops: wheat, corn, barley, rye, potatoes.

The gentle slopes are occupied by annual plants, fruit trees and vine. Some meadow and hill areas are occupied by hayfields and grasslands.

The coal deposits in Rovinari are situated in the forest area, namely on the sessile oak floor, but the valleys are occupied by *Fagus silvatica* (Munteanu, 1998).

7.2. Soil sampling

The present paper focuses on the technogenic soil around Cicani pit in Rovinari, Gorj county resulted from surface brown coal mining activities. Cicani pit was localized on the left bank of the Jiu River, within the radius of Bâlteni Township. The proceedings for its opening were initiated in 1963 and the coal excavations started in 1965. Mining operations were run between 1965 and 1974. The pit was closed because of reserve exhaustion in 1974. In the 1970's, the dump of Cicani pit was used for experimental grain cultivation. Today, it has been recultivated with trees, vine and grain, the production being comparable to that of regular lands. The evolution of the soil, which reflects the biological recultivation efficiency, was studied using microbiological and enzymological methods.

The experiments were conducted between 2002 and 2006.

Analyses were conducted on seasonal soil samples collected from seven experimental versions at depths of 5-15 and 20-40 cm respectively. Each soil sample was introduced in Erlenmeyer flasks sealed with cotton wool wads. Both the Erlenmeyer flasks and the cotton wool wads had been sterilized in advance. A label containing the sampling site, date and depth was attached on each of the flasks. The tested soils were the following:

I - soil cultivated with fruit trees (apple trees);

II - soil cultivated with alfalfa;

III - soil cultivated with hazelnut and birch trees;

IV - soil cultivated with fruit trees (plum trees);

V - soil cultivated with young vines;

VI - soil cultivated with bearing vines (over 20 years old);

VII - unfertilized (meadow) soil collected from a nearby area unaffected by mining activities.

All experimental versions (I-VI) were fertilized with farmyard manure. All the soil samples were transferred to the Microbiology laboratory within Babeş-Bolyai University, Cluj-Napoca and preserved in the refrigerator at a temperature of 4° C until processed.

7.3. Methods of analysis

Bacteria from different eco-physiological groups were determined using the methods of analysis: aerobic heterotrophic bacteria, ammonifying bacteria, denitrifying bacteria, desulfating bacteria, iron-reducing bacteria, levan synthesizing bacteria, levan lytic bacteria.

7.4. Enzymatic methods

The following quantitative enzymatic activities were determined in the seven soil samples: phosphatase (PA), catalase (CA), non-enzymatic cathalysis (NCA), actual dehydrogenase (ADA), potential dehydrogenase (PDA) and urease.

In addition, the following five qualitative polyase activities were determined: amylase (AA), dextranase (DA), levanase (LA), cellulose (CA) and inulinase (IA).

CHAPTER 9. RESULTS AND DISCUSSIONS

9.1. Research on the ecological groups of bacteria

The results of the bacteriological analyses conducted on the technogenic soil in Rovinari, Gorj county in 2002-2006 indicated that the bacteria in the 7 physiological groups were identifiable in almost all the experimental versions studied. This proves that the soil microbiota is complex and the microbiological potential of the technogenic soil in Rovinari is recovering and evolving toward that of the unfertilized soil (unaffected by mining activities). As expected, the aerobic heterotrophic bacteria are best represented, being present in all versions. These bacteria are responsible with the mineralization of organic substances and influenced by the amount of vegetal waste introduced in the soil, respectively the organic fertilization using farmyard manure (Drăgan and Kiss, 1986).

The yearly analysis indicates a general increase in the values between 2002 and 2006, which indicates the evolution of the technogenic soil toward a fertile soil.

The seasonal analysis indicates that the number of bacteria is high in spring and autumn, slightly lower in summer and low in winter. However, a significant increase in the number of bacteria was observed between the winters of 2002 and 2006.

As far as the depth analysis is concerned, the 5-15 cm deep layer contains a larger number of bacteria than the 20-40 cm deep layer.

As expected, the highest values – for all groups of bacteria – corresponded to the unfertilized soil, regardless of the year or depth, because it was unaffected by mining activities. The significant difference between the values associated with the unfertilized soil and those associated with other technogenic soil versions indicates that the soil in Rovinari still has a relatively low microbiological potential. However, it can evolve into a fertile agricultural soil if proper agro-chemical and agro-technical methods are used.

9.2. The determination of the soil quality bacteriologic index (BI)

The biological potential of the soil samples was estimated by calculating the soil quality bacteriologic index. The soil quality bacteriologic index, which was calculated for the first time for a technogenic soil, indicates that this is an important parameter characterizing the bacterial population of the soil and allows a better tracking of its bioremediation by comparison with the unfertilized soil.

The 7 eco-physiological groups of bacteria were used to estimate the quality of the technogenic soil in Rovinari, Gorj county or the BI.

The soil quality bacteriologic index (BI) was calculated based on the absolute values of the number of bacteria (per 10 g of soil) according to the following formula:

$$BI=\frac{1}{n}\sum \log_{10} N$$

with:

n = number of physiological groups

N = number of bacteria in each physiological group (Muntean, 1995-1996).

The yearly analysis indicated a general increase in the BI values between 2002 and 2006, which suggests the evolution of the technogenic soil toward a fertile soil.

The seasonal analysis indicated that the BI values were high in spring and autumn and slightly lower in summer. A slight decrease in the BI values could also be observed in winter all throughout 2002-2006.

As far as the depth analysis is concerned, the BI values were higher at depths of 5-15 cm than they were at depths of 20-40 cm.

The highest values corresponded to the unfertilized soil, which was unaffected by mining activities, regardless of the season or depth. Also, there was a slight increase in the BI values of the unfertilized soil between 2002 and 2006.

The BI values vary depending on both the sampling site and the season.

The data obtained indicates that the soil quality bacteriologic index is an important parameter characterizing the richness of the bacterial population of the soil, there being significant differences between the unfertilized soil version and the technogenic soil versions.

Fig.6 The seasonal variation of the soil quality bacteriologic index (BI) of the technogenic soil in Rovinari, Gorj county at depths of 20-40 cm in 2002

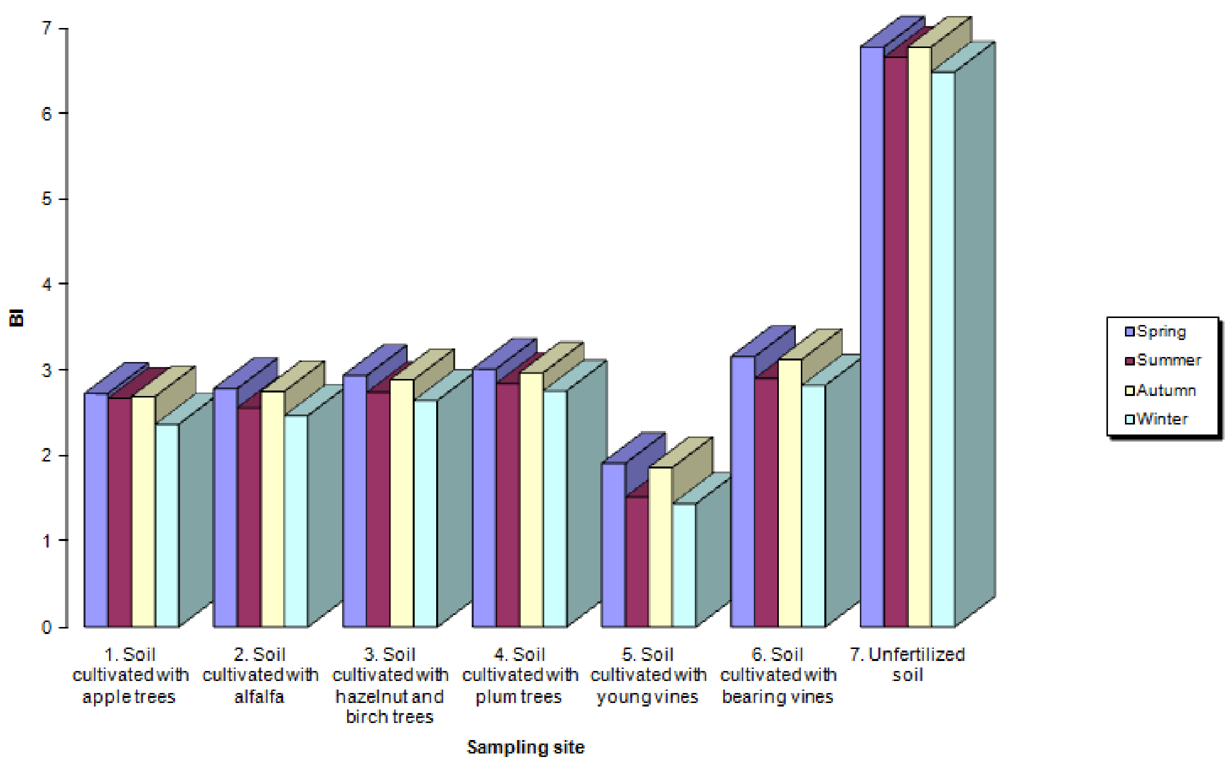
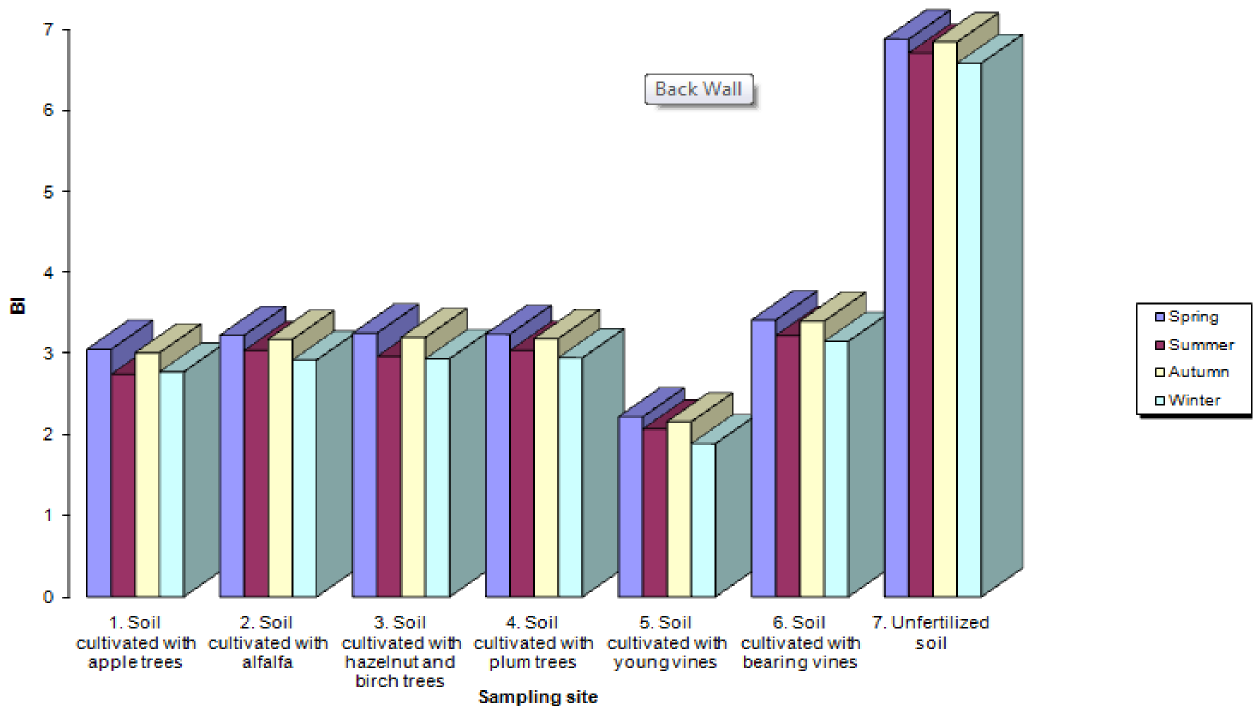


Fig.14 The seasonal variation of the soil quality bacteriologic index (BI) of the technogenic soil in Rovinari, Gorj county at depths of 20-40 cm in 2006



9.3. The analysis of the enzymatic activity in the technogenic soil in Rovinari, Gorj county

9.3.1. The quantitative enzymatic activity

9.3.1.1. The phosphatase activity

Phosphatase activity was identified in all soil samples tested. In the main, it was higher in 2006 than in 2002-2005, the tests indicating elevated numerical values. The seasonal analysis indicated that the phosphatase activity fluctuated, maximum values being registered in spring and sometimes in autumn. The maximum value registered in spring was due to the application of manure whereas the elevated values sometimes registered in autumn were due to the accumulation of organic waste at the end of the vegetation period. These results are accountable as the biological activity is much higher in the warm season. The phosphatase activity was lower in summer and relatively low in winter all throughout 2002-2006. The maximum values were associated with the unfertilized soil, which was unaffected by mining activities. The phosphatase activity was higher in the soil collected from depths of 5-15 cm than in the soil collected from depths of 20-40 cm.

Fig. 15 The evolution of the phosphatase activity in the technogenic soil in Rovinari, Gorj county at depths of 5-15 cm in 2006

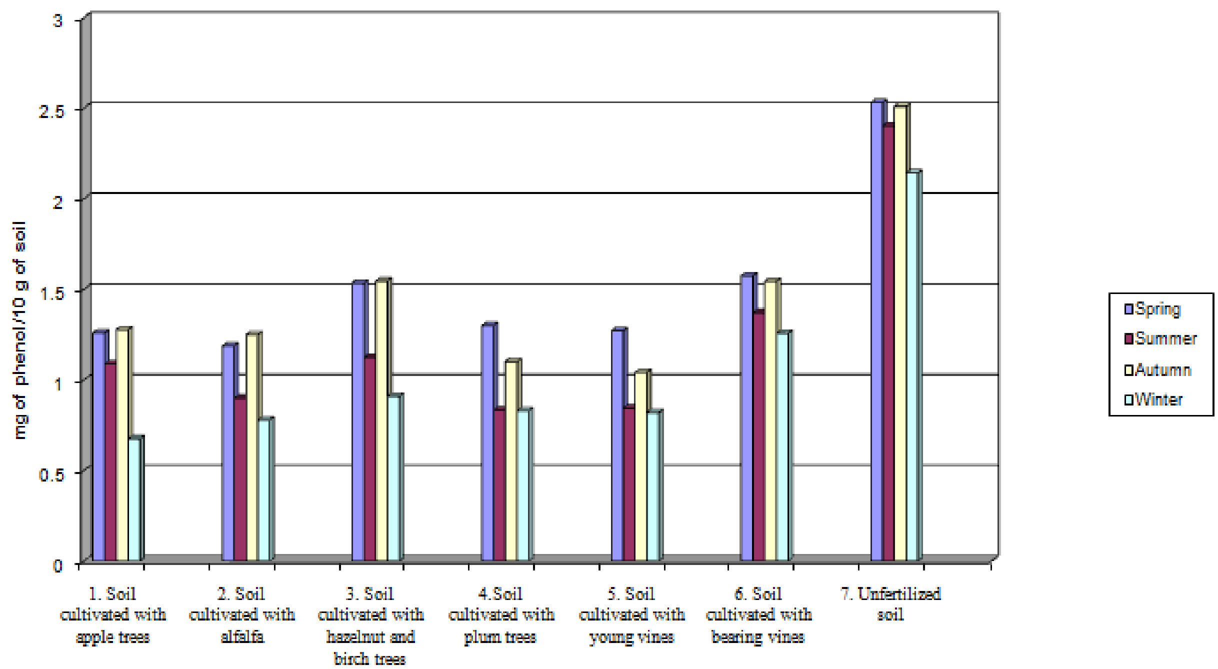
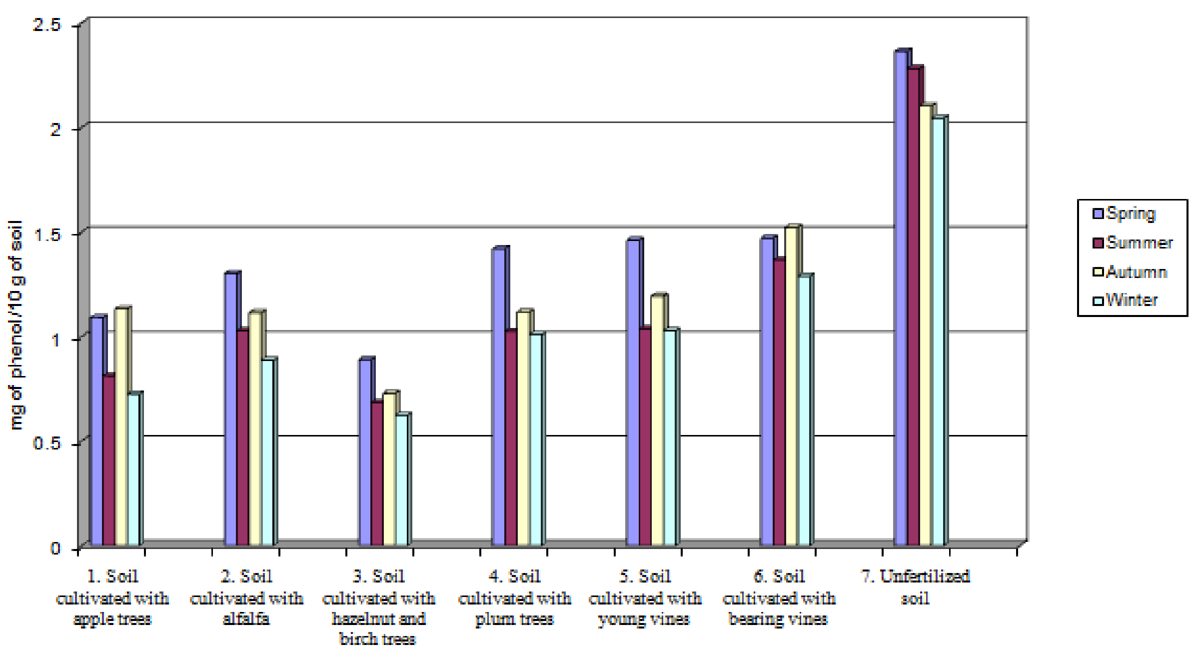


Fig. 24 The evolution of the phosphatase activity in the technogenic soil in Rovinari, Gorj county at depths of 20-40 cm in 2006



9.3.1.2. The catalase and non-enzymatic cathalysis activities

In the main, the catalase and non-enzymatic cathalysis activities were higher in 2006 than in 2002-2005, the tests indicating elevated numerical values. In general, the non-enzymatic H_2O_2 splitting activity was low, but higher than the catalase activity. The higher values associated with the non-enzymatic cathalysis activity could be due to the presence of highly reduced substances (sulphites, iron oxides). The seasonal analysis indicated that both the catalase and the non-enzymatic cathalysis activities fluctuated, maximum values generally being registered in spring and only rarely in autumn all throughout 2002-2006. They were lower in summer and relatively low in winter all throughout 2002-2006. The analysis revealed that the catalase activity was higher in the unfertilized soil than in the other experimental versions all throughout 2002-2006. The same situation presented itself in the case of the non-enzymatic cathalysis activity. Both activities were the highest in the soil collected from depths of 5-15 cm all throughout 2002-2006.

Fig. 25 The evolution of the catalase activity in the technogenic soil in Rovinari, Gorj county at depths of 5-15 cm in 2002

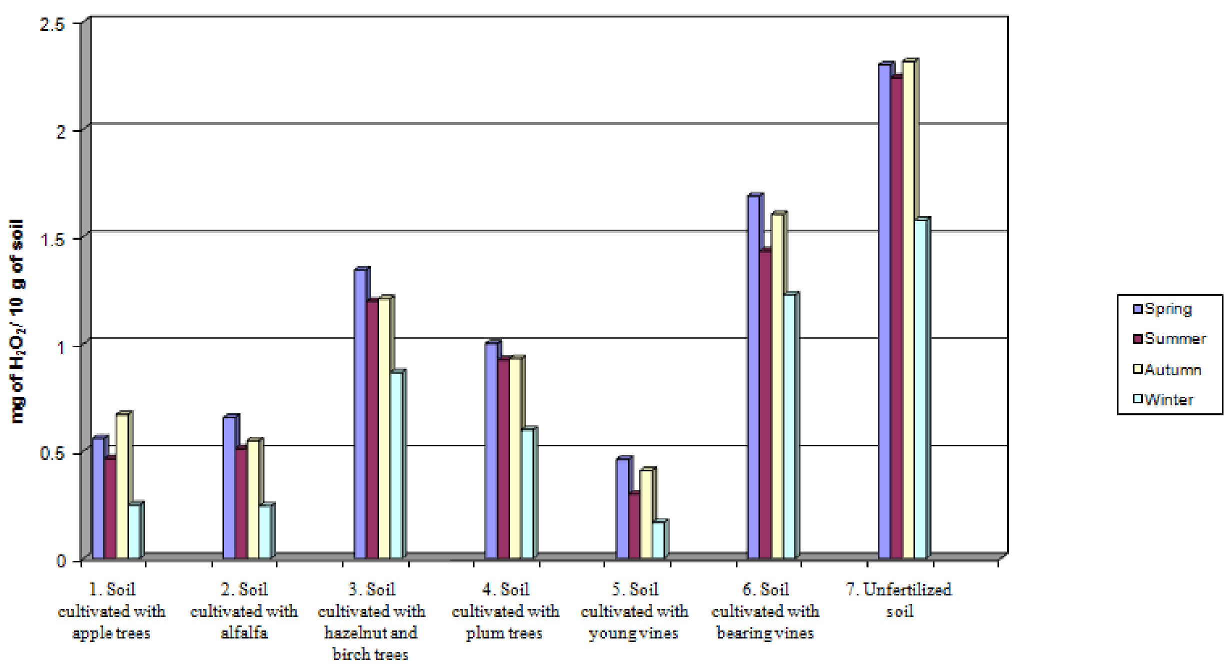
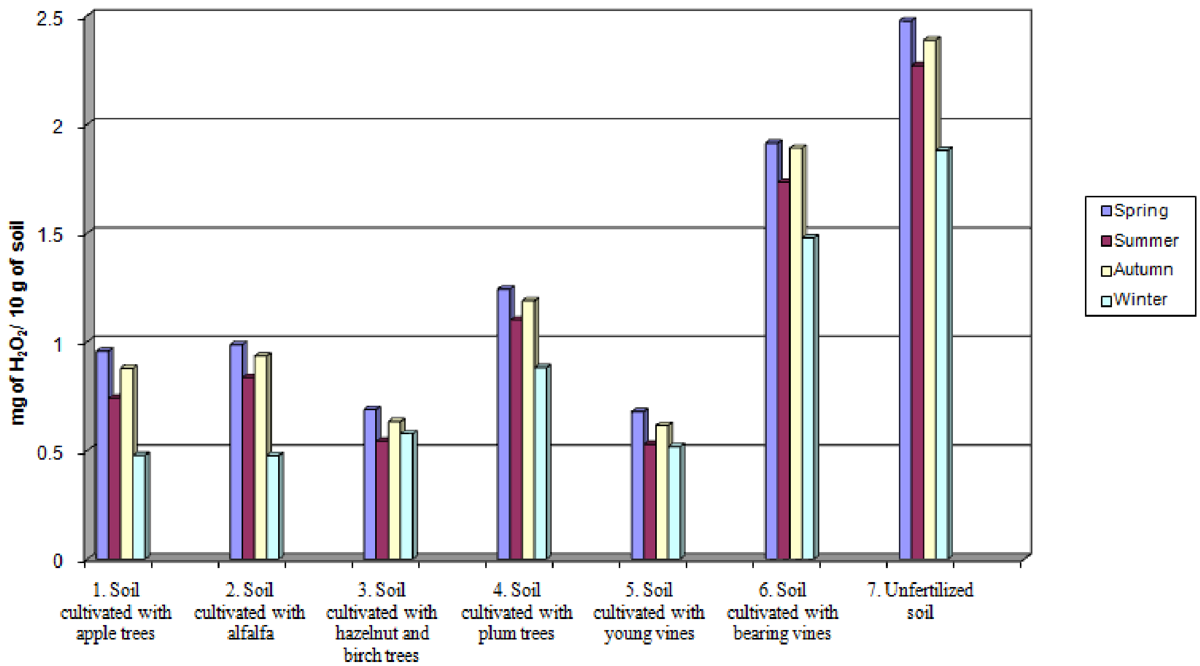


Fig.44 The evolution of the non-enzymatic cathalysis activity in the technogenic soil in Rovinari, Gorj county at depths of 20-40 cm in 2006



9.3.1.2. The actual and potential dehydrogenase activities

In the main, both the actual and potential dehydrogenase activities were higher in 2006 than in 2002-2005, the tests indicating elevated numerical values both on the lower and upper limits. In general, the potential dehydrogenase activity was lower than the actual one. This revealed the stimulant action of the easily assimilable carbon source on the enzyme synthesis carried out by micro-organisms. The differences were not very significant, which indicates a relatively high microbial respiratory activity in the tested soils even in the absence of a supplementary infusion of nutrients, respectively hydrogen donors (Muntean and collab., 2001). The high values associated with the actual dehydrogenase activity prove the existence of a high microbial potential in the technogenic soil in Rovinari. The seasonal analysis indicated that both the actual and the potential dehydrogenase activities fluctuated, maximum values generally being registered in autumn and only rarely in spring all throughout 2002-2006. They were lower in summer and relatively low in winter all throughout 2002-2006. The actual dehydrogenase activity was the highest in the unfertilized soil as compared to the other experimental versions all throughout 2002-2006. The same situation presented itself in the case of the potential dehydrogenase activity. Both activities were the highest in the soil collected from depths of 5-15 cm all throughout 2002-2006.

Fig.58 The evolution of the actual dehydrogenase activity in the technogenic soil in Rovinari, Gorj county at depths of 20-40 cm in 2006

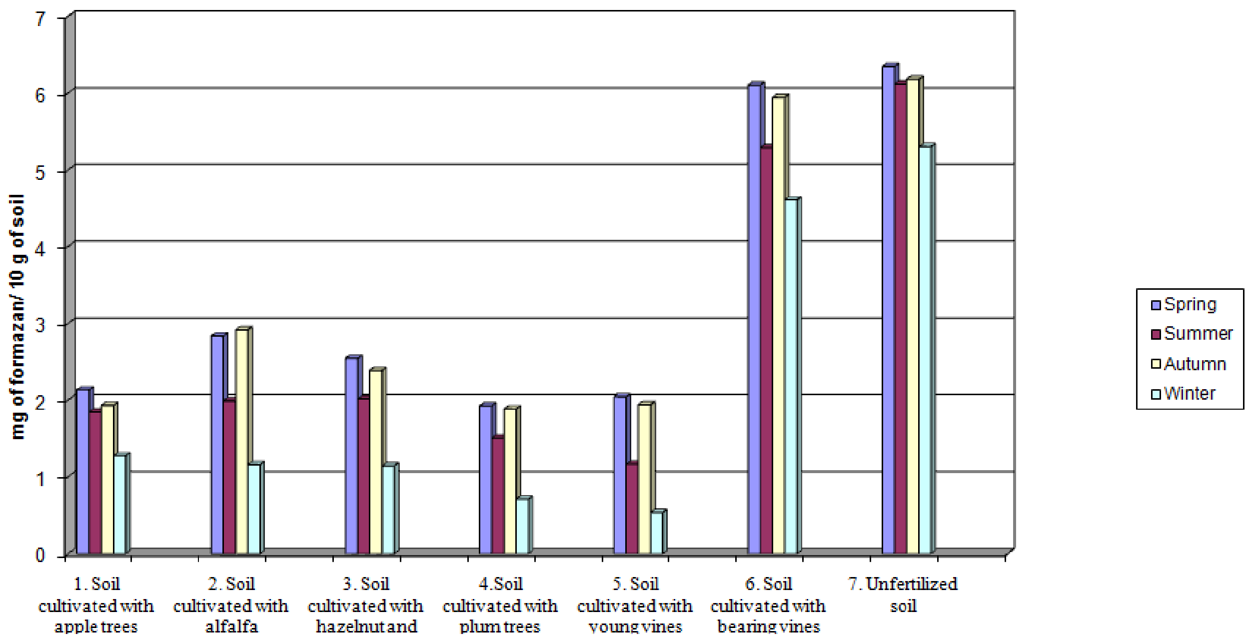
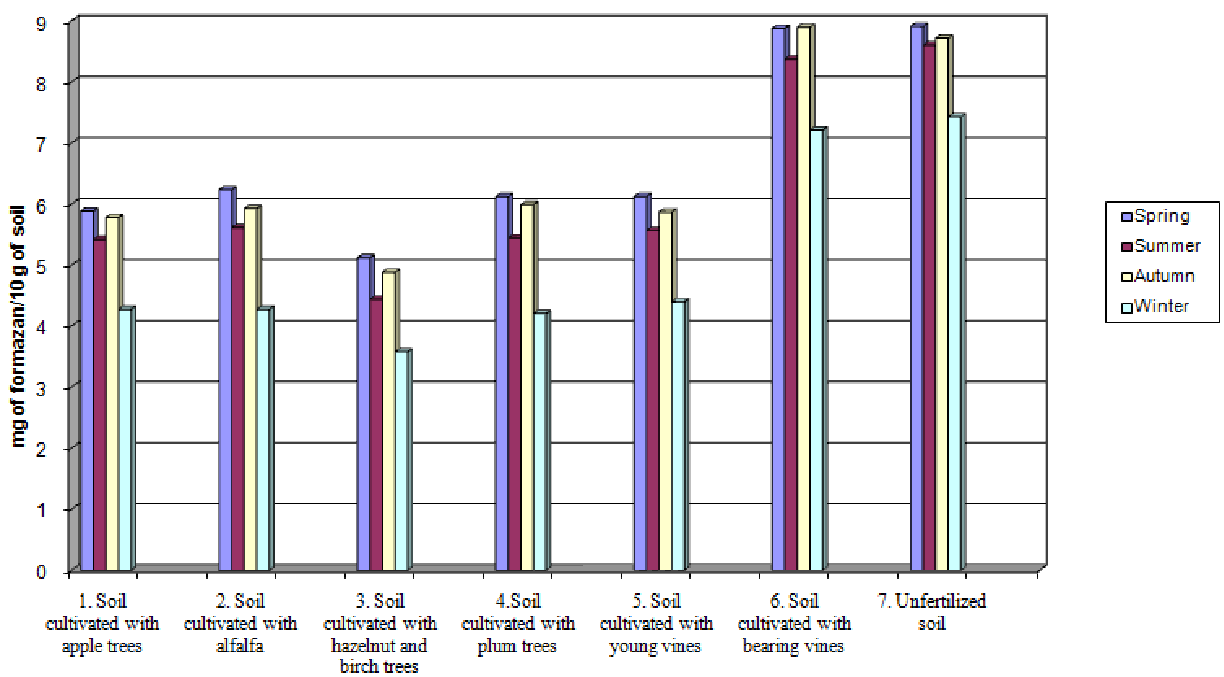


Fig.64 The evolution of the potential dehydrogenase activity in the technogenic soil in Rovinari, Gorj county at depths of 20-40 cm in 2006



9.3.1.4. The urease activity

The urease activity was higher in 2006 than in 2002-2005, the tests indicating elevated numerical values. The seasonal analysis indicated that the urease activity fluctuated, maximum values being registered in spring and sometimes in autumn all throughout 2002-2006. The maximum value registered in spring was due to the application of manure whereas the elevated values sometimes registered in autumn were due to the accumulation of organic waste at the end of the vegetation period. The urease activity was lower in summer and relatively low in winter all throughout 2002-2006. These results are accountable as the biological activity is very low in winter. In 2002-2006, the maximum values associated with the urease activity corresponded to the unfertilized soil, which was unaffected by mining activities. The urease activity was higher in the soil collected from depths of 5-15 cm than in the soil collected from depths of 20-40 cm all throughout 2002-2006.

Fig.69 The evolution of the urease activity in the technogenic soil in Rovinari, Gorj county at depths of 5-15 cm in 2004

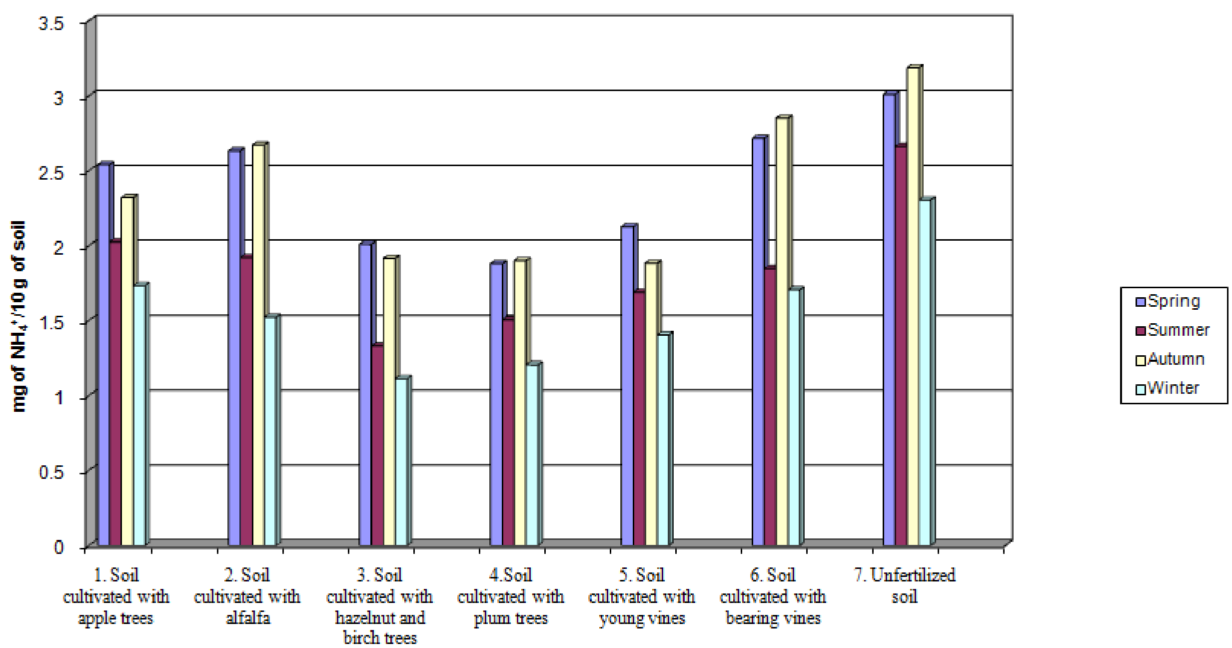
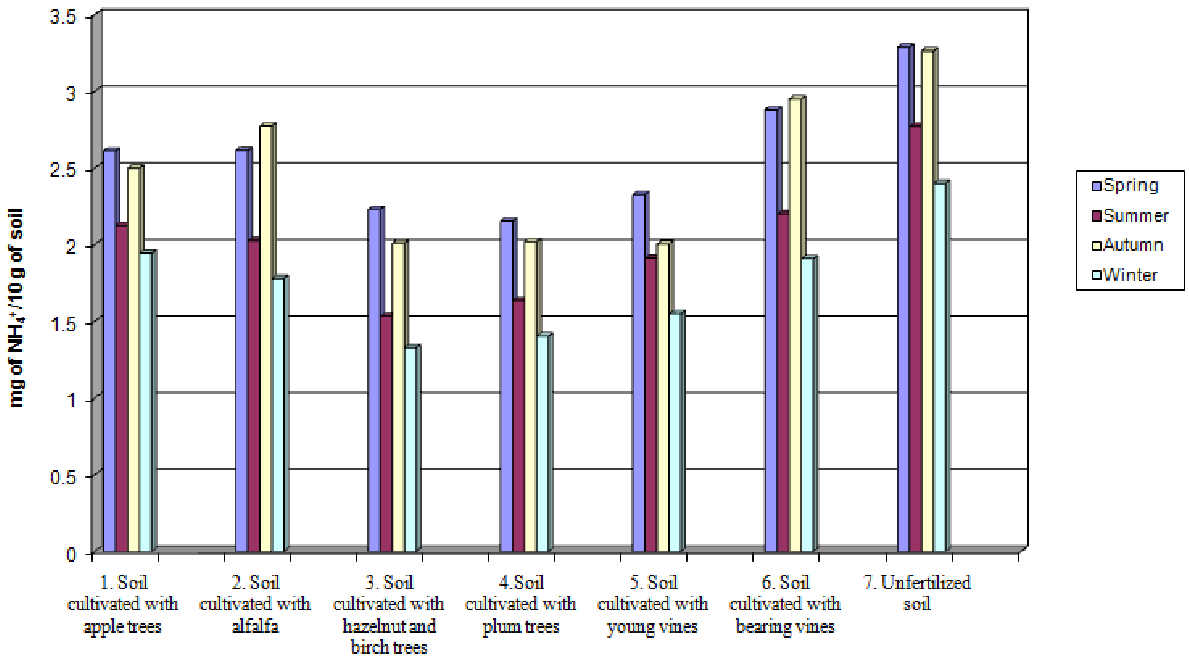


Fig.70 The evolution of the urease activity in the technogenic soil in Rovinari, Gorj county at depths of 5-15 cm in 2005



9.3.1.5 The soil quality enzymatic index (EI)

No such research has been conducted on the technogenic soils in Rovinari, Gorj county, so our mission was to follow the values of the soil quality enzymatic index of the technogenic soil here with the purpose of estimating the soil quality.

On an enzymological level, the quality of the technogenic soil is characterized by the intensity of the enzymatic activities as defined by the quality enzymatic index.

The enzymatic potential of the technogenic soil increases in direct relation to the enzymatic index. The soil quality enzymatic index (EI) offers a perspective over the enzymatic potential of the soil and is determined according to the following formula elaborated by Muntean and collaborators in 1996:

$$EI = 1/n \sum V_r(i)/V_{max}(i)$$

with:

EI = soil quality enzymatic index

n = number of activities

$V_r(i)$ = individual real value

$V_{max}(i)$ = individual maximum theoretical value

In theory, the enzymatic index can vary between 0 (in case there is no activity in the samples) and 1 (in case the individual real values and the individual maximum theoretical values associated with all activities are equal).

The values of the soil quality enzymatic index were maximum in spring and autumn, lower in summer and low in winter. A significant increase in the soil quality enzymatic index was observed between 2002 and 2006.

Fig.76 The seasonal variation of the soil quality enzymatic index (EI) of the technogenic soil in Rovinari, Gorj county at depths of 20-40 cm in 2002

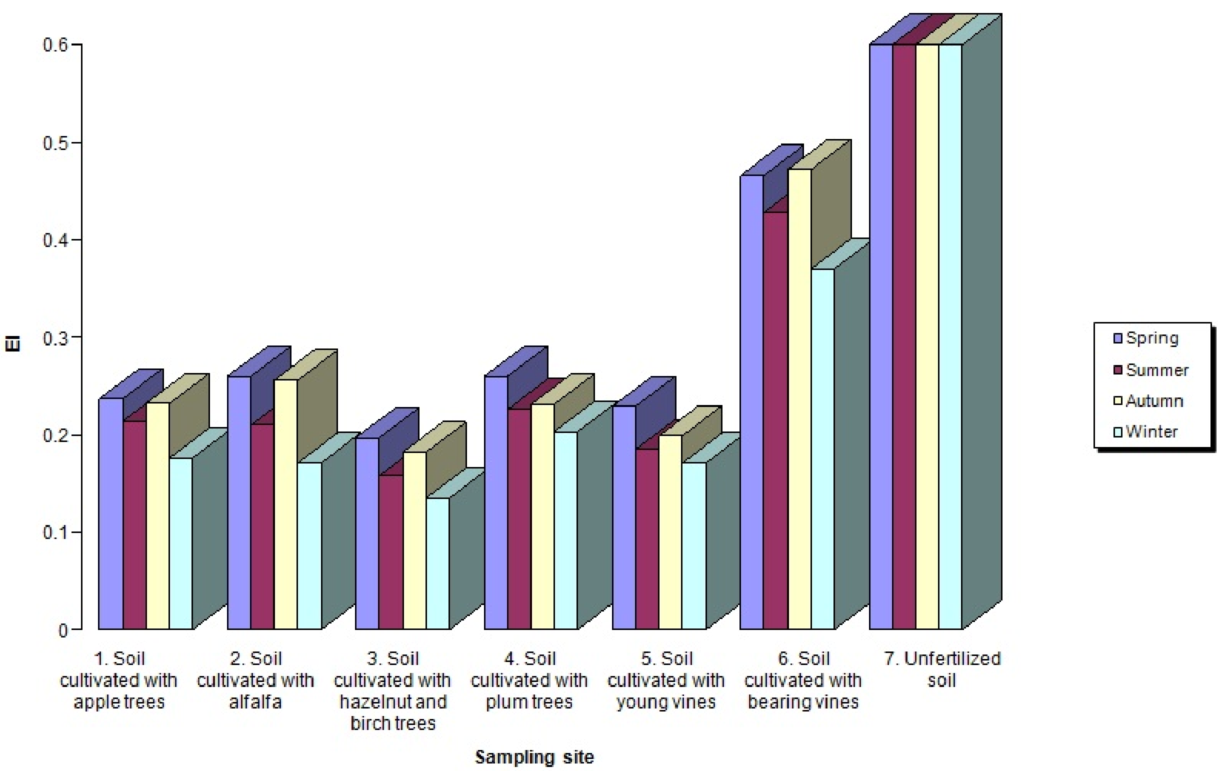
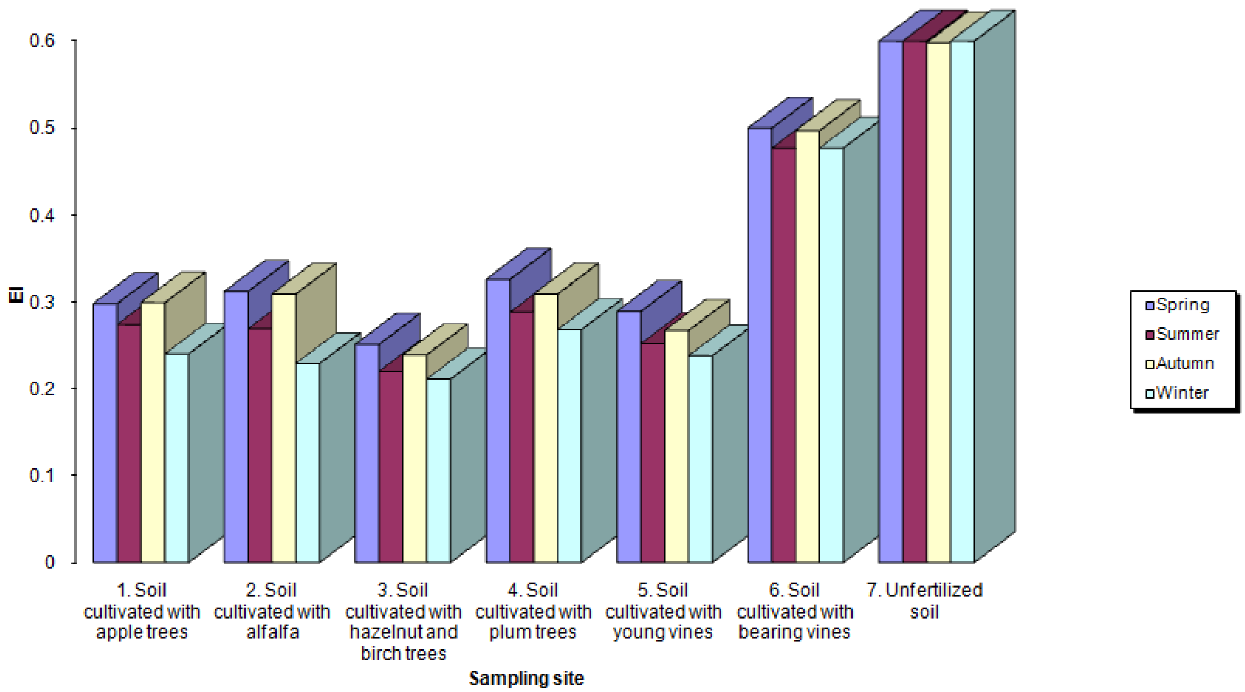


Fig. 84 The seasonal variation of the soil quality enzymatic index (EI) of the technogenic soil in Rovinari, Gorj county at depths of 20-40 cm in 2006



9.3.2. The qualitative enzymatic activity

Five qualitative polyase activities were determined in the seven soil samples: amylase (AA), dextranase (DA), levanase (LA), cellulose (CA) and inulinase (IA). The reducing hydrolysis products are highlighted using the paper chromatography method. The polyase activity increases in direct relation to the hydrolysis spot intensity. The assessment includes comparison between the spots corresponding to the experimental samples and the spots corresponding to the unfertilized ones. The intensity of the enzymological analyses as determined based on the color spots was marked with "+".

The qualitative enzymatic (polyase) activities were poorly represented. The analyses indicated that the highest activity levels were registered in spring and autumn. The intensity of the activities was lower in summer and some of them were non-existent in winter (LA, CA, IA). Concurrently, the tests indicated a variation of the intensity of the enzymatic activity according to depth, the maximum values being registered at depths of 5-15 cm.

***Table 64*. The evolution of the qualitative enzymatic (polyase) activities in the technogenic soil in Rovinari, Gorj county at depths of 5-15 cm in 2002**

Sampling time	Enzymatic activity	Soil cultivated with apple trees	Soil cultivated with alfalfa	Soil cultivated with hazelnut and birch trees	Soil cultivated with plum trees	Soil cultivated with young vines	Soil cultivated with bearing vines	Unfertilized soil
Spring	AA	+ + +	+ +	+ +	+ +	+ +	+ +	-
	DA	+	+ +	+	+/-	+/-	+	+
	LA	+/-	-	-	+/-	-	+	+
	CA	-	-	-	-	-	+/-	+
	IA	+ +	+	+	+	+	+ +	+
Summer	AA	+	+ +	+	+/-	+/-	+ +	-
	DA	+/-	+	+/-	+/-	+/-	+	+
	LA	+/-	-	-	-	-	+/-	-
	CA	-	-	-	-	-	+/-	+
	IA	+	+/-	+/-	+	+/-	+	+
Autumn	AA	+ +	+	+	+	+	+ +	-
	DA	+	+	+/-	+/-	+/-	+	+/-
	LA	-	-	-	+/-	-	+	-
	CA	-	+/-	-	-	-	+/-	+/-
	IA	+	+ +	+	+	+	+ +	+/-
Winter	AA	+/-	+/-	+/-	+/-	+/-	+	-
	DA	+/-	-	-	-	-	+/-	+/-
	LA	-	-	-	-	-	-	-
	CA	-	-	-	-	-	-	+/-
	IA	-	-	-	-	-	+/-	-

***Table 72*. The evolution of the qualitative enzymatic (polyase) activities in the technogenic soil in Rovinari, Gorj county at depths of 5-15 cm in 2006**

Sampling time	Enzymatic activity	Soil cultivated with apple trees	Soil cultivated with alfalfa	Soil cultivated with hazelnut and birch trees	Soil cultivated with plum trees	Soil cultivated with young vines	Soil cultivated with bearing vines	Unfertilized soil
Spring	AA	+ + + + +	+ + +	+ +	+ + +	+ +	+ + +	-
	DA	+	+	+	+ +	+	+	+
	LA	-	+/-	-	+/-	-	+	+
	CA	-	+/-	-	+/-	-	+/-	+
	IA	+ + +	+ +	+ +	+ + +	+ +	+ + + + +	+
Summer	AA	+ +	+ +	+ +	+ +	+	+ +	-
	DA	+	+	+	+	+	+	+
	LA	-	-	-	-	-	+/-	+
	CA	-	-	-	-	-	+/-	+
	IA	+ +	+	+	+	+	+ + +	+
Autumn	AA	+ + +	+ + +	+ +	+ +	+ +	+ + +	-
	DA	+	+ +	+	+ +	+	+	+
	LA	-	-	-	+/-	-	+	+
	CA	-	+/-	-	-	-	+/-	+
	IA	+ + +	+ +	+ +	+ +	+ +	+ + + +	+
Winter	AA	+/-	+/-	+/-	+/-	+/-	+	-
	DA	-	+/-	-	-	-	+/-	+/-
	LA	-	-	-	-	-	-	+/-
	CA	-	-	-	-	-	-	+/-
	IA	-	-	-	-	-	+/-	-

CONCLUSIONS

1. We have tracked the interactions between the micro-organisms, soil and plants in the experimental versions of the technogenic soil in Rovinari, Gorj county resulted from surface brown coal mining activities and subjected to biological recultivation. Soil samples from seven experimental versions were collected seasonally and tested: I - soil cultivated with fruit trees (apple trees); II - soil cultivated with alfalfa; III - soil cultivated with hazelnut and birch trees; IV - soil cultivated with fruit trees (plum trees); V - soil cultivated with young vines; VI - soil cultivated with bearing vines (over 20 years old); VII - unfertilized (meadow) soil collected from a nearby area unaffected by mining activities.

2. The results of the analyses indicate that bacteria from all seven groups (aerobic heterotrophic bacteria, ammonifying bacteria, denitrifying bacteria, desulfating bacteria, iron-reducing bacteria, levan synthesizing bacteria, levan lytic bacteria) were present in almost all the experimental versions studied. This proves that the soil microbiota is complex and the microbiological potential of the technogenic soil in Rovinari is recovering and evolving toward that of the unfertilized soil (unaffected by mining activities).

3. The bacteria in the technogenic soil in Rovinari, Gorj county presented an evident fluctuation in the numerical density according to the nature of the cultivated plants, season, depth and sampling site.

4. The large presence of bacteria in the unfertilized soil version is explained by its provenance from a grassland area unaffected by mining activities with high biological activity. The aerobic heterotrophic bacteria were the most common in all soil samples tested. They are responsible with the mineralization of organic substances and influenced by the amount of vegetal waste introduced in the soil, respectively the organic fertilization using farmyard manure.

5. The tests revealed that the number of bacteria was higher in spring and autumn, slightly lower in summer and low in winter with all soil versions.

6. The unfertilized soil, which was unaffected by mining activities, presented the highest values of the soil quality bacteriologic index (BI) regardless of the season or depth.

7. Six enzymatic activities were identified in the soil samples collected seasonally to calculate the soil quality enzymatic index (EI). They were the following: phosphatase, catalase, non-enzymatic cathalysis, actual and potential hydrogenase and urease.

8. Quantitative enzymatic activities (phosphatase, catalase, non-enzymatic cathalysis, actual and potential dehydrogenase, urease) were detected in all soil samples with the values increasing between 2002 and 2006.

9. The absolute values associated with these quantitative enzymatic activities fluctuated according to the sampling site, season and depth. The highest values corresponded to the unfertilized soil, which was unaffected by mining activities. The seasonal analysis indicated that maximum values were registered in spring and sometimes in autumn. The intensity of the activities was higher in the soil collected from depths of 5-15 cm than in the soil collected from depths of 20-40 cm.

10. The values of the soil quality enzymatic index were maximum in spring and autumn, lower in summer and low in winter. A significant increase in the soil quality enzymatic index was observed between 2002 and 2006.

11. The qualitative enzymatic (polyase) activity – amylase, dextranase, levanase, cellulose and inulinase –, allows a more complex appreciation of the general enzymatic potential of the technogenic soil in Rovinari. The amylase, dextranase and inulinase activities were well represented whereas the levanase and cellulose activities were poorly represented or even undetectable in winter. The intensity of the activities was higher particularly in spring and autumn.

12. The enzymatic potential of the soil quality allowed a differentiation being made between the technogenic soil and the unfertilized one (unaffected by mining activities). Thus, it has been estimated that technogenic soil is evolving into an agricultural soil as a result of the agro-chemical and agro-technical methods used.

13. The soil constitutes an irreplaceable source of energy for live forms and the speed at which soils degrade as a result of industrial activities being performed is higher than the speed at which natural balance is restored. Therefore, there is a stringent need to recover the degraded lands which can lead to the restoration of the natural balance and the formation of new ecologically balanced landscapes compliant with the demands of society in the opencast coal mining areas.

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