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## THE NUMBER OF MICROORGANISMS IN DEPTS IN ALLUVIAL SOIL IN SKOPJE REGION

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### Abstract

The soil represents living and dynamic environment for different types of microorganisms. With their enzyme activity microorganisms participate in the creation of plant nutrients. Thus, microorganisms participate in the creation and maintenance of soil fertility and are considered as biogenic indicators. In this paper, the total number of microorganisms, actinomyces, diazotrophs, fungi and cellulolytic, denitrifying, ammonifying and nitrifying microorganisms was examined in alluvial soil in Jurumleri locality, Skopje. The number of microorganisms was studied at three different depths (0-10 cm, 10-20 cm and 20-30 cm). The examined soil has favorable physical and chemical properties for growth and development of microorganisms. It was found that most of the studied groups of organisms are present at a depth of 0-10 cm, while ammonifying bacteria are mostly present at a depth of 20 - 30 cm.

**Key words:** soil, bacteria, actinomyces, diazotrophs, yeasts, cellulolytic, denitrifying, ammonifying, nitrifying microorganisms, molds.

### Introduction

Soil represents a complex system composed of solid, liquid and gaseous phase. The solid phase consists of organic and mineral matters, liquid of water and substances dissolved in it, and the gaseous phase consists of oxygen, carbon dioxide, nitrogen, hydrogen, ammonia, etc. All three phases are interrelated and their relationship affects fertility and soil microbial activity (Filipovski G, 1974). The quantitative and qualitative composition of soil microorganisms is different in different types of soils and depends on abiotic (temperature, moisture, acidity, content of organic and mineral substances) and biotic factors (microorganisms, plants, animals and humans and their interaction). The number of microorganisms and their activities in the soil is an important indicator of soil fertility since in the soils with formed profile they maintain its structure, the level of organic matter and through the transformation of materials they provide plants with soil nutrients. Microorganisms in the soil are deployed in horizontal and vertical direction, depending on the latitude, climatic factors, soil type, type of microorganisms and according to the depth of the profile and the altitude. Target of this research is to examine the number of different groups of microorganisms by depth of the profile on alluvial soil in the region of Skopje.

### Material and methods

Research are made on alluvial soil in the vicinity of Skopje, particularly in Jurumleri with geographical position of the soil profile on 41 ° 58'20, 84 "north and 21 ° 33'24, 44" east on 276 m altitude. Soil analysis are made for the mechanical composition (Table 1) and chemical properties of the soil (Table 2).

The mechanical composition of the soil depends from the subsoil and the processes that occur during soil genesis and the evolution of soil (Mitrikeski J., Mitkova T, 2006).

In terms of mechanical composition of the soil dominates the fraction sand and dust, which reflects very well on the physical properties of the soil. According to the classification of soils by Scheffer and Schachtschabel, examined soil belongs to the group of loam.

Table 1. Mechanical composition of the soil

Depth in cm	Coarse sand 0,2-2 mm	Cramp (fine) sand 0,02- 0,2	Total	Dust 0,02-0,002	Clay <0,002	Clay + dust
0-20	2.87	56.53	59.40	34.60	6,00	40.60
20-40	3.46	60.24	63.70	34.30	2.00	36.30
40-60	3.09	50.11	53.20	42.40	5.00	47.40
60-80	5.18	41.72	46.90	45.66	7.44	53.10
80-115	1.78	42.15	43.93	45.95	10.12	

Table 2. Chemical composition of the soil

Depth in cm	pH		Total N %	Humus %	CaCO <sub>3</sub>	Readily available nutrients in the soil	
	H <sub>2</sub> O	n KCl				P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
0-20	8.1	7.2	0.1698	2.83	9.9	16.47	20.10
20-40	8.2	7.2	0.0708	1.18	10.83	15.29	18.10
40-60	8.7	7.3	0.066	1.10	11.66	4.31	18.20
60-80	8.7	7.3	0.051	0.85	9.99	5.29	20.40
80-115	8.7	7.3	0.0378	0.63	10.83	3.72	22.10

Based on the presented data about the chemical composition of the soil was determined that the soil according to U.S. classification has neutral to moderately alkaline reaction of the soil solution and in the deeper layers (strata) alkalinity increases. The presence of carbonates in the soil is important because it strongly influences on the chemical composition of the soil, particularly on the reaction of the soil solution, so according to Penkov (1996) the soil belongs to carbonate rich soils.

Examined soil is poor to moderately rich with humus; total N content is in correlation with the level of humus in the soil and its presence decreases with increasing of the depth. The presence of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O in the top layers shows that the soil is middle provided, and in the deeper layers these values are declining.

The samples for microbiological analysis of the soil are taken aseptically on depth of 0-30 cm. The soil samples are taken in spring and are analyzed in the microbiological laboratory of the Faculty of Natural Sciences and Mathematics.

In the soil are studied different groups of microorganisms according to standard methods (Jarak and Govedarica, 1997).

Tests are made with the method of sowing on adequate dilution of soil on selective nutrient substrates and incubation on temperature of 28 °C and the total number of microorganisms and ammonia forming bacteria 3-4 days, nitrifying bacteria (nitric forming bacteria), nitrate forming bacteria and nitrate reducing bacteria 5-7 days, fungi and actinomyces 2-3 days.

The total number of microorganisms in the soil is examined on substrate of soil agar ( $10^{-6}$ ); nitrifying bacteria (nitric forming bacteria) are grown on a substrate esbi agar ( $10^{-6}$ );

nitrate forming bacteria are determined on mineral substrate with composition:  $(\text{NH}_4)_2\text{SO}_4$  2g,  $\text{K}_2\text{HPO}_4$  1g,  $\text{MgSO}_4$  0,5 g,  $\text{FeSO}_4$  0,4 g, NaCl 0,4 g,  $\text{CaCO}_3$  1g,  $\text{MgCO}_3$  1 g, agar 1,5% ,distilled water;

nitrate reducing bacteria are studied on mineral substrate with composition:  $\text{KNO}_3$  2g,  $\text{K}_2\text{HPO}_4$  1g,  $\text{MgSO}_4$  0,5 g,  $\text{FeSO}_4$  0,4 g, NaCl 0,4 g,  $\text{CaCO}_3$  1g,  $\text{MgCO}_3$  1 g, agar 1,5%, distilled water;

ammonia forming bacteria are examined by the method of titer on liquid substrate- nutrient broth, using a reagent- leaves soaked with Krupov reactive which is prepared from one part 3%  $\text{H}_2\text{SO}_4$  and two parts aqueous solution of fuchsin; cellulose splitting microorganisms are studied using carboxyl methyl cellulose ( $10^{-2}$ ); yeasts are tested on the substrate Czapek-Dox Agar with composition:  $\text{NaNO}_3$  2g,  $\text{KH}_2\text{PO}_4$  1g,  $\text{MgSO}_4$  0,5 g, KCl 0,5 g,  $\text{FeSO}_4$  0,1 gr, sucrose 300 g, agar 200 g) ( $10^{-4}$ ); molds are determined on the substrate Czapek-Dox Agar with composition:  $\text{NaNO}_3$  2g,  $\text{KH}_2\text{PO}_4$  1g,  $\text{MgSO}_4$  0,5 g, KCl 0,5 g,  $\text{FeSO}_4$  0,1 g, sucrose 300 g, agar 200 g) ( $10^{-4}$ ); actinomyces are determined on synthetic agar according to Krasilnjnikov (composition:  $\text{K}_2\text{HPO}_4$  5g,  $\text{MgCO}_3$  5g, NaCl 5g,  $\text{KNO}_3$  10g,  $\text{FeSO}_4$  10 g,  $\text{CaCO}_3$  150g, sucrose 200 g, agar 160 g), ( $10^{-5}$ ).

### Results and discussion

The results from the microbiological tests are shown in Table 3.

in the surface layer, and with increasing of the depth their number decreases.

Bacteria are prokaryotic organisms that have a key role in the process of circulation of matter. Fertile soils contain between one hundred million and a billion bacteria in one gram of soil, their biomass is between 300 - 3000 grams per  $\text{m}^3$  of soil, their presence entirely depends on the amount of organic matter, pH, the content of water, air, warmth, light, crop rotation and other agro-technical measures.

The average number of bacteria in the examined soil at a depth of 0-10cm is 88 per gram of soil, at a depth of 10-20cm is 132 per gram of soil and at depth of 20-30 cm is 64. Most bacteria per gram of soil are noted at a depth of 10-20 cm.

Actinomyces are heterotrophic organisms; saprophytes with developed digestive system with ability for decomposition of the organic complex such as humus, chitin, cellulose that for other organisms is difficult to decompose (Waksman, 1945).

Environmental conditions in the soil are suitable for the development of actinomyces and they are most numerous in the surface layer of 0-10 cm.

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Table 3. Number of microorganisms by depth in alluvial soil ( $10^x$  /g soil)

Soil layer (strata)	Total no. of bacteria	Actinomyces	Nitrifying bacteria	Yeasts	Cellulose splitting microorganism	Nitrate reducing bacteria	Molds	Ammonia forming bacteria	Nitrate forming bacteria
0-10 first iteration	81	139	230	70	144	221	16	+++	184
0-10 second iteration	61	193	111	95	180	213	38	++	228
0-10 third iteration	122	161	150	95	134	233	33	+	195
10-20 first iteration	79	22	57	30	77	102	16	+++	137
10-20 second iteration	110	35	80	24	53	110	18	++	107
10-20 third iteration	209	20	88	14	42	82	5	+	83
20-30 first iteration	15	42	74	34	83	93	6	+++	59
20-30 second iteration	36	15	102	45	107	83	11	+++	97
20-30 third iteration	141	66	100	87	52	82	6	++	65

Physiological groups of microorganisms - ammonia forming bacteria, nitrate reducing bacteria, nitrate forming bacteria and nitrifying bacteria (nitric forming bacteria) are important for the circulation of nitrogen, carbon, sulfur, phosphorus and other elements. The ammonia forming bacteria with a role to decompose organic nitrogen compounds are numerous in the examined soil, so at a depth of 0-10 cm and 10-20 cm the number of ammonia forming bacteria is equal, and their number is highest at a depth of 20-30 cm. Microorganisms that oxidize ammonia up to nitrates are nitrate forming bacteria and they are most numerous in the surface layer, with increasing of the depth their number declines. Nitrate reducing bacteria are microorganisms that reduce the nitrates up to elemental nitrogen. They are most numerous in the surface layer of the soil.

Cellulose splitting microorganisms decompose the cellulose in the soil, thus creating oxy-acids that enrich the soil with nitrogen substances. The number of cellulose splitting microorganisms is greatest in the surface layer of 0-10 cm (up to 152 per gram of soil).

Molds grow better in acidic soils, because they are acid-fast, although they are numerous in soils with neutral reaction. The number of molds in the examined soil is higher.

### Conclusions

Based on the results of the research we concluded that:

The mechanical structure and chemical properties of the soil are favorable for development of all studied groups of microorganisms.

The depth of the soil affects the number of microorganisms.

The total number of microorganisms is highest in the soil layer at a depth of 10-20 cm.

The ammonia forming bacteria are most frequent in the soil layer of 20-30 cm, while all other groups of microorganisms – actinomyces, nitrifying bacteria (nitric forming bacteria), nitrate forming bacteria, nitrate reducing bacteria, yeasts, molds and cellulose splitting microorganisms are most numerous in the surface layer of 0-10 cm.

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### **БРОЈНОСТА НА МИКРООРГАНИЗМИТЕ НА РАЗЛИЧНА ДЛАБОЧИНА ВО АЛУВИЈАЛНА ПОЧВА ВО СКОПСКО**

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#### **Апстракт**

Почвата претставува жива, динамична средина во која живеат различни видови микроорганизми. Преку ензимската активност микроорганизмите учествуваат во создавањето на хранливите материи за растенијата. Оттука, микроорганизмите учествуваат во создавањето и одржувањето на плодноста на почвата и претставуваат индикатори на нејзината биогеност. Во овој труд се испитувани вкупниот број на микроорганизми, актиномицетите, азотофиксаторите, габите, целулолиотичките микроорганизми, денитрификаторите, амонификаторите, и нитрификаторите во алувијална почва, во н. Јурумлери, Скопско. Бројноста на микроорганизмите е испитувана во три различни длабочини ( 0-10 см, 10-20см, и 20-30см ). Испитуваната почва има поволни физичко - хемиски својства за живот и развој на микроорганизмите. Констатирано е дека најголем број на испитуваните групи микроорганизми е на длабочина од 0-10 см, а амонификаторите на длабочина од 20 – 30 см.

**Клучни зборови:** микроорганизми, алувијални почви.