

ISSN 1821-1046

UDK 630

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SUSTAINABLE FORESTRY ODRŽIVO ŠUMARSTVO

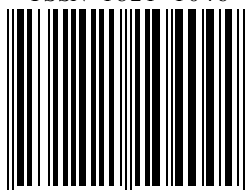
COLLECTION
TOM 63-64

ZBORNİK RADOVA
TOM 63-64



BELGRADE BEOGRAD
2011.

ISSN 1821-1046



9 771821 104000

ISSN 1821-1046
UDK 630

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PROCEEDINGS

ZBORNIK RADOVA

Publisher

Institute of Forestry
Belgrade, Serbia

Izdavač

Institut za šumarstvo
Beograd, Srbija

For Publisher

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Printed in

150 copies

Tiraž

150 primeraka

Printed by

Klik tim DOO
Beograd

Štampa

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Beograd

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Belgrade, 2011

Preuzimanje članaka ili pojedinih delova ove publikacije u bilo kom obliku nije dozvoljeno bez odobrenja

Beograd, 2011

Cover Page: Author of the Photos Tatjana Ćirković-Mitrović, M.Sc.
Naslovna strana: Autor fotografije mr Tatjana Ćirković-Mitrović

CIP – Каталогизација у публикацији
Народна библиотека Србије, Београд

630

SUSTAINABLE Forestry : collection =
Održivo šumarstvo = zbornik radova / chief
editor = glavni i odgovorni urednik Snežana
Rajković. - 2008, T. 57/58- . - Belgrade
(Kneza Višeslava 3) : Institute of forestry,
2008- (Beograd : Klik tim). - 24 cm

Godišnje. - Je nastavak: Zbornik radova -
Institut za šumarstvo = ISSN 0354-1894
ISSN 1821-1046 = Sustainable Forestry
COBISS.SR-ID 157148172

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UDK 630*114.1/.2+630*114.6=111
Original scientific paper

SOIL EXAMINATION FOR THE PURPOSE OF FORECASTING OCCURRENCE OF ENTOMOPATHOGENIC AND BENEFICIAL MICROORGANISMS

Vesna GOLUBOVIĆ ĆURGUZ¹, Zoran MILETIĆ²

Abstract: *In two sample plots, located at the site of Hungairan Oak and Turkey Oak forest (*Quercetum frainetto cerris*) and at the site of montane beech forest (*Fagetum montanum*), in which a mass natural perishing of gypsy moth larvae occurred in spring of 2011, the principal characteristics of soil – a natural development environment for certain stadia of possible entomopathogenic and other microorganisms, and a potential cause for the collapse, were analysed. Physical and chemical properties, as well as the abundance of principal physiological groups of soil microorganisms, were examined. The existence of similarity in soil physical properties was revealed at both sites, whereas the differences between chemical properties were far more significantly pronounced. The differences in chemical properties of the soil and the organic layer resulted in manifestation of differences with respect to abundance and relations among principal physiological groups of soil microorganisms.*

Key words: Soil physical properties, soil chemical properties, soil microorganism abundance

PREGLED ZEMLJIŠTA U FUNKCIJI PROGNOZE POJAVE ENTOMOPATOGENIH I KORISNIH MIKROORGANIZAMA

Izvod: *Na dva ogledna polja na staništu sladuna i cera (*Quercetum frainetto cerris*) i staništu brdske bukove šume (*Fagetum montanum*), gde je konstatovan masovan pomor gubara, a da nije bilo tretiranja nikakvim hemijskim i biološkim preparatima*

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Translation: Dejan Arsenovski

analizirano je zemljište. Ispitane su fizičke i hemijske osobine kao i brojnost osnovnih fizioloških grupa zemljišnih mikroorganizama. Konstatovano je da postoji slinost u fizičkim svojstvima zemljišta na oba lokaliteta, a da su razlike u hemijskim svojstvima značajnije izražene. Razlike u hemijskim svojstvima zemljišta i organske prostirke su rezultirale ispoljavanjem razlika u brojnosti i odnosima osnovnih fizioloških grupa zemljišnih mikroorganizama.

Ključne reči: Fizička svojstva zemljišta, hemijska osobine zemljišta, brojnost zemljišnih mikroorganizama.

1. INTRODUCTION

A large number of beneficial entomopathogenic microorganisms spend one period of their development cycle in soil. Soil properties (soil solution reaction, nature of organic matter, presence of mineral plant assimilatives, humidity, aeration and oxidisability of pedochemical environment, as well as thermal conditions), to a large extent determine the species of soil microorganism that inhabit the soil, as well as their activity. Allelopathic relations of symbiosis and antagonism are established within a microorganism community that inhabits one pedochemical environment. That is performed through creation of matters by means of which microorganisms act upon each other, such as antibiotics, fungistatics, etc. The survival of entomopathogenic and other beneficial microorganisms, which spend only a part of their development cycle in soil, depends on such relations established within a soil microorganism community, as well as on physical and chemical properties of the environment in which they are active.

2. WORK METHODS

Sample plots were established at two sites in which a mass perishing of gypsy moth was observed. The first sample plot was established in the Hungarian Oak and Turkey Oak forest in the area of Barajevo, while the other was set up in a montane beech forest at Kosmaj. Pedological profiles were created in sample plots, the type of soil was determined and the samples, according to pedogenetic horizons, were taken for laboratory analysis.

The analysed soil properties are the following:

- Active and substitution acidity (pH u H₂O i pH u KCl), - potentiometrically ,
- Hidrolytic acidity (Y1) and the sum of adsorbed base cations (S), by Kappenn's method (Živković 1966). Based on those values, the unsaturation of adsorptive complex (T-S), total capacity of adsorption (T) and the degree of adsorptive complex saturation by base cations (V) were calculated, according to the following formulae:

$$T-S = 0.65 \cdot Y1 \quad [\text{ekv. mmol/100 g soil}]$$

$$T = S + (T-S) \quad [\text{ekv. mmol/100 g soil}]$$

$$V = \frac{S}{T} \cdot 100 \quad [\%]$$

- The total humus content was determined by wet combustion in the potassium dichromate ($K_2Cr_2O_7$) and sulphuric acid (H_2SO_4) compound, by Tjurin method (Škorić, Racz 1966).
- The total nitrogen content, by Kjeldahl method (Džamić 1966)
- The content of plant accessible forms of phosphorus and potassium, using AL-method by Egner-Richm (Džamić et al 1996); whereby phosphorus was determined by a colorimetric method and potassium by a flame-photometric method.
- Soil particle size composition was determined by a sedimentation method with the application of Na- pyrophosphate as a peptisation agent. Removal of organic matter, carbonates and gypsum was not performed during the process (Racz 1971). Based on the soil textural composition, a textural class was determined, according to a Ferre's triangle.

For the purpose of examination of the abundance of soil microorganisms, samples of the horizon organic layer and the humus-accumulative horizon were taken. The determination of soil microorganism abundance was performed on samples in natural wetness condition. Nutrient media were sown by soil suspension 0.1 ccm in 10^{-3} dilution. The sowing was repeated three times, and the number of microorganisms was calculated per 1g of absolutely dry soil. Four different media were used: Čapek's agar (3 gr. $NaNO_3$, 1 gr. KH_2PO_4 , 0.5 gr. KCl, 0.5 gr. $MgSO_4$, 0.01 gr. $FeSO_4$, 20.0 gr. agar, 3.0 gr. sucrose, distilled water up to 1000 ml), MPA (meso-peptonic agar- 41.3 gr. nutrient agar, distilled water up to 1000 ml), Erzbi's agar (0.2 gr. KH_2PO_4 , 0.2 gr. $MgSO_4$, 0.2 gr. NaCl, 0.1 gr. K_2SO_4 , 5.0 gr. $CaCO_3$, 20.0 gr. agar, 20.0 gr. malt sugar (glucose), distilled water up to 1000 ml) and synthetic agar with sucrose (0.5 gr. KH_2PO_4 , 0.5 gr. $MgCO_3$, 0.50 gr. NaCl, 1.0 gr. KNO_3 , 0.01 gr. $FeSO_4$, $CaCO_3$ in excess, 20.0 gr. agar, 25 gr. sucrose, distilled water up to 1000 ml). The media were sterilised in an autoclave at the temperature of $120^\circ C$, pressure of 1.5 at, in duration of 20 minutes. Following the autoclaving, the media were poured in petri dishes. After sowing, all petri dishes were placed in a thermostat at the temperature of $22 \pm 1^\circ C$. The determination of the total number of fungi, bacteria and actinomyces, developed on nutrient media, was performed after 5 and 10 days. The obtained measurement data were presented in graphs and tables, according to their site of origin.

The following physiological groups were determined:

- The number of ammonifying microorganisms on meso-peptonic agar;
- The number of oligonitrofilic microorganisms on Esbhi's agar;
- The number of actinomyces on synthetic agar;
- The number of fungi on Čapek's agar;
- The total number of microorganisms on soil agar;

3. RESEARCH RESULTS

The soil type in the sample plot 1 is luvisol, which is a soil type typical of oak forests. The physical properties of this soil are characterised by a large depth of solum, which in the analysed profile amounts to 100 cm (Table 1).

Table 1. *Soil-particle size composition and textural class*

Horizon	Depth	Coarse sand	Fine sand	Silt	Clay	Total sand	Total clay	Textural class
	cm	%	%	%	%	%	%	
Illimerised soil - Luvisol								
A	0-5	3.70	49.90	25.20	21.20	53.60	46.40	Silty loam
E	5-20	0.60	27.30	34.00	38.10	27.90	72.10	Clay loam
Bt	20-100	0.40	26.10	32.00	41.50	26.50	73.50	Clay
Brown loessial soil - illimerised cambisol								
A	0-2/8	1.00	32.00	42.60	24.40	33.00	67.00	Loam
E	2/8-30	0.90	28.40	44.80	25.90	29.30	70.70	Loam
(B)/Bt	30-100	1.10	28.10	39.00	31.80	29.20	70.80	Clay loam

This is a deep soil of a high capacity for absorption and retention of water. The skeleton is completely absent in the entire depth of solum. The humus-accumulative horizon belongs to a silty loam textural class. It is well water-permeable and aerated. As the soil depth increases, so does the content of clay, while the textural class in the eluvial horizon transforms into clay loam, and in the illuvial horizon it changes into clay. Such profile differentiation, based on textural composition, causes blocking of gravitational water runoff into the deeper layers of soil.

The chemical properties of the analysed luvisol are characterised by moderately acid reaction of the humus-accumulative horizon. As the depth of solum increases, so does the pH value and in the eluvial horizon active acidity of soil solution is in transition between moderately acid and highly acid. In the illuvial horizon the acidity slightly decreases. The adsorptive complex is characterised by a high total adsorption capacity, particularly in the humus-accumulative horizon. Such high total adsorption capacity in the humus horizon is a result of a high content of humus, while in the deeper horizons it is a result of a high content of clay. The sum of adsorbed base cations is highest in the humus-accumulative horizon, which is a result of a release of bases by mineralisation of organic matter. In the eluvial horizon, the sum of basis abruptly decreases and then again increases in the illuvial horizon. The degree of the adsorptive complex base cations saturation follows a similar pattern. According to the content of total humus in the humus-accumulative horizon, the soil belongs to a type with a high content of humus. Still, the thickness of the humus horizon is very low. The content of total nitrogen is high, while the carbon nitrogen ratio in the humus horizon is very broad, which indicates a quite high content of non-decomposed organic matter. As the solum depth abruptly decreases, so does the content of humus and nitrogen, while the C/N ratio is becoming narrower. According to the limit value for AL-method, the content of plant accessible phosphorus forms is within the limits of well supplied. However, it is quite sufficient for the needs of forest tree species. The humus horizon is well supplied with plant accessible potassium forms, while eluvial and illuvial horizons are average supplied.

Table 2. Soil chemical properties

Horizon	Depth	pH		Adsorptive complex					Total		Accessible		
		H ₂ O	KCl	T	S	T-S	V	Y1	humus	N	P ₂ O ₅	K ₂ O	
	cm			ekv.m.mol/100g			%	cm ³	%	%	C/N	mg/100g	
Illimerised soil - Luvisol													
A	0-5	5.96	5.38	109.40	58.01	51.39	53.03	79.06	20.92	0.96	12.58	7.36	31.61
E	5-20	4.46	3.39	41.02	11.78	29.24	28.71	44.98	1.18	0.21	5.73	5.39	14.39
Bt	20-100	4.79	3.54	36.08	16.14	19.94	44.74	30.67	0.91	0.18	5.15	6.03	14.58
Brown loessial soil - illimerised cambisol													
A	0-2/8	4.70	3.59	34.79	13.09	21.71	37.61	33.40	2.10	0.20	10.54	0.00	7.63
(E)	2/8-30	4.88	3.52	28.61	11.78	16.83	41.16	25.90	0.84	0.28	3.03	0.00	4.61
Bt(B)	30-100	5.06	3.60	27.67	15.27	12.40	55.17	19.08	0.53	0.19	2.81	0.00	5.44

At the sample plot at Kosmaj, in a montane beech forest, a brown loessed soil was identified. The solum depth is 100cm. According to the textural composition, the humus-accumulative and cambic horizon belong to a loam class, while the illuvial horizon is composed of clay loam. Although signs of illimerisation are present in the soil profile, the clay transition index is less than 1.5, which classifies this soil as cambic (Škorić et al 1985). The soil is weakly skeletal, well-structured and well-aerated. The water-absorption and retention capacity is high.

The humus-accumulative and eluvial horizons are characterised by a very high acidity of soil solution, whereas the acidity of illuvial horizon is high. The sum of adsorbed base cationes is quite low. According to the degree of adsorptive complex base cationes saturation, the humus-accumulative and eluvial horizons are district, while the illuvial horizon possesses a slightly higher degree of base cationes saturation of 50%, which classifies it as eutric. According to the content of total humus in the humus-accumulative horizon, the soil belongs to a class with a low content of humus. The amount of total nitrogen is high in relation to such a low humus content, which results in a narrow carbon nitrogen ratio. The narrow C/N ratio indicates the intensity of organic matter decomposition processes and the occurrence of rapid transition of plant assimilatives from organic to mineral and plant accessible forms. The quantity of plant accessible phosphorus forms is extremely low and it is below the limit for an AL-method detection, in the entire depth of soil solum. Additionally, the supply of plant accessible potassium forms is very weak in the entire depth of solum.

In the organic layer of beech forest, the abundance of ammonifying microorganisms is nearly equal to the total number of microorganisms on soil agar (Table 3). The equal number of mineralogenic and ammonifying microorganisms indicates that the processes of organic matter decomposition occur in the organic layer, with a part of decomposition inter-products entering into mutual synthesis by creating humus matter, and a part of them is decomposed to final products, whereby plant assimilatives are released in easily soluble and plant accessible forms. In the humus-accumulative horizon of the identical profile, mineralogenic organisms, which use plant assimilatives in the same form as higher plants,

significantly prevail over ammonifying microorganisms. This is a result of spreading of soluble plant assimilatives from the organic layout into the soil surface layers. Oligonitrophile microorganisms are weakly represented, both in the organic layer and in the humus-accumulative horizon. Actinomyces are not identified in the organic layer, i.e., fungi are dominant in dehumidification processes. That indicates that the decomposition of hard-degradable matters, such as lignin or humus, is not performed to the final products and that the decomposition products have an acid reaction. Decomposition processes of hard-degradable matters take place more intensely in the humus-accumulative horizon, where actinomyces, capable of decomposing humus matters, appear in more significant numbers. Decomposition of hard-degradable organic compounds is performed already in the organic layer, where more significant abundance of actinomyces was observed.

Table 3. *Abundance of soil microorganism principal groups (1000 units/1gr dry soil)*

Horizon	Soil agar	MPA	Erzbi's agar	Synthetic agar		Čapek's agar	
	Total number of microorganisms	Ammonifiers	Oligonitrophiles	Actinomyces	Fungi	Actinomyces	Fungi
OP 1 Oak forest							
O lfh	57,761	57,760	17,205	-	31,952	-	3,686
A	151,920	40,902	9,089	5,194	19,477	5,843	25,320
OP 2 Montane beech forest							
O lfh	11,464	93,078	25,280	4,596	29,877	-	16,087
A	71,666	28,779	63,201	-	12,415	-	10,722

In the organic layer horizon of the sample plot at Kosmaj in a montane beech forest, the number of ammonifying microorganisms significantly prevails over the total number of microorganisms on soil agar. That means that a large amount of mineral forms of plant assimilatives are not released in processes of decomposition of organic matter in the organic layer. In the humus-accumulative horizon, the total number of microorganisms on soil agar significantly prevails over the ammonifying microorganisms. This is a result of a higher presence of soil mineral component in surface layers of soil. In comparison to the first sample plot, the total number of microorganisms on soil agar is significantly lower in the beech forest, both in the organic layer and in the humus horizon. That indicates a lower soil fertility in the second sample plot and a lower amount of plant accessible forms of nitrogen, phosphorous and potassium. Fungi prevail in the humus horizon, while actinomyces are not identified either on a synthetic or Čapek's agar. This is a result of a significantly lower humus content in comparison to the first sample plot.

4. DISCUSSION

The rhizosphere is the narrow region of soil that is directly influenced by root and it presents a dynamic system with a specific physical, chemical and biological characteristics. The biological component of this region consists of plant roots and rhizospheral microorganisms. In addition to root secretions, the microbe population in rhizosphere is influenced by soil parameters (soil type, pH, structure,

content of nutrients). Root age also influences composition and diversity of microbe populations. During the period of intensive root growth, bacterial populations are dominant, whereas mature root provides better conditions for development of fungi and actinomyces (Raičević i sar., 2010). The activity of soil microbe communities contributes, in a long-term, to stability and sustainability of ecosystem.

Numerous and diverse microbe populations play the role in a large number of processes, primarily in the processes of organic matter transformation, creation of plant assimilatives, maintaining a soil structure, degradation of pollutants, but also in biological control of plant and animal pathogens. Great abundance of microbe populations and their large diversity enable them a full participation in those processes.

Populations dominant in the soil are those participating in the transformation of easily degradable organic compound, monosaccharides, organic acids, amino acids and, under a large presence of these compounds, dominant fungi genera are *Mucor*, *Pythium*, *Penicillium*, while the dominant bacteria genus is *Pseudomonas*. In the second phase, dominant species are of the genera *Trichoderma*, *Fusarium*, *Chaetomium*, and bacteria *Bacillus* spp., which participate in cellulose and hemicellulose decomposition processes. The third phase is far slower, the transformation of hard-degradable organic matters, lignin and polyphenol takes place in this phase and it is dominated by fungi, primarily basidiomycete, but also cellulolytic fungi *Fusarium* i *Trichoderma*.

Large variances in the diversity of microbe populations occur as a response to edaphic conditions, with excessive humidity stimulating growth of anaerobic bacteria. The increased acidity favours the growth of certain microbe populations, and in current conditions, fungi are generally dominant.

5. CONCLUSION

Based on the conducted investigations, it can be concluded that there are certain similarities, but also differences, in the soil characteristics at the sites in which a mass perishing of gypsy moth occurred, with no treatment by bio-pesticides and chemo-pesticides.

Both investigated soils are characterised by a great depth of solum, high capacity for water absorption and retention, good aeration and favourable oxidising conditions of surface layers. A profile differentiation according to textural composition is evident in both investigated soils.

The chemical properties of soils differ significantly. The acidity of soil solution is particularly pronounced in both sites, but the total content of humus and nitrogen is significantly higher in the oak forest in comparison to beech forest. The soil in the oak stand has a higher total adsorption capacity and the higher sum of adsorbed base cationes. Moreover, it is better supplied with accessible forms of phosphorous and potassium.

The differences in chemical characteristics of the soil, along with the differences in characteristics of beech and oak litter, resulted in differences in the abundance ratio of certain physiological groups of soil microorganisms, both in the organic layer horizon and in the humus-accumulative horizon.

ACKNOWLEDGEMENTS

The study was partly financed by the Ministry of Science of the Republic of Serbia, the Project 31070 – SUBPROJECT: New technological methods in the integral protection of forests with the focus on the entomopathogenic fungus *Entomophaga maimaiga*, as the possible solution to the problem of the frequent occurrences of the outbreak of gypsy moth in the forest ecosystems of Serbia.

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SOIL EXAMINATION FOR THE PURPOSE OF FORECASTING OCCURRENCE OF ENTOMOPATHOGENIC AND BENEFICIAL MICROORGANISMS

Vesna GOLUBOVIĆ ĆURGUZ, Zoran MILETIĆ

Summary

In two sample plots, located at the site of Hungairan Oak and Turkey Oak forest (*Quercetum frainetto cerris*) and at the site of montane beech forest (*Fagetum montanum*), in which a mass natural perishing of gypsy moth larvae occurred in spring of 2011, the principal characteristics of soil – a natural development environment for certain stadia of possible entomopathogenic and other microorganisms, and a potential cause for the collapse, were analysed. Physical and chemical properties, as well as the abundance of principal physiological groups of soil microorganisms, were examined.

Both investigated soils are characterised by a great depth of solum, high capacity for water absorption and retention, good aeration and favourable oxidising conditions of surface layers. A profile differentiation according to textural composition is evident in both investigated soils

The chemical properties of soils differ significantly. The acidity of soil solution is particularly pronounced in both sites, but the total content of humus and nitrogen is significantly higher in the oak forest in comparison to beech forest. The soil in the oak

stand has a higher total adsorption capacity and the higher sum of adsorbed base cations. Moreover, it is better supplied with accessible forms of phosphorous and potassium.

The differences in chemical characteristics of the soil, along with the differences in characteristics of beech and oak litter, resulted in differences in the abundance ratio of certain physiological groups of soil microorganisms, both in the organic layer horizon and in the humus-accumulative horizon.

